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The effects of group II mGluR agonists on short-term depression and neural coding in the rat dentate gyrus *in vitro*.

by

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Thesis submitted for the degree of Doctor of Philosophy at the University of Dublin, Trinity College.

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III Declaration

I declare that this work has not been submitted previously as a thesis for a degree at this or any other institution and that it is entirely my own work. The Trinity College Dublin Library may lend this thesis without restriction.

John Kilbride

John J. Kilbride BSc.

IV Summary

Short-term depression is an activity-dependent reduction in synaptic efficacy which has been observed at a wide variety of synapses in the CNS, and is often attributed to a depletion of the available store of neurotransmitter. At the medial and lateral perforant path-granule cell synapses, short trains of stimuli at a range of frequencies between 0.5 Hz and 200 Hz resulted in a reversible depression of the evoked EPSP amplitude. A steady-state level of short-term depression was attained after 5-10 stimuli. In both pathways the steady-state EPSP amplitude decreased with increasing stimulation frequencies. In the medial perforant path the onset of depression was well fitted (r^2 = 0.935) with a single exponential function over all frequencies tested, with a time constant of decay ranging from 5 ms at 200 Hz to 1470 ms at 0.5 Hz.

The time-averaged postsynaptic response (steady-state EPSP amplitude x frequency), which is a reliable estimate of transmitter mobilisation (Curtis and Eccles, 1960), increased linearly with frequency up to a limiting frequency of 10 Hz - 20 Hz in the medial and 20 Hz - 50 Hz in the lateral perforant paths. Short-term depression was found to impart a gain control mechanism on synapses in the medial perforant path. Specifically, the existence of a limiting frequency reduced the rate coding capabilities of these synapses, in so far as the postsynaptic response to high-frequency stimulation was independent of stimulation frequency for rates above ~20 Hz. This was accompanied by an increased sensitivity to transient rate changes which enables these synapses to respond to relative rather than absolute changes in firing rate and to discriminate preferentially between small fluctuations in the firing rate of slowly as opposed to rapidly firing afferents.

The effect of metabotropic glutamate receptor (mGluR) activation on low frequency evoked EPSPs and on short-term depression was examined. At the test frequency (0.0166 Hz), perfusion of the potent and specific group II

mGluR agonist LY354740, induced a reversible, dose-dependent depression of the EPSP amplitude in the medial perforant path. The EC_{50} value was found to be 115 ± 16 nM whilst the maximal depression observed was ~80 % with a concentration of 10 μM. In the lateral perforant path, L-AP4 induced a dose-dependent reversible depression of synaptic transmission, with an EC_{50} value of 2.4 μM and a maximal depression of ~80 % with 10 μM.

Modulation of short-term plasticity by selective activation of either group II or group III mGluRs was examined over a range of frequencies between 1 and 200 Hz. Short-term depression was not altered by mGluR activation up to 20 Hz and 10 Hz in the medial and lateral perforant paths respectively. At frequencies greater than these, a dose-dependent inhibition of short-term depression was observed. The selective activation of group II and III mGluRs extended the linear range, over which transmitter mobilisation was independent of stimulation frequency, and as such is further evidence of the attenuation of synaptic depression at high frequencies by LY354740 and L-AP4 respectively. The most likely mechanism through which short-term depression is attenuated by the activation of groups II and III mGluRs during HFS, is through a reduction in presynaptic Ca^{2+} influx which leads to a lessening of neurotransmitter depletion and a concomitant enhancement of facilitation. This theory is strengthened by the findings that lowering extracellular Ca^{2+} resulted in a similar reduction of short-term depression to that resulting from activation of group II mGluR.

In the dentate gyrus, long-term depression (LTD) does not rely on presynaptic mechanisms for its induction and did not alter the balance between rate and temporal coding. In keeping with the observed attenuation of short-term depression, activation of group II mGluRs, extended the range over which the coding of synaptic transmission was possible on the basis of a mean firing rate code, whilst reducing the responsiveness to transient changes in firing rate. These observations may point towards a physiological role for presynaptic mGluRs in the living brain.

V Abbreviations

(1S,3S)-ACPD	(1S,3S)-1-Aminocyclopentane-1,3-dicarboxylic acid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxalone proprionic acid
cAMP	cyclic 3',5'-adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CNS	Central nervous system
Cyclothiazide	6-chloro-3,4-dihydro-3-(2-norbornen-5-yl)-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide
DAG	Diacylglycerol
DCG - IV	(2S,2'R,3'R)-2-(2',3'-Dicarboxycyclopropyl)glycine
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
GABA	Gamma-aminobutyric acid
IP3	inositol (1,4,5)-triphosphate
L-AP4	L(+)-2-Amino-4-phosphonobutyric acid
LCCG-1	(2S,1'S,2'S)-2-carboxycyclopropyl)glycine
LFS	Low frequency stimulation
L-SOP	L - serine o - phosphate
LTD	Long - term depression
LTP	Long - term potentiation
L-*trans*-2,4,PDC	*trans*-4-Carboxy-L-proline / L-*trans*-pyrrolidine-2,4-dicarboxylic acid.
LY354740	(+)-2-aminobicyclo[3.1.0]hexane-2-6-dicarboxylic acid
mGluR	Metabotropic glutamate receptor
MSOP	(RS)-alpha-Methylserine-O-phosphate
MSOPPE	(RS)-alpha-Methylserine-O-phosphate monophenyl ester)
NMDA	N-methyl-D-aspartic acid
NO	Nitric Oxide
PI	Phosphatidylinositol

Picrotoxin	(a 1:1 mixture of picrotoxin and picrotin)
PLC	Phospholipase C

Chapter 1

General Introduction

1.1 Preface

One of the most intriguing questions in science is how the brain represents the environment around us. What we do know is that the brain uses a combination of electrical and biochemical signals to represent and convey information internally. What remains more of a mystery is exactly how these signals are encoded to represent an accurate picture of the external world. It does appear that the intervals between the electrical spikes are every bit as important as the spikes themselves. We can only guess at the degree of accuracy which our brains achieve, because we have nothing to compare them against. This illustrates part of the problem facing the scientific community in so far as the instrument we are forced to use in seeking to understand the nervous system, is the very object we wish to comprehend and therefore it's possible we don't have the processing power to understand our own hardware.

The nervous system is huge, there are literally billions of synaptic connections. Trying to describe this connectivity in a definitive manner is near impossible, as many of the connections are constantly changing. Which is why it is all the more remarkable that we have the ability to retain and subsequently reproduce mental impressions years after they were first laid down. Learning is one thing but to subsequently recall is another matter altogether. The ability to do so must necessitate some physical representation in the brain. This is what we mean by an engram or trace. Its existence has only ever been inferred, as no-one has as yet managed to directly demonstrate its existence. The trace cannot be seen, or touched and yet it is apparently stored everywhere throughout the brain, its existence defines our lives, our idea of a self, more than anything else (Rose, 1987).

Synaptic transmission involves dynamic, plastic processes. The fact that some of these processes have been proposed as the cellular correlates of learning and memory (Stevens, 1988; Bliss and Collingridge, 1993; Martinez and Derrick, 1996), in time, may or may not be proven correct. The general public are largely interested in disorders of the mind or of mental processes, that is, they only want to know why things go wrong and how they can be fixed. The greatest incentive to study synaptic transmission lies not in finding out why it all goes wrong but rather in discovering how it works so well for so long.

1.2 Synaptic transmission

Within the CNS, neurons are connected into vast networks at specialised junctions called synapses. Successful transmission of signals across the synapse requires a series of discrete steps. Firstly within the presynaptic terminal the arrival of an action potential (electrical signal) serves as the signal for an influx of calcium via N- P- and Q type voltage-gated calcium channels (Borst and Sakmann, 1996: Igelmund et al., 1996). The consequent rise in the intraterminal Ca^{2+} concentration increases the likelihood that transmitter-containing vesicles will fuse with the cell membrane and release their contents into the synaptic cleft. Subsequently the interaction between the neurotransmitter substance (chemical signal) and specific receptors located on the postsynaptic cell membrane alters transmembrane conductance's and the activity of second messenger systems. Excitatory neurotransmitters, such as glutamate, induce excitatory postsynaptic potentials (EPSPs) in the postsynaptic cell, whereas inhibitory transmitters like GABA induce inhibitory postsynaptic potentials (IPSPs). The textbook idea of an integrate-and-fire neuron maybe somewhat of an oversimplification, as it appears that the digital action potentials are subject to (analogue) linear and non-linear

(amplification, saturation, thresholding) processing once in the domain of the postsynaptic cell (Koch, 1997). Nevertheless the net effect is to alter the postsynaptic membrane potential such that at the cell soma when the threshold for action potential generation is surpassed, a "spike" is produced and propagated both down the axon and back into the dendrites. In this way information is passed from neuron to neuron throughout the neural network.

Implicit in this classical view of chemical synaptic transmission is the assumption that information flows in one direction only, that is from presynaptic to postsynaptic neuron. During the last 30 years, it has become apparent that the processes involved in synaptic transmission are not constant, but are in fact subject to ongoing activity-driven modulation. A prime example of this is the ability of a neuron to modulate the release of neurotransmitter through receptors situated on the presynaptic membrane of either its own or other local neurons (Thompson et al., 1993; Wu and Saggau, 1997). The activation of these "autoreceptors" and "heteroreceptors" might serve to reduce transmitter release and cause synaptic depression or possibly to prevent an excessive release of neurotransmitter, which might prove detrimental to cellular homeostasis (Hille, 1994, Langer, 1997). The hippocampus contains a wealth of presynaptic receptors for many of the major transmitter systems, such as glutamate, GABA, acetylcholine, and adenosine and is therefore an ideal preparation for observing these processes.

1.3 Hippocampus

The hippocampus is a curved mass of cortical tissue and it extends the length of the floor of the temporal horn of the lateral ventricle in the limbic system. The hippocampus consists of Ammon's horn, the dentate gyrus and the subiculum. In all mammals two interlocking c-shaped principal cell layers make up the hippocampus. They are the granule cell layer of the dentate gyrus and the pyramidal cell layer of Ammon's horn. The latter may be subdivided into four subfields: CA1 to CA4 (Cornu Ammonis) (Witter, 1989; Patton and McNaughton, 1995)

The hippocampus has proven to be an attractive area of the brain to study on account of its distinctive and easily identifiable shape and structure. Both the cell bodies and the connecting fibres between the different areas are laminated, that is they are arranged in orderly layers. Furthermore the hippocampus has been known to play a role in memory formation since the 1950's. The study of patients who suffer from a debilitating condition known as anterograde memory impairment, (a condition in which the ability to store new memories is lost, whilst old, established ones remain intact) has shed much light on the role of the hippocampal formation in memory. Patients who have bilateral damage to the hippocampi or have had them removed to treat a severe form of epilepsy, as was the case with the long-suffering patient H.M., report such memory impairment. (Scoville and Milner, 1957; Milner, 1972; Rolls, 1996). The susceptibility of the hippocampus to the neuronal degeneration associated with Alzheimer's disease, anoxia and ischemia as well as its low seizure threshold, as evidenced in patients with epilepsy have all attracted much research interest.

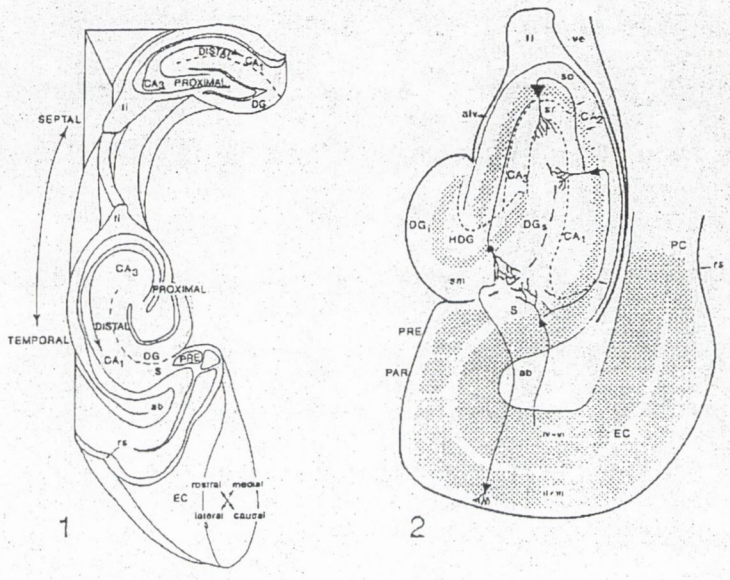
1.3.1 Principal hippocampal neurons

Dentate granule cells

The granule cell layer consists of small (< 10 μM wide) spherically shaped cell bodies, arranged 4 - 6 cells deep. In the rodent it has a distinctive U - shaped structure (Fig.1.1). Each granule cell gives rise to several dendrites, which branch extensively and are densely covered with spines. The dendritic tree extends from the apical portion of the cell body into the molecular layer where connections are made with many afferents from several sources. The axon extends from the basal pole of the cell body towards the hilar region, from where many collaterals emerge. The thin unmyelinated axons are referred to as mossy fibres on account of the characteristic appearance of their synaptic terminals, which exit the hilus and enter stratum lucidum of CA3 where they form synaptic connections. (Witter, 1989; Patton and McNaughton, 1995).

CA3 pyramidal cells

The pyramidal neurons in CA3 are distinguishable from their counterparts in CA1 by the occurrence of large, sometimes branched spines or excrescence's on their proximal dendrites. These excrescence's form synaptic contacts with the mossy fibres originating from the dentate gyrus. The axon emerges from the large cell body and extends through the stratum oriens into the alveus of the hippocampus, with collaterals extending towards the stratum radiatum of CA1, where synapses are formed with the apical dendrites of CA1 pyramidal cells. (Witter, 1989; Patton and McNaughton, 1995).



Abbreviations for Figures

II–VI	cortical layers
ab	angular bundle
alv	alveus
CA1,2,3	subfields of Ammon's horn
DG	dentate gyrus
DG_1	infrapyramidal blade of the dentate gyrus
DG_2	suprapyramidal blade of the dentate gyrus
EC	entorhinal cortex
fi	fimbria
HDG	hilus of the dentate gyrus
hf	hippocampal fissure
PAR	parasubiculum
PC	perirhinal cortex
PRE	presubiculum
rs	rhinal sulcus
S	subiculum
sm	molecular layer
so	stratum oriens
sr	stratum radiatum
ve	lateral ventrical

Fig.1.1 Artist's representation of the hippocampus and entorhinal cortex of the rat. The longitudinal or septotemporal axis, which runs from the septal pole to the temporal pole, is indicated. In the two transverse sections at the temporal and septal extreme the proximodistal axis of Ammon's horn and the subiculum is illustrated. This axis runs from the hilus of the dentate gyrus toward the subiculum. From Witter (1989)

CA1 pyramidal cells

The cell bodies of the pyramidal layer are arranged 3 - 6 cells deep. They are multi-polar neurons with an elaborate dendritic tree extending perpendicular to the cell body layer in both directions. Thick apical dendrites pass through the cell sparse stratum oriens, stratum radiatum and stratum lacunosum-moleculare, where numerous synaptic connections are made with afferent fibres. The axon and basal dendrites originate from the base of the cell body, and after an initial spine-free segment they become richly coated with spines. (Witter, 1989; Patton and McNaughton, 1995).

Interneurons

By and large, interneurons have a restricted axonal plexus, are without dendritic spines and release GABA as their neurotransmitter. At the border between the granule cell and polymorphic cell layers lie at least five types of pyramidal-shaped basket cells. They are the most numerous interneurons in the dentate gyrus region and they synapse back onto the granule cells. Near the pyramidal cell layer, three groups of interneurons predominate: basket cells, axo-axonic cells and bistratified cells. The multisynaptic basket cells surround the soma of the pyramidal cells. Action potential initiation is inhibited by the axo-axonic cells which synapse onto the initial segment of the pyramidal cells. Whilst both apical and basal dendrites are innervated by the bistratified cells. All these cell types extend towards the strata radiatum and oriens, where they form synaptic connections with the excitatory Schaffer collaterals and commissural-associational fibres (Witter, 1989; Patton and McNaughton, 1995).

1.3.2 Trisynaptic circuitry

The basic circuitry of the hippocampus has been known since the time of Cajal at the turn of the century (Cajal, 1901). Arbitrarily, the entorhinal cortex is considered to be the starting point in a unidirectional network of excitatory connections through the dentate gyrus, CA3, CA1 and subiculum. Functionally this means information originating in other cortical areas can enter the hippocampus via the entorhinal cortex, be filtered and processed throughout the hippocampal circuit before being returned to the neocortex via the entorhinal cortex (Jones, 1993).

The primary source of hippocampal afferents is from the entorhinal cortex. Originating in layer II of the entorhinal cortex, the perforant pathway projects through the subiculum to the dentate gyrus and CA3. The perforant path may be differentiated into medial and lateral components. Fibres which originate in the medial entorhinal cortex terminate in the middle one-third (medial perforant path) whilst those that originate from the lateral entorhinal cortex terminate in the outer one-third (lateral perforant path) of the molecular layer of the dentate gyrus (McNaughton, 1980). The fibres originating in layer III of the entorhinal cortex terminate in the dendritic zone of area CA1 and the subiculum. Although this projection is not laminar, it is topographical in nature. The dentate granule cells give rise to mossy fibres which terminate on the proximal dendrites of CA3 pyramidal cells. Furthermore the granule cells also synapse with inhibitory cells in the polymorphic layer, which feed-back onto the granule cells and serve as a brake on cell excitability in the granule cell layer (Jones, 1993). The pyramidal cells of area CA3 project heavily within the CA3 region itself and also to CA1 via the Schaffer collateral afferents, which form synapses within the stratum radiatum and stratum oriens. CA1 pyramidal

cells project to the subiculum and to the deep layers of the entorhinal cortex. (Witter, 1989; Patton and McNaughton, 1995; Jones, 1993).

1.4 Glutamate receptors

1.4.1 AMPA / kainate receptors

Glutamate is the endogenous agonist at the AMPA / kainate, NMDA, and metabotropic glutamate receptors. The AMPA receptor takes its name from the specific agonist, α-amino-3-hydroxy-5-methyl-4-isoxalone proprionic acid, which activates this receptor. AMPA receptors are oligomers; that is the functional receptor may be comprised of either four or five subunits (GluR1 - GluR4) which are coded for by four genes. Each gene can code for two splice variants, namely 'flip' and 'flop'. The topology is similar across all subunits, consisting of a long extracellular N-terminal region and a shorter intracellular C-terminal. There are three hydrophobic transmembrane (TM) domains with an intramembrane loop connecting TM1 to TM2 (Conti and Weinberg, 1999). AMPA receptors open rapidly (< 1ms) although the affinity for glutamate is low (EC_{50} ~ 3 - 30 μM), the deactivation and desensitisation properties of the receptor varies according to its subunit composition, for instance GluR1 and GluR4 deactivate and desensitise more quickly than GluR2. Those receptors that are composed of flop-rich splice variants deactivate and desensitise more quickly than those comprised of flip-rich variants (Conti and Weinberg, 1999). The role of AMPA receptors in mediating fast excitatory neurotransmission in the CNS occurs via the activation of a ligand gated cation channel. AMPA receptors are primarily selectively permeable to Na^+, (although lack of GluR2 confers Ca^{2+} permeability) at the resting membrane potential (-60 mV to -80 mV) they provide the majority of the current responsible for generating synaptic responses. Specific

antagonists at the AMPA receptor include 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2,3-dihydroxy-6-nitro-7-sufamoyl-benzo (F) quinoxaline (NBQX) (Honore et al., 1988; Sheardown et al., 1990).

The existence of another class of glutamate receptor, which is differentiated on the basis of its selectivity for kainate, has been known for many years. However it is only recently that their functional significance has become a little more clearer, due to the development of more specific pharmacological compounds which enable the smaller kainate currents to be teased out from the larger AMPA receptor mediated currents (Lerma et al., 1997; Chittajallu et al., 1999). To date five kainate receptor subtypes have been identified, namely GluR5,6,7 and KA1 and KA2. All share the same topology, with an extracellular terminus, three transmembrane domains (TM1, TM3 and TM4) leading to an intracellular C-terminal domain. Tetanic stimulation delivered to the mossy fibre pathway into the CA3 region in the hippocampus elicits detectable kainate currents, which suggests that the conductance through the channel is very small and is not observable during low frequency test stimulation (Chittajallu et al., 1999).

1.4.2 NMDA receptors

The NMDA receptor takes its name from the specific agonist, N-methyl-D-aspartic acid. The NMDA receptor is composed of either 4 or 5 subunits, for a functional receptor the essential NR1 subunit must be co-expressed with at least one NR2 subunit. The NR2 subunit exists in 4 isoforms, namely (NR2A-D), and this bestows a potential for diversity between NMDA receptors composed of different combinations of subunits. NMDA and AMPA receptors share the same topology; that is an extracellular N-terminal domain, an intracellular C-terminal domain, connected by three

transmembrane domains with a loop between the first and second domains. In comparison to AMPA receptors, the affinity for glutamate is higher at NMDA receptors (EC_{50} ~ 0.4 - 1.7 μM), and although they are slower to open (> 2 ms), once open, they remain open for longer (Conti and Weinberg, 1999). This receptor is coupled to cation channels which allow the flux of Na^+, K^+ and most pertinently, Ca^{2+}, across the cell membrane. However, unlike the AMPA ligand-gated channels, these channels are also voltage-gated, that is they must be depolarised before the Mg^{2+} which blocks the channel at the resting membrane potential is removed. Therefore these receptors are usually activated by depolarisation arising from AMPA receptor-coupled cation channels and the NMDA receptor mediated current then enhances this excitatory synaptic transmission. It is these two properties (high Ca^{2+} permeability and voltage dependent channel block by Mg^{2+}) that underlie its involvement in the induction of LTP. (Collingridge et al., 1983a,b Malenka and Nicoll, 1993).

In addition to the essential glycine binding site (Johnson & Asher, 1987), several co-factors can modulate the channel conductance, for example Zn^{2+} and opioids. (D)-2-amino-5-phosphonopentanoic acid (D-AP5) and (R)-3-(2-carboxypiperazin-4-yl)propyl-l-phosphonic acid ((R)-CPP) (Watkins et al., 1990) act as effective competitive blockers at the NMDA receptor, whilst the polycyclic amine, 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801) is a potent, non competitive, open - channel, NMDA receptor antagonist (Wong et al., 1986).

1.4.3 Metabotropic glutamate receptors (mGluRs)

The classical model of fast excitatory synaptic transmission at glutamatergic synapses involves glutamate acting on ionotropic receptors such as the AMPA and NMDA receptors. During the 1980s, it was discovered that excitatory synaptic transmission could be modulated by glutamate receptors linked directly to second messenger systems via GTP -binding proteins with the discovery that of glutamate receptors coupled to activation of phosphoinositide hydrolysis (Sladeczek, et al., 1985). Prior to this, the prevailing wisdom held that such modulation of cell excitability and synaptic transmission could only occur from extrinsic afferents such as acetylcholine, serotonin and dopamine, all of which activate G -proteins. (Hille, 1994; Conn and Pin, 1997). As glutamate is the ubiquitous principle excitatory neurotransmitter in the brain, and synaptic transmission across glutamatergic circuits is at the heart of a number of physiological processes such as learning and memory, then it is not surprising that a mechanism exists whereby glutamate can regulate its action on ionotropic receptors.

Disorders of glutamatergic transmission may contribute to a number of pathological conditions; Alzheimer's disease, stroke, anxiety, epilepsy, pain (Conn and Pin, 1997). The existence of presynaptic mGluRs offers researchers a target for the development of specific compounds which might selectively alter the imbalance that leads to the pathological condition without disturbing the normal physiological function at glutamatergic synapses. Such compounds have been developed to act at other presynaptically located receptors. For instance mirtazepine is an antidepressant which modulates the release of serotonin and noradrenaline from central synapses (Langer, 1997). The desire to

develop such compounds underlies much of the research interest in this area.

Metabotropic glutamate receptors do not share any sequence homology and are larger than all other G-protein coupled receptors (GPCR). As such they may be regarded as a new family of GPCRs. All mGluRs consist of a large extracellular domain (N-terminal) which houses the glutamate binding site. The intracellular loops separating the seven transmembrane domains (TMDS) corresponds to the G-protein coupling sites. Within the cytoplasm, there is a carboxyl-terminal domain whose function is as yet unknown (Fig. 1.2) (Conn and Pin, 1997).

The functional diversity of the mGluR family is reflected by the identification of 8 subtypes (mGluR 1-8) to date. They have been classified into three groups (I, II, and III) based on their amino acid sequence homology, transduction mechanisms and pharmacological profile.

(I) Group I mGluR

Group I mGluR (mGluR 1 and 5) are selectively activated by 3,5 - dihydroxyphenylglycine (DHPG). They are coupled to phospholipase C which induces the hydrolysis of phosphoinositide (PI) and as a consequence the intracellular levels of IP_3 and DAG are elevated. IP_3 acts on receptors on the intracellular calcium stores, releasing sequestered calcium into the cyctoplasm. DAG activates protein kinase C (PKC) which in turn phosphorylates numerous substances.

Binding studies have shown that mGluR 5 is distributed throughout all dendritic regions of the hippocampus and the strongest immunolabelling is in the dendritic fields of area CA1. In dentate granule and CA3 pyramidal

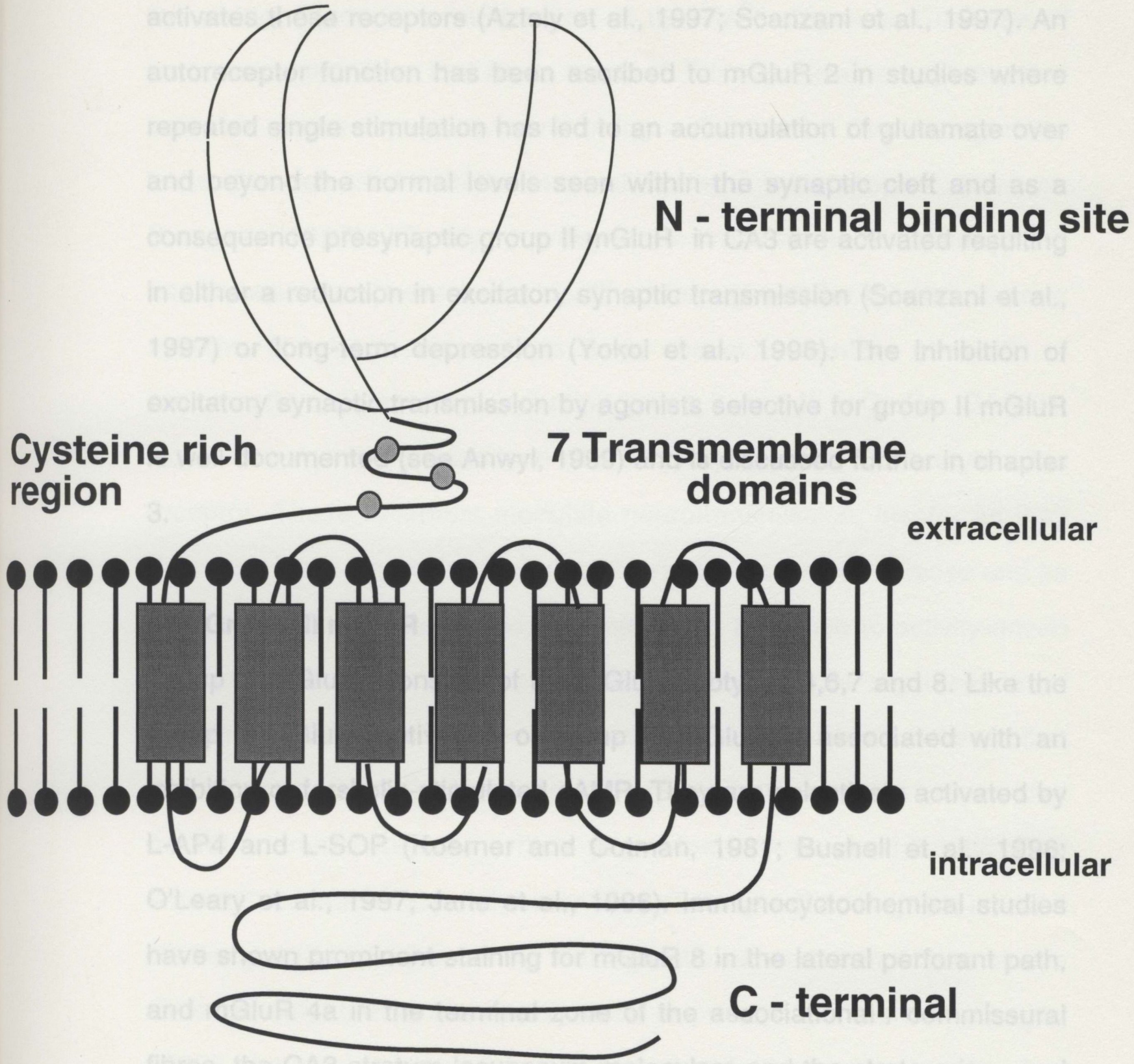
cells the strongest labelling is for mGluR 1, which seem to be localised to postsynaptic elements throughout the hippocampus. (Shigemoto et al., 1997). In the hippocampus, group I mGluRs mediate the inhibition of excitatory synaptic transmission at the Schaffer collateral - CA1 synapses (Gereau and Conn, 1995).

(II) Group II mGluR

Group II mGluR consists of the mGluR 2 and 3 subtypes, which are about 70 % identical to each other in their amino acid sequence. Both receptors are negatively coupled to adenylate cyclase. Although the activation of these receptors with pharmacological agonists is usually associated with an inhibition in forskolin -stimulated cAMP levels (Pin and Duvoisin, 1995), a number of studies have found that cAMP levels are elevated after group II mGluR activation (Reid et al., 1996).

Immunocyctochemical studies have shown that in hippocampus the areas most densely populated with group II mGluR include the neuropil of the stratum lacunosum moleculare (CA1) and the medial perforant path of the dentate gyrus (Shigemoto et al., 1997). Immunocytochemistry for mGluR 2 / 3 has also been found in the neuropil at the mossy fibre terminal zone (Yokai et al., 1996) and the inner layer of stratum lacunosum moleculare of CA3. Immunoelectron microscopy suggests that of the two subtypes, mGluR 3 is present at a lower density in the hippocampus. (Petralia et al., 1996; Shigemoto et al., 1997). In contrast to group III mGluRs, group II (and most likely mGluR 2) mGluRs are often found at the preterminal region of small unmyelinated axons. Indeed they are rarely located at the presynaptic membrane specialisation (Otterson and Landsend, 1997). The location of mGluR 2 away from the release site coupled to the low affinity of these receptors for glutamate make it hard to see how they could be

Fig.1.2 metabotropic glutamate receptor



(From Conn and Pin, 1997)

activated by glutamate released from homologous release sites at low firing rates. One possibly that has been suggested, is that glutamate originating from heterologous presynaptic terminals "spills over" and activates these receptors (Aztely et al., 1997; Scanzani et al., 1997). An autoreceptor function has been ascribed to mGluR 2 in studies where repeated single stimulation has led to an accumulation of glutamate over and beyond the normal levels seen within the synaptic cleft and as a consequence presynaptic group II mGluR in CA3 are activated resulting in either a reduction in excitatory synaptic transmission (Scanzani et al., 1997) or long-term depression (Yokoi et al., 1996). The inhibition of excitatory synaptic transmission by agonists selective for group II mGluR is well documented (see Anwyl, 1999) and is discussed further in chapter 3.

(III) Group III mGluR

Group III mGluRs consists of the mGluR subtypes 4,6,7 and 8. Like the group II mGluR, activation of group III mGluR is associated with an inhibition of forskolin-stimulated cAMP. They are selectively activated by L-AP4 and L-SOP (Koerner and Cotman, 1981; Bushell et al., 1996; O'Leary et al., 1997; Jane et al., 1996). Immunocyctochemical studies have shown prominent staining for mGluR 8 in the lateral perforant path, and mGluR 4a in the terminal zone of the associational / commissural fibres, the CA3 stratum lacunosum moleculare and the strata oriens and radiatum of CA1 (Shigemoto et al., 1997). In contrast to group II mGluR, ultrastructural localisation of immunoreactivity for group III mGluR was predominantly restricted to axon terminals close to the release sites in both glutamatergic and nonglutamatergic synapses (Shigemoto et al., 1997).

1.5 Presynaptic Inhibition

The term presynaptic receptor refers to a receptor located on the presynaptic neuron which when activated by a neurotransmitter or a co-transmitter leads to a modulation of the Ca^{2+} dependent release of neurotransmitter from that nerve terminal. When it became apparent that some of these receptors were being activated by the endogenous transmitter released from the terminal, the term "autoreceptor" was born (Langer, 1997). Today presynaptic receptors for almost all neurotransmitters have been identified throughout the central nervous system. The hippocampus has been shown to contain a wealth of presynaptic autoreceptors including group II and III mGluRs, $GABA_B$, Adenosine A_1, NPY, Dopamine (D2 and D3), and the M2 Acetylcholine receptor. These receptors modulate neurotransmission, insofar as they decrease (or in some circumstances increase) transmitter release and as a consequence alter synaptic transmission in response to activity-driven demands (Wu and Saggau, 1997). The functional significance of presynaptic receptors is not entirely understood although it is believed that they serve to adjust synaptic strength or prevent excessive neurotransmitter release which might lead to cerebral injury. One important consequence of the existence of presynaptic receptors is that it challenged the traditional view that synaptic transmission occurs in one direction only, that is from presynaptic to postsynaptic neuron (Langer, 1997). The mechanism underlying the action of presynaptic receptors has been the subject of much debate over recent years. It appears that the transduction mechanisms which are responsible for the inhibition of neurotransmitter release involve either an inhibition of Ca^{2+} channels or of the exocytotic machinery or alternatively a facilitation of presynaptic K^+ channels. The regulation of synaptic transmission by autoreceptors is frequency-dependent. At low frequencies, presynaptic receptors are

usually inhibitory, that is, they exert a powerful negative feedback effect on synaptic transmission. However at higher stimulation frequencies, their action is less certain. There is a growing body of evidence that suggests that they are less effective at reducing synaptic transmission and may in fact actually enhance it. The action of baclofen on $GABA_B$ receptors at the nMAG (Brenowitz et al., 1998), cultured hippocampal neurons (Isaacson and Hille, 1997) and visual cortex (Varela et al., 1997) or the action of acetylcholine at neocortical synapses (Tsodyks and Markram, 1997), and ventral striatum (Pennartz and Lopes da Silva, 1994) have all been found to display this context - dependent activity.

The inhibitory neurotransmitter GABA plays an important role in stabilising synaptic transmission in the CNS. A loss of GABAergic inhibition may lead to seizure activity as well as interacting with normal brain functions like synaptic plasticity and memory storage (Davies et al., 1993). Presynaptic $GABA_B$ autoreceptors may regulate the release of GABA (Deisz and Prince, 1989) which like other neurotransmitter systems, may or may not be of physiological significance. Nevertheless it is believed that the role of $GABA_B$ autoreceptors is to regulate the level of postsynaptic depolarisation and consequently reduce the likelihood of paroxysmal activity developing (Ben - Ari et al., 1979). The mechanism by which $GABA_B$ receptors cause their effects is unclear. On the one hand there is evidence that they are coupled via G-proteins to the inhibition of presynaptic calcium channels (Isaacson, 1998), in contrast other evidence points towards an action on potassium channels (Andrade et al., 1986). Presynaptic $GABA_B$ receptors like most presynaptic receptors are a potential target for the development of therapeutic agents, which would be hoped to have anxiolytic or anticonvulsant properties.

1.6. Short-term plasticity

Synaptic transmission was once thought to be governed by static processes of fixed strength. Today we know that this is not the case, chemical synapses are dynamic structures whose post-synaptic output is a variable function of both the strength or "weight" of the synaptic connection, determined postsynaptically and the immediate history of the presynaptic firing rate (Markram et al., 1998b) These processes are regulated across both short and long-term timescales (Davis and Murphy, 1994).

Short- term plasticity refers to changes in synaptic efficacy that last from a few milliseconds to tens of seconds and are induced by repetitive synaptic stimulation (Zucker, 1989; Capogna, 1998). Short-term plasticity endows synapses with the ability to make rapid transient alterations in their information processing capabilities and it is for this reason that short-term plasticity is said to have a pivotal role in regulating synaptic communication across the neural network (Koch, 1997; Abbott et al., 1997; Gerstner et al., 1997). At a behavioural level, many creatures depend on short-term plasticity for their survival, for example, short-term depression underlies sensory adaptation, contrast adaptation (Chance et al., 1998) and the habituation of the escape response exhibited by many fish and insects (Zucker, 1989).

Short-term plasticity may be differentiated into processes that cause either a facilitation or a depression in the postsynaptic response to repeated stimulation (Zucker, 1989; Stevens and Wang, 1994). These activity dependent changes occur essentially as a result of fluctuations in the concentration of intracellular calcium in the presynaptic terminal (Fisher et al., 1997). It would be an oversimplification to think that synapses display

one or other of these processes to the exclusion of all others. In the main, they do not, rather a given synapse is likely to display a heterogeneity of release probabilities and therefore may exhibit a blend of facilitation and depression (Rosenmund et al., 1993; Dobrunz and Stevens 1997; Markram et al., 1998b). Depression of excitatory and inhibitory synapses may occur simultaneously albeit to different extents (Varela et al., 1999, Galarreta and Hestrin, 1998). In studies which have employed poisson-distributed spike trains which resemble more closely the natural stimulation paradigms, the dynamic processes of facilitation and depression co-exist, manifested as a postsynaptic potential which rises and falls over time (Varela et al., 1997; Dobrunz and Stevens, 1999).

The balance between depression and facilitation has been shown to be partly dependent on changes in the probability of neurotransmitter release from presynaptic terminals. (Dobrunz and Stevens, 1997, 1999; Tsodyks and Markram, 1997). Experimental conditions which manipulate the release probability affects the time course and expression of short-term plasticity. Reducing the probability of release by lowering extracellular calcium (Varela et al., 1997; Barnes-Davies and Forsythe, 1995; Thies 1965) or by presynaptic inhibition of calcium entry has been shown to reduce synaptic depression and reveal an underlying facilitation (Barnes-Davies and Forsythe, 1995).

1.6.1 Short-term depression

Short-term depression or synaptic depression refers to a gradual decline in the postsynaptic response that is observed during repetitive stimulation (Zucker, 1989). Although depression was first observed at the neuromuscular junction (Del Castillo and Katz, 1954), subsequent studies have shown that it is a characteristic of many central synapses; the

Schaeffer-collateral input to pyramidal cells in area CA1 of the rat hippocampus (Dobrunz and Stevens, 1997; Debanne et al., 1996), CA3 (Scanzani, 1995), dentate gyrus (McNaughton 1980, Debanne et al., 1996), calyx of Held (Von Gersdorff et al., 1997), neocortex (Varela et al., 1997; Abbott et al., 1997; Markram and Tsodyks, 1996; Galerreta and Hestrin, 1998), ventrial striatum (Pennartz and Lopes da Silva, 1994), nucleus magnocellularis (nMAG) (Brenowitz et al., 1998), medial nucleus of the trapezoid body (MNTB) (Wang and Kaczmarek., 1998).

In the 1950s, Del Castillo and Katz (1954) discovered that during a train of stimuli delivered to the neuromuscular junction there is a reduction in the number of quanta or "packets" of neurotransmitter released which leads to the observed short-term depression. This depletion model of synaptic depression is still widely accepted today (Betz, 1970; Kasuno and Landau, 1975; Varela et al., 1997). Contemporary thinking holds that there is a ready releasable pool or store of neurotransmitter available for release after an approaching action potential signals the calcium influx necessary to trigger release. The physical interpretation of this pool is as yet unclear, although there is evidence to suggest that it corresponds to the morphologically defined pool of docked vesicles (Rosenmund and Stevens, 1996; Dobrunz and Stevens, 1997). For depression to occur the initial probability of neurotransmitter release from the presynaptic terminal must be high, if repetitive stimulation results in a depression of the postsynaptic response due to depletion of the available pool of neurotransmitter, then it follows that the probability of release will decline until a steady-state level of synaptic transmission is achieved where the depletion and subsequent recovery processes are balanced (Betz, 1970; Markram and Tsodyks, 1996). Furthermore both the depletion and replenishment of this store of neurotransmitter are exponential processes,

whose time courses are dependent on the frequency of stimulation such that replenishment of this store is faster at high frequencies where depression is greater (Wang and Kaczmarek, 1998).

1.6.2 Release Statistics

At a presynaptic release zone, there are a given number of release sites (N) and the probability that any given site will release a quantum of neurotransmitter is often denoted by (P). It is believed that during depletion, the fraction of release sites loaded with a quantum (one vesicle) drops, therefore the probability of release depends not only on an approaching action potential liberating a docked vesicle (P_A) but also that the quantum is actually filled with transmitter (P_F) (Zucker, 1973). Therefore the probability of release may be expressed in the following way: $P = P_F.P_A$. As such, P should fall during depletion as the fraction of release sites which are primed for release falls. If the number of release sites (N) falls, the explanation maybe that the action potential was either, blocked (signal or conduction failure) or else it did not activate all the release sites. Alternatively a reduction in the number of primed release sites due to transmitter depletion might explain a reduction in the number of release sites (Zucker, 1989; Copogna, 1998)

Other mechanisms which have been proposed to underlie short-term depression include a calcium-dependent adaptation of neurotransmitter release (Hsu et al., 1996) and autoreceptor activation (Forsythe and Clements, 1990; Von Gersdorff et al., 1997). There is some evidence to support postsynaptic AMPA receptor desensitisation (Otis et al., 1996) as an alternative explanation.

1.6.3 Synaptic depression and neural coding

In the cortex, there is a evidence that synaptic depression imparts a "gain control" mechanism whereby afferents that are very active are handled at a lower gain. This ensures that afferents that fire at low frequencies are not drowned out by the noise created by more active afferents. In contrast to inhibitory or adaptive mechanisms, synaptic depression is input specific (Abbott et al., 1997; Koch, 1997; Gerstner et al., 1998). The postsynaptic response increases linearly with presynaptic firing rate, up to a certain limiting frequency, above which the postsynaptic response becomes inversely proportional to the stimulation rate, so that at firing rates greater than this limiting frequency, the time averaged steady-state postsynaptic response becomes independent of firing rate (Tsodyks and Markram, 1997; Abbott et al., 1997; Markram et al., 1998a). Effectively this puts a ceiling on the range of frequencies over which the postsynaptic neuron can decode information carried within the presynaptic firing rate (rate coding). Even though the steady-state postsynaptic response to all firing rates above the limiting frequency is equal, there exists a time-window before synaptic depression takes effect, where the *change* in firing rate is signalled by a transient change in the postsynaptic response. Furthermore the response to fixed percentage rate changes is equal for rates greater than the limiting frequency, this bestows depressing synapses with the ability to respond to fractional rather than just absolute changes in firing rate, analogous to the way the auditory system codes changes in loudness. Fixed amplitude changes are preferentially monitored on slowly as opposed to rapidly firing afferents, which makes common sense as a 2 Hz change is more likely to be represent a significant change in content on a neuron whose average input is 4 Hz then from one which is driven at 50 Hz. (Tsodyks and Markram, 1997; Abbott et al., 1997; Koch 1998; Gerstner et al., 1998) .

1.6.4 Short - term facilitation

Short-term facilitation refers to the increase in the postsynaptic response observed during repetitive stimulation (Zucker, 1989). Although short-term facilitation is common at most synapses, it may only be revealed when a more prominent depression is relieved. (Copogna, 1998). Short-term facilitation may be further differentiated on the basis of its temporal characteristics. If the increase in synaptic efficacy is very brief, in the vicinity of 1 second, then it is usually referred to as just facilitation. Whilst more prolonged increases in efficacy include, augmentation, which has an intermediate lifetime of a few seconds, and post-tetanic potentiation which refers to the increase and subsequent decay of the synaptic response after prolonged stimulation (Zucker, 1989). Both augmentation and post-tetanic potentiation seem to rely on an elevation in the intracellular calcium concentration, which originates from stores which are distinct from the readily-releasable pool of neurotransmitter (Fisher et al., 1997). Although the exact mechanisms underlying facilitation are poorly understood, there is a general agreement that the residual calcium hypothesis is perhaps the most plausible explanation (Katz and Miledi, 1968). Basically this theory as proposed by Katz and Miledi suggested that the relationship between transmitter release and intracellular calcium is not linear, and facilitation results from this. Briefly, an approaching action potential causes Ca^{2+} influx into the presynaptic terminals, this serves as the signal for the exocytotic machinery to release a quantum of neurotransmitter. Afterwards the level of intracellular calcium subsides to the previous level as Ca^{2+} is sequestered into the intracellular stores. However if a second action potential enters the terminal before the intracellular Ca^{2+} level has returned to its resting level then the residual calcium which persists at the

release sites facilitates the fusion of vesicles and subsequent release of neurotransmitter (Stanley, 1986).

1.6.5 Paired-pulse stimulation

As an experimental paradigm, paired - pulse stimulation has proven to be a valuable tool for electrophysiologists over the last 30 years. It involves delivering pairs of stimuli (separated by an interstimulus interval of between 10 and 400 ms) to the preparation and measuring the ratio of the second EPSP / EPSC with respect to the first. (McNaughton, 1980). The paired-pulse ratio is inversely correlated to the release probability, therefore paired-pulse depression/facilitation is indicative of synapses which display a high/low probability of release respectively. In the hippocampus, under normal conditions, paired-pulse depression is observed in the medial perforant path of the dentate gyrus (McNaughton, 1980; Kahle and Cotman, 1993; Kilbride et al., 1998), conversely the lateral perforant path (McNaughton 1980; Harris and Cotman., 1981; Andreson and Hablitz, 1994), CA3 (Scanzani et al., 1997) and Schaffer collateral-CA1 synapses (Nathan and Lambert, 1991; Manabe et al., 1993) are all associated with paired-pulse facilitation. Herein lies one of the advantages of using this stimulation paradigm over single pulses; that is it may be used, in conjunction with other cues, to verify the location from which the electrophysiological recording is being made. This is especially valuable when recording from the neighbouring and possibly overlapping, medial and lateral perforant paths. Furthermore a change in the paired-pulse ratio is indicative of a presynaptic locus of the induction of either short or long-term synaptic plasticity (Kirkwood et al., 1993).

1.7 Long-term plasticity

As communication between neurons occurs at synapses, then this would appear to be the obvious place for neural plasticity to occur. The linkage of synaptic strength to memory storage is an elaboration of the Hebbian synapse: that is when an axon of one cell repeatedly excites another cell, then some biochemical change or growth process occurs in one or both cells, so as to make the first cell more efficient at firing the second cell than before. (Hebb, 1949; Lisman, 1989; Koch, 1997). Over the past thirty years, two experimental models; long - term potentiation (LTP) and long - term depression (LTD) have become widely accepted as the cellular correlates of learning and memory. For instance in the rat amygdala, training by pairing a conditioned stimulus (an audible tone) with an unconditioned stimulus (a footshock) subsequently led to a potentiated response following the conditioned stimulus alone, in a manner which was similar to associate LTP (Rogan et al., 1997). Furthermore in slices taken from the lateral amygdala, fear conditioning was found to induce LTP of synaptic currents (McKenna and Shinnick - Gallagher, 1997).

1.7.1 Long-term potentiation

Long-term potentiation (LTP) is defined as a stable, relatively long - lasting (hours *in vitro*, days or weeks *in vivo*) enhancement in synaptic transmission that occurs throughout the CNS, following a brief conditioning period of presynaptic stimulation (Malenka et al., 1989; Malinow, 1994). In 1973 Bliss and Lomø were the first to induce this phenomenon in the rabbit dentate gyrus *in vivo*. Since then LTP has been induced in all areas of the hippocampus as well as other brain regions (Nicoll et al., 1988; Bliss and Collingridge, 1993; Robertson et al., 1996; Bear, 1995; Malenka and Nicoll, 1993; Malinow, 1994). LTP is typically induced by delivering brief high-frequency stimulation to the afferent input (50 - 100 pulses at a

frequency > 100 Hz). Alternative protocols include theta - burst stimulation (several short trains of 4 pulses at 100 Hz every 200 ms) (Larson et al., 1986) and pairing a short train of high - frequency presynaptic stimulation with intracellular depolarisation (Gustafsson et al., 1987). LTP can be NMDA receptor dependent or independent, homosynaptic (where the LTP is confined to the synapses that have received the conditioning stimulus) and heterosynaptic (where the LTP is not), all share the three basic properties of co-operativity, associativity, and input specificity. Co-operativity means that there is a certain threshold number of afferent inputs which must be active in order to produce LTP, i.e. weak tetani alone will not induce LTP (McNaughton et al., 1978). However a weak input may elicit LTP if it is active at the same time as a strong input, this is what is meant by associativity (Levy and Steward, 1979). Finally the input specificity of LTP ensures that only inputs that are active at the time of the tetanus will undergo LTP (Andersen et al., 1977). Regardless of the protocol employed, the period after the induction of LTP is divisible into a number of distinct stages. Universal agreement on what constitutes the different stages of LTP (as well as what cellular events underlie them) is not forthcoming; for example researchers who work *in vivo* may not agree that the relatively short experiments carried out *in vitro* constitute LTP at all. Nevertheless it is generally agreed that the potentiation immediately following the tetanus results from a surge in the amount of neurotransmitter substance released and is known as post-tetanic potentiation (PTP). Potentiation lasting up to 10 - 15 minutes may be regarded as short-term potentiation (STP) and anything longer than 10 - 15 minutes is LTP (Bliss and Collingridge, 1993).

1.7.2 Long-term depression

Long-term depression (LTD) is a long lasting decrease in synaptic efficacy which follows certain types of electrical stimulation or pharmacological manipulation (Bear and Abraham, 1996). Functionally it is the converse to LTP and as such has been embraced as part of the cellular model of learning and memory (Bear, 1996; Stevens, 1998). LTD in tandem with LTP endows neural networks with the ability to modify synaptic strength in both directions; specifically it allows depotentiation of previously potentiated synapses and prevents a saturation of synaptic strength by LTP. LTD has been reported at several synapses *in vitro* including in the hippocampus: CA1 (Dudek and Bear, 1992; Mulkey et al., 1992), CA3 (Yokai et al., 1996), and the medial perforant path of the dentate gyrus (O' Mara et al., 1995) and in the cerebellum (Boxal et al., 1996). The induction of LTD *in vivo* has proved to be more problematic, with several groups finding conflicting results (Manahan-Vaughan, 1997). Although several forms of LTD have been described on the basis of the stimulation paradigm used: homosynaptic, heterosynaptic, associative and cerebellar LTD all appear to result from an increase in the postsynaptic intracellular calcium concentration (Mulkey and Malenka, 1992; Debanne et al., 1994; Reyes and Stanton, 1996). Homosynaptic LTD in the medial perforant path is usually induced with a low-frequency stimulation protocol of 900 pulses delivered at 1Hz or by pairing mild postsynaptic depolarisation (~ 40 - 50 mV) with brief low frequency stimulation (20-60 pulses at 1 - 10 Hz; Wang et al., 1997).

Chapter 2

Materials and Methods

2.1 Animals

The regulations as issued by the Department of Health on the use of animals in scientific research were followed throughout the course of these experiments. Male Wistar rats weighing between 40-100g (3 and 5 weeks) were used throughout this study. The animals were supplied by the BioResources Unit, Trinity College Dublin. Every effort was made to ensure that the conditions under which the animals were kept, remained stable on a day to day basis. The animals were housed in a cage containing 5-10 rats, with food and water available at all times. The animal house in the BioResources unit was on a twelve hours light / dark cycle, with a constant temperature of 21°C - 23 °C.

2.2 Artificial cerebro-spinal fluid (ACSF)

The incubation and experimental solution contained the following unless otherwise stated (in mM): NaCl, 120; $NaHCO_3$, 26; NaH_2PO_4, 1.25; KCl, 2.5; Mg_2SO_4, 2; $CaCl_2$, 2; glucose, 10. The pH was adjusted to 7.35 - 7.45 by gassing with 95 % O_2 / 5 % CO_2. The saline was prepared each morning before experiments commenced.

2.3 Preparation of slices

Rapid decapitation was achieved using a guillotine without anaesthesia. To begin with, the excess skin and muscle were cut away from the skull using a pair of dissecting scissors. An incision was then made at the base of the skull, which facilitated its removal with a pair of forceps. Subsequently a second incision along the midline of the skull was made to allow the removal of the remaining bones with the forceps. Following this the dura was cut using a pair of spring scissors before removing the cerebellum with a scalpel. The brain was removed quickly and submerged in cold (< 5°C) oxygenated (95 % O_2 / 5 % CO_2) saline for the remainder

of the dissection. This part of the dissection was accomplished within 1.5 mins. The brain was trimmed to size and fixed on a stage with superglue before being placed in the cutting chamber of a Campden Vibroslice. The vibroslice was set at a fast (8) vibration speed and a slow (2) advance speed. On the first advance, the top 4mm of the brain was removed and discarded, exposing the hippocampus. Subsequently several transverse hippocampal slices, cut to a thickness of 350 μm, were removed. These were then transferred to and maintained at room temperature in an submersion type chamber (Medical Systems Corporation, New York) with normal ACSF, gassed with a mixture of 95 % O_2 / 5 % CO_2 passing over them.

2.4 Electrophysiological recording

2.4.1 Experimental set-up

Slices were placed in a purpose built submersion type recording chamber with a volume of 0.5ml and secured down with a nylon mesh. The saline was heated to 32°C, unless otherwise stated, via a stirring heater device (Grant Instruments, Ltd. Cambridge, UK) and gassed with a mixture of 95 % O_2 / 5 % CO_2 for 30 minutes before being delivered to the slice via a gravity feed system at rates of up to 5ml per minute. The slices were visualised under illumination, with a Nikon microscope with a magnification of 25 X, which was set on a steel plate on a wooden table and surrounded by a Faraday cage. The recording and stimulation electrodes were manoeuvred and positioned with two Narishige (Japan) manipulators.

2.4.2 Electrodes

The bipolar stimulating electrodes were made by twisting two strands of insulated tungsten wire (diameter 0.01mm) together. The recording electrodes were low resistance (~ 1MΩ) glass microelectrodes which were pulled using a Flaming / Brown micropipette puller, P-87 (Sutter Instrument Corp. CA., U.S.A.). The recording electrodes were filled with normal ACSF.

2.4.3 EPSP recording

The peak amplitude of the field or population excitatory postsynaptic potential (EPSP) was used as an index of excitatory synaptic transmission in the hippocampus. Both the recording and stimulation electrodes were positioned in the medial / lateral perforant paths of the dentate gyrus or area CA1 of the hippocampus, at a distance of 1-2mm from each other. EPSPs were evoked by a brief single square wave voltage pulse (0.1ms in duration). The stimulation intensity was adjusted to evoke a test EPSP which was about 30 % (1 - 1.5mV) of the maximal EPSP amplitude at the test frequency (either 0.033Hz or 0.0166Hz). These stimulation pulses were generated by either a Master - 8, eight channel, programmable pulse generator (A.M.P.I., Jerusalem, Israel) or a Grass S-48 stimulator (Grass Instruments, Quincy, Mass., U.S.A.) which were driven by IBM compatible PC and Apple Macintosh computer protocols respectively. The stimulation pulses were fed through a stimulation isolation unit before entering the stimulating electrode, to isolate the electrode from ground. The EPSPs were monitored on the screen of a storage oscilloscope (Neltronic Ltd., Dublin 12, Ireland), whilst records of EPSPs were generated by pCLAMP 6 computer software (Axon instruments Inc. Foster city, CA, USA) or Maclab Scope V3.5.6 software (AD instruments Ltd., Hastings, U.K.). The EPSPs were amplified by a Grass P16 microelectrode DC amplifier (Grass

Instruments, Quincy, Mass., U.S.A.), converted from analogue to digital form (Axon instruments, Digidata 1200 or a MacLab/2e, AD/DA converter) before being stored on either a Dell dimension 466 personal computer or a Macintosh Performa 450 for subsequent off - line data processing.

2.5 Data analysis and Statistics

The "normalised" EPSP was calculated by normalising every value in a particular condition to the 1st EPSP amplitude in that condition. However in chapters 4 and 5, where short trains of 10-20 stimuli were delivered to the medial and lateral perforant paths of the dentate gyrus, the EPSP (%) was calculated by expressing each EPSP as a percentage of the 1st control EPSP. Data was analysed using student's paired t-test and analysis of variance (ANOVA) at the 5 % level of significance.

2.6 Compounds

Unless otherwise stated the compounds used in this study were dissolved into the ACSF directly. The vehicle used to dissolve picrotoxin was dimethyl sulphoxide (DMSO), however the final concentration of the solvent was less than 0.05 % and no effect on synaptic transmission was observed at that concentration. Cyclothiazide was dissolved in absolute ethanol, LY354740, MSOP, MSOPPE, L-AP4 and L-trans-PDC were dissolved in freshly made NaOH (0.1N). After all final dilutions were made, there was no observable change in the pH of the ACSF.

Cyclothiazide:	Tocris Cookson
DCG - IV:	Tocris Cookson
L-AP4:	Tocris Cookson
L-*trans*-2,4,PDC:	Tocris Cookson
LY354740:	Eily Lily, (Indianapolis)
MSOP:	Tocris Cookson
MSOPPE:	Tocris Cookson
Picrotoxin:	Sigma

Chapter 3

Neurophysiological studies of the presynaptic inhibitory action of the group II metabotropic glutamate receptor agonists, LY354740 and DCG-IV, in the rat hippocampus, *in vitro*.

3.1 Abstract

Electrophysiological studies were carried out on the presynaptic inhibitory action of the group II metabotropic glutamate receptor (mGluR) agonists (+)-2-aminobicyclo[3.1.0]hexane-2-6-dicarboxylic acid (LY354740) and ((2S,2'R,3'R)-2-(2',3'-Dicarboxycyclopropyl)glycine) (DCG-IV) in three paths of the rat hippocampus *in vitro*, the medial and lateral perforant paths to the dentate gyrus, and the Schaffer collateral/commissural path to CA1. LY354740 caused a dose-dependent reversible inhibition of the field excitatory postsynaptic potential (EPSP) in the medial and lateral perforant paths, with an EC_{50} of 115 ± 16 nM and 230 ± 58 nM respectively. Maximal inhibition by LY354740 was much greater in the medial path (about 80 %) than in the lateral path (about 50 %). No inhibition was observed in field recordings from the Schaffer collateral input to CA1. A presynaptic locus of inhibition was confirmed by LY354740 inducing dose-dependent changes in paired-pulse depression / facilitation. DCG-IV had a similar action to LY354740, but with a lower potency.

3.2 Introduction

As the principal excitatory neurotransmitter in the mammalian central nervous system, glutamate plays a pivotal role in a number of physiological and pathological conditions. Although glutamate has long been known to activate the AMPA and NMDA ionotropic receptors (iGluRs), thus altering transmembrane conductances through cation channels, it was only in the 1980s that glutamate was observed to activate a family of receptors which are linked to second messenger systems via g-proteins. These metabotropic glutamate receptors (mGluRs) have been differentiated into 3 groups on the basis of their pharmacology, signal transduction pathways and molecular biology.

Group I (mGluRs 1 and 5) mGluR are linked to phosphoinositide hydrolysis, group II (mGluRs 2 and 3) and group III (mGluRs 4,6,7 and 8) are negatively coupled to adenylate cyclase and are further differentiated on the basis of their sensitivity to pharmacological agonists. The activation of mGluRs has been associated with a range of physiological and pathological effects at both presynaptic and postsynaptic locations throughout the CNS (reviewed by Conn and Pin, 1997). Presynaptic inhibition of excitatory synaptic transmission by mGluRs was first established in hippocampal CA1 using the non-selective agonist trans-aminocyclopentane-1,3-dicarboxylic acid (t-ACPD) (Baskys and Malenka, 1991; Desai and Conn, 1991). Since then a number of studies established that presynaptically located mGluRs perform a similar function in other brain regions, such as the hippocampal dentate gyrus (Macek et al., 1996; Bushell et al., 1996), Striatum (Lovinger, 1991) and the neocortex (Sladeczek et al., 1993; Burke and Habilitz, 1994).

The presynaptic locus of inhibition was established on the basis of the following observations. Firstly no postsynaptic changes were associated with the mGluR induced inhibition (Lovinger, 1991; Baskys and Malenka, 1991; Burke and Hablitz, 1994). Secondly, t-ACPD did not have a differential effect on either the AMPA or NMDA receptor mediated components of excitatory synaptic transmission. This would suggest that mGluR activation was depressing synaptic transmission by reducing the level of glutamate release from the presynaptic terminals (Baskys and Malenka, 1991; Lovinger, 1991). Thirdly, a change in a form of short-term plasticity known as paired-pulse depression / facilitation is indicative of a presynaptic modulation of neurotransmitter release. Several studies have shown that mGluR agonists enhanced paired-pulse facilitation in CA1 (Baskys and Malenka, 1991); CA3 (Kamiya, 1996) and the lateral perforant path of the dentate gyrus (Kahle and Cotman, 1993; Dietrich et al., 1997) whilst paired-pulse depression in the medial perforant path was reduced (Brown and Reyman, 1995; O'Leary et al., 1997).

The development of pharmacological agents selective for mGluR subtypes has revealed the presence of presynaptic group II mGluR in the hippocampus. In young CA1; (1S,3S)-1-aminocyclopentane-1,3-dicarboxylic acid (1S, 3S) - ACPD (Vignes et al., 1995) and DCG-IV (Yokai et al., 1996) have been shown to depress excitatory synaptic transmission, although these receptors appear to be absent in the mature CA1 (Kilbride et al., 1998). In the medial perforant path, (2S,1'S,2'S)-2-carboxycyclopropyl)glycine LCCG-1 (Ugolini and Bordi 1995); DCG - IV (Macek et al., 1996; Huang et al., 1997) and LY354740 (Kilbride et al., 1998) have all been shown to reduce excitatory synaptic transmission.

These findings have been supported by binding studies which have localised mGluR 2 and 3 to presynaptic elements within the hippocampus via subtype specific antibodies, in particular the medial perforant path of the dentate gyrus was found to be densely populated with what appears to be mGluR 2 (Shigemoto et al., 1997).

LY354740 is a recently synthesised high affinity efficacious and selective group II mGluR agonist. (Bond et al., 1997: Monn et al., 1997; Schoepp et al., 1997a,b). LY354740 suppressed forskolin-stimulated cAMP production at group II mGluR with a nanomolar potency, but crucially it did not have any agonist or antagonist action at groups I and III mGluRs. Interest in LY354740 is partly because it has potential as a therapeutic tool in a number of conditions. LY354740 counteracted the haloperidol-induced muscle rigidity in an animal model of Parkinson's disease, (Konieczny et al., 1998). An anti-convulsive action against ACPD-induced limbic seizures was reported by Monn and his colleagues in 1997. Furthermore it has been shown to have anxiolytic activity in the fear potentiated startle and elevated plus maze models, without producing the unwanted secondary effects such as memory impairment, that established agents such as Diazepam produce. (Helton et al., 1998; Monn et al., 1997).

In this study, I have investigated the presynaptic inhibitory action of LY354740 in the medial and lateral perforant paths of the hippocampal dentate gyrus, as well as area CA1, comparing its action against that of the established group II mGluR agonist DCG-IV.

3.3 Materials and methods

3.3.1 Slice preparation

All experiments were carried out on hippocampal slices obtained from Wistar rats (50 -70g) (BioResources Unit, Trinity College, Dublin, Ireland). Slices were obtained as described previously (Huang et al., 1997). Briefly, the brain was rapidly removed after decapitation and placed in cold (5°C) oxygenated (95 % O_2, 5 % CO_2) artificial cerebro-spinal fluid (ACSF) containing in mM: NaCl, 120; $NaHCO_3$, 26; NaH_2PO_4, 1.25; KCl, 2.5; Mg_2SO_4, 2; $CaCl_2$, 2; glucose, 10). Hippocampal slices (350 μM) were cut using a Campden vibroslice (Campden Group Instruments, London, U.K.) and transferred immediately to an incubation chamber, maintained at room temperature, for a period of at least 60 minutes. Single slices were then transferred to a submersion type recording chamber at 30-31°C.

3.3.2 Electrophysiology

Field excitatory postsynaptic potentials (EPSPs) were recorded using standard glass electrodes filled with ACSF. Both recording and stimulating electrodes were placed in either the middle or outer third of the molecular layer of the dentate gyrus in order to stimulate and record from either the medial or lateral perforant path respectively, and in the Schaffer collateral / commissural path in the stratum radiatum of CA1. Test EPSPs were evoked using a Grass S48 stimulator (0.0166 Hz, pulse width 0.1 ms) via a bipolar insulated tungsten wire electrode, adjusted to give about 30 % of the maximal response (~1 mV). EPSP amplitude was measured using Maclab Scope v3.5.6. Stimulation and recording from the medial or lateral perforant pathway was verified by the use of paired-pulse stimulation (interstimulus interval 40 ms). Paired-pulse depression (~30 %) was indicative of the medial perforant path, and paired-pulse facilitation (~15

%) was indicative of the lateral perforant path. Further verification was obtained by observing the switch from "source" to "sink" upon moving the recording electrode from one pathway to the other, and the pharmacological selectivity of each pathway was used as a post-experimental cue to the identity of the recorded pathway.

3.3.3 Data analysis and statistics

For each EPSP pair, the second EPSP amplitude was divided by the first and multiplied by 100 to give the paired-pulse "percentage". A paired-pulse percentage of less than 100 was indicative of paired-pulse depression whereas a percentage greater than 100 was indicative of paired-pulse facilitation. The effect of LY354740 in the lateral perforant path and CA1 are presented in this way, in the text and in figures 3.3 and 3.5. However the effects of LY354740 and also DCG - IV on paired-pulse depression in the medial perforant pathway have been normalised to the baseline or control period. This was done so that the dose-dependent change in paired-pulse could be easily visualised in a figure displaying four concentrations of an agonist. Summarised results are expressed as normalised EPSP mean amplitude ± S.E.M. Data was analysed using student's paired t-test, and repeated measures analysis of variance (ANOVA) at the 5% level of significance. Dose-response curves were constructed using Graphpad Prism software where curves were best fitted using a one-site binding hyperbola $Y=B_{max}*X / (K_d+X)$ where B_{max} is the maximal binding and K_d is the concentration of ligand required to reach half-maximal binding.

3.3.4 Compounds

All experiments in the dentate gyrus were carried out in the presence of 100 μM picrotoxin to block $GABA_A$ receptor-mediated inhibition. Drugs were added directly to the perfusate after establishing a steady baseline. Each slice was exposed to only one concentration of either LY354740 or DCG-IV except in some of the experiments carried out using LY354740 in the lateral perforant pathway, when cumulative doses were tested. LY354740 was a generous gift from Eli Lily, USA. DCG-IV was obtained from Tocris Cookson.

3. 4 Results

3.4.1 LY354740 and DCG-IV inhibit the EPSP in the medial perforant path.

The placement of the recording and stimulating electrodes in the medial perforant path was verified by the presence of paired-pulse depression of EPSPs in response to paired-pulse stimulation. After establishing a stable amplitude test EPSP for at least 20 minutes, bath application of LY354740 (20-25 min) caused a dose-dependent and reversible inhibition of the EPSP amplitude in the medial perforant path. The approximate threshold concentration of LY354740 , which induced a small inhibition of the EPSP amplitude of 11 $\pm$ 4 % ($P < 0.05$, $n = 5$) was 10 nM. LY354740 at higher concentrations of 100 nM and 300 nM reduced the EPSP amplitude by 36 % $\pm$ 2 % ($P < 0.05$, $n = 7$) and 62 $\pm$ 6 % ($P < 0.05$, $n = 4$) respectively (Fig. 3.1B). Maximal inhibition of 85 $\pm$ 3 % ($p < 0.05$, $n = 4$) was evoked with 5 μM LY354740. The dose-response curve for LY354740 (Fig. 3.6A) was best fitted with a one-site binding hyperbola. The EC_{50} was estimated to be 115 $\pm$ 16 nM from this dose-response curve (see methods and legend).

Evidence that the LY354740-evoked inhibition of the EPSP amplitude was presynaptic was shown by an accompanying reduction in paired-pulse depression. In control media, EPSPs evoked in pairs, at an interval of 40ms, resulted in paired-pulse depression of about ~16 %. Paired- pulse depression was reduced by LY354740 in a dose-dependent manner. Thus 10 nM, 100 nM, 300 nM and 5 μM caused absolute percentage changes in paired-pulse depression of 8 $\pm$ 4 % ($P < 0.05$, $n = 5$), 21 $\pm$ 12 % ($P < 0.05$, $n = 4$), 56 $\pm$ 9 % ($P < 0.05$, $n = 4$) and 78 $\pm$ 11.7 % ($P < 0.05$, $n = 5$) respectively (Fig. 3.1C).

Perfusion of DCG-IV also resulted in a dose-dependent and reversible inhibition of the EPSP amplitude, although less potently than LY354740. In the presence of 100 nM, 200 nM, 500 nM and 5 μM DCG-IV, the EPSP amplitude was reduced by 21 ± 4 % ($P < 0.05$, $n = 6$), 36 ± 2 % ($P < 0.05$, $n = 8$), 47 ± 3 % ($P < 0.05$, $n = 7$) and 82 ± 4 % ($P < 0.05$, $n = 4$) of control values respectively (Fig. 3.2B). The EC_{50} value was estimated to be 317 ± 54 nM from the dose-response curve of figure 3.6A fitted with a one-site binding hyperbola. Similar to that observed with LY354740, the inhibition of the test EPSP by DCG-IV was accompanied by a reduction in paired-pulse depression. For example, in 100 nM, 200 nM, 500 nM and 5 μM, the absolute percentage changes in paired-pulse were 11 ± 3 % ($P < 0.05$, $n = 6$), 29 ± 6 % ($P < 0.05$, $n = 7$), 42 ± 8 % ($P < 0.05$, $n = 7$) and 89 ± 19 % ($P < 0.05$, $n = 4$) respectively (Fig. 3.2C).

3.4.2 LY354740 and DCG-IV inhibit the EPSP in the lateral perforant path.

The placement of the recording and stimulating electrodes in the lateral perforant path was verified by the presence of paired-pulse facilitation of EPSPs in response to paired-pulse stimulation. Both LY354740 and DCG-IV inhibited the EPSP in the lateral perforant path, but with a lower potency and lower maximal inhibition than in the medial perforant path. The EPSP amplitude was significantly reduced by 15 ± 2 %, 28 ± 3 %, 46 ± 4 % and 48 ± 4 % in 100 nM, 500 nM, 1 μM and 5 μM LY354740 respectively (Fig.3.3B). All values were significant ($P < 0.01$, $n = 5$) when tested using a repeated measures analysis of variance. The EC_{50} value was estimated to be 230 ± 58 nM from the dose-response curve (Fig. 3.6B). A dose-dependent increase in paired-pulse facilitation accompanied the LY354740-induced depression of the EPSP amplitude, in the lateral perforant path. In control conditions the paired - pulse facilitation was 7 ±

4%. This increased to 15 ± 4 %, 20 ± 4 %, 25 ± 3 % and 25 ± 4 % in 100 nM, 500 nM, 1 μM and 5 μM respectively. All values were significant when tested using the repeated measures analysis of variance ($p < 0.01$, $n = 5$) (Fig. 3.3C).

DCG-IV also inhibited the EPSP in the lateral perforant path, but as was the case in the medial perforant path, with a lower potency than LY354740. Perfusion of DCG-IV reduced the EPSP amplitude by 7 ± 3 %, 16 ± 5 %, 17 ± 5 % and 39 ± 7 % in 100 nM, 200 nM, 500 nM and 5 μM respectively. All values were significant when tested using the student's paired t-test ($p < 0.01$, $n = 5$) (Fig. 3.4B). The estimated EC_{50} value was 334 ± 69 nM (Fig. 3.6B). In a manner similar to LY354740, the increase in paired-pulse facilitation with DCG-IV was relatively small and was dose-dependent. DCG-IV caused an absolute percentage increase in paired-pulse facilitation of 7 ± 4 %, 9 ± 4 %, 13 ± 7 % and 14 ± 6 % in 100 nM, 200 nM, 500 nM and 5 μM respectively. All values were significant when tested using the student's paired t-test ($p < 0.01$, $n = 5$), (Fig. 3.4C).

3.4.3 LY354740 has no effect on EPSP amplitude in CA1.

LY354740 was applied at a concentration which was sufficient to maximally depress synaptic transmission in both pathways of the dentate gyrus (2 μM). At this concentration LY354740 had no discernible effect on the EPSP amplitude, 98.9 ± 2.6 % ($P > 0.5$, $n = 4$, Fig. 3.5).

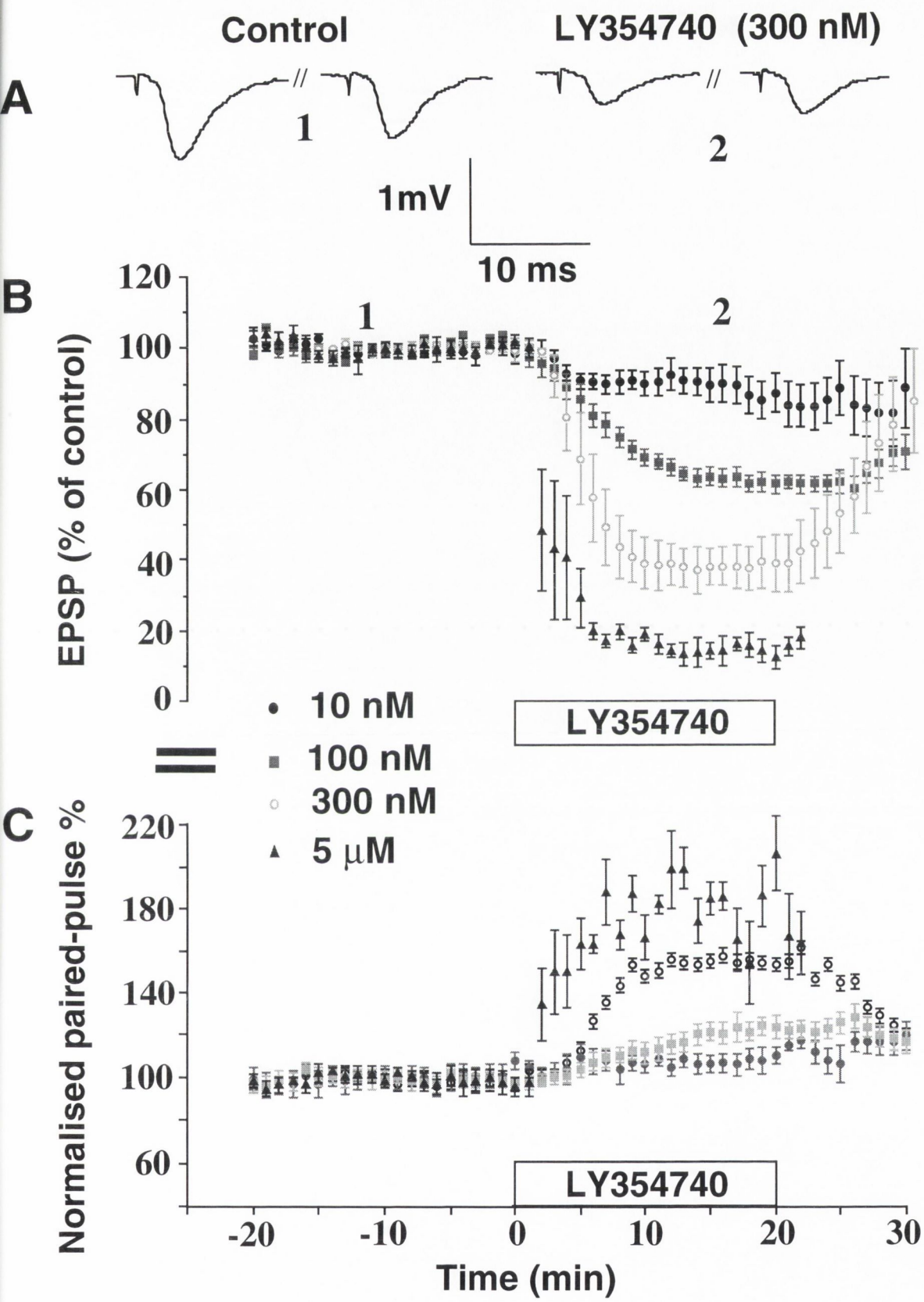


Figure 3.1 LY354740 evokes a dose-dependent inhibition of excitatory synaptic transmission evoked in the medial perforant path of the dentate gyrus *in vitro.* (A)Typical example of pairs of EPSPs (25 Hz) recorded at 0.0166 Hz in control (left) and in the presence of 300 nM LY354740. (B) LY354740 at doses of 10 nM, 100 nM, 300 nM and 5 μM resulted in an increasing inhibition of the field EPSPs. (C) LY354740 at doses of 10 nM, 100 nM, 300 nM and 5 μM caused a dose-dependent reduction in (normalised) paired-pulse depression.

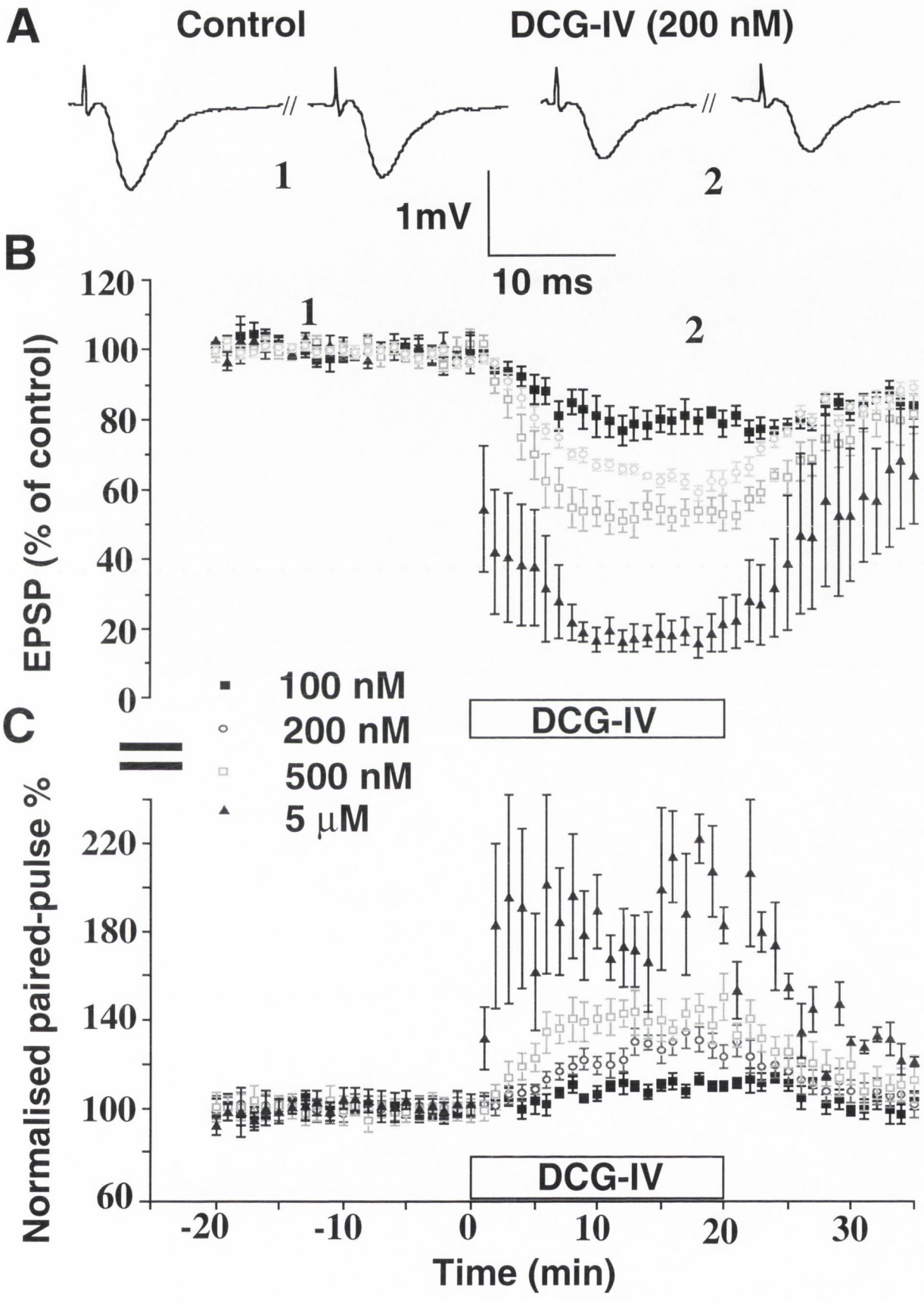


Figure 3.2 DCG-IV evokes a dose-dependent inhibition of excitatory synaptic transmission in the medial perforant path of the dentate gyrus *in vitro*. (A) Typical example of pairs of EPSPs (25 Hz) recorded at 0.0166 Hz in control (left) and in the presence of DCG-IV (200 nM) (B) DCG-IV at doses of 100 nM, 200 nM, 500 nM and 5 μM resulted in an increasing inhibition of the field EPSPs. (C) DCG-IV 100 nM, 200 nM, 500 nM and 5 μM caused a dose-dependent reduction in (normalised) paired-pulse depression.

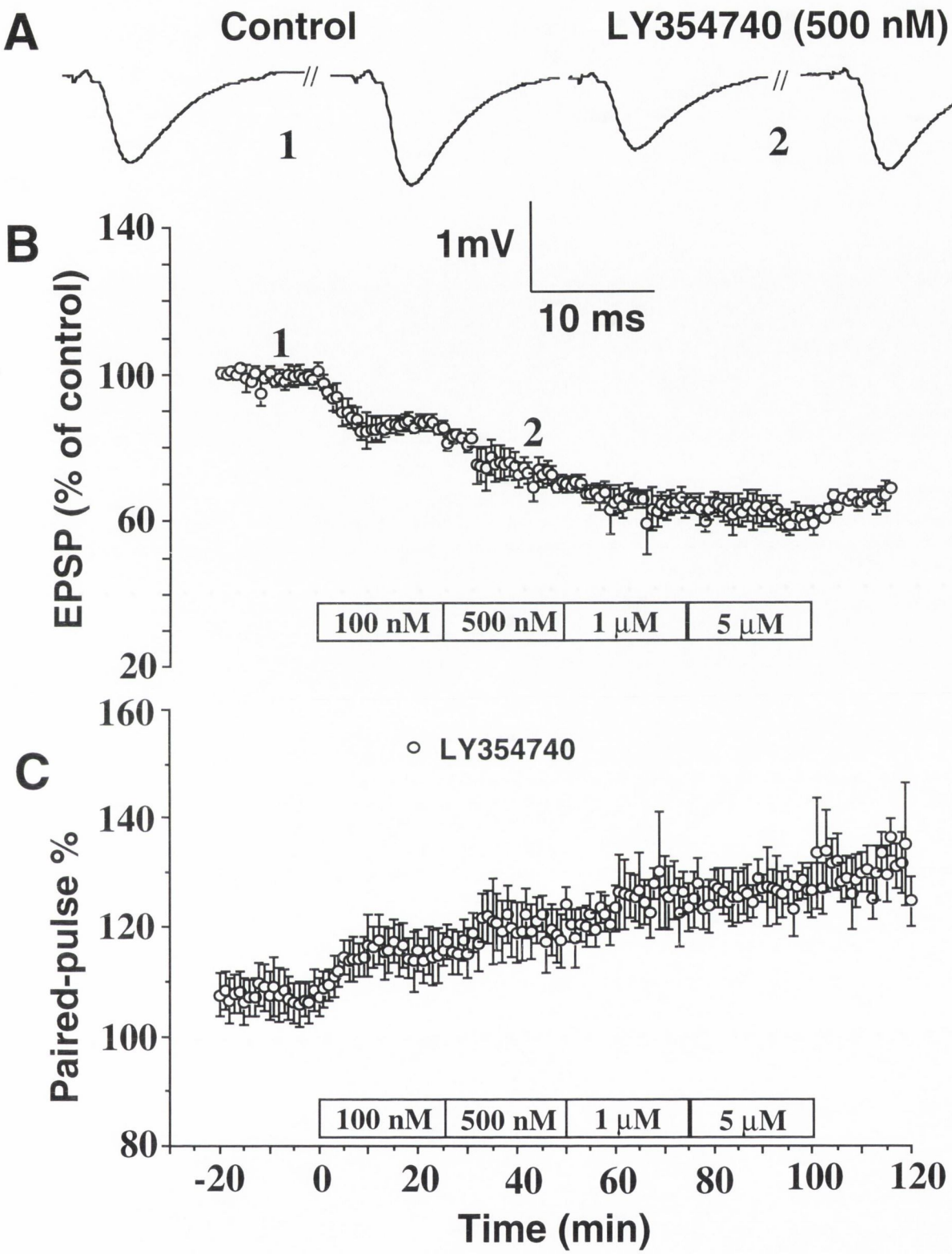


Figure 3.3 LY354740 causes a dose-dependent inhibition of excitatory synaptic transmission evoked in the lateral perforant path of the dentate gyrus *in vitro*. (A) Typical example of pairs of EPSPs (25 Hz) recorded at 0.0166 Hz in control (left) and in the presence of LY354740 (500 nM). (B) LY354740 100 nM, 500 nM, 1 μM and 5 μM caused a dose-dependent reduction in the EPSP amplitude. (C) LY354740 100 nM, 500 nM, 1 μM and 5 μM caused a dose-dependent increase in the absolute level of paired-pulse facilitation.

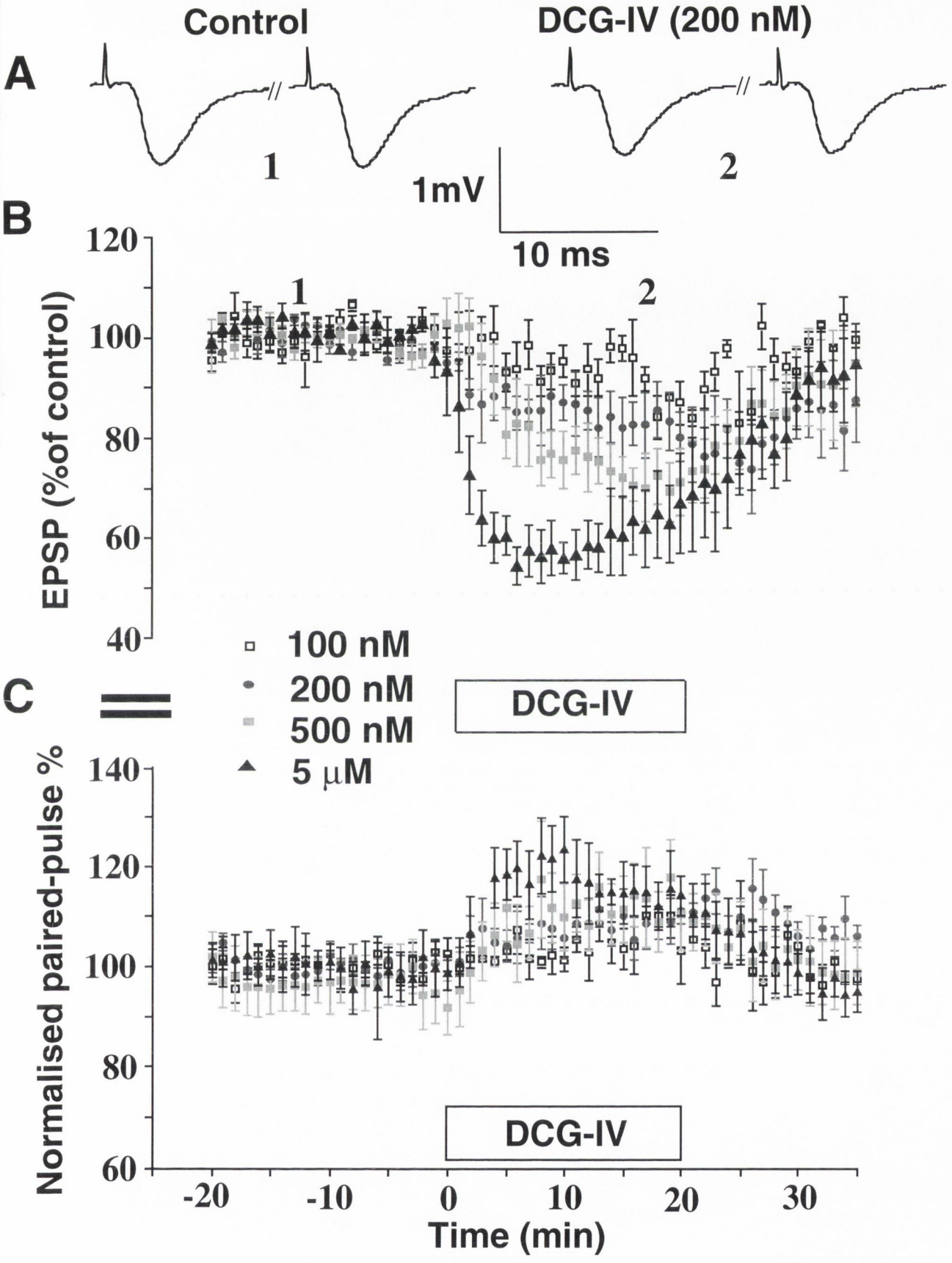


Figure 3.4 DCG-IV causes a dose-dependent inhibition of excitatory synaptic transmission evoked in the lateral perforant path of the dentate gyrus *in vitro*. (A) Typical example of pairs of EPSPs (25 Hz) recorded at 0.0166Hz in control (left) and in the presence of DCG-IV (200 nM). (B) DCG-IV 100 nM, 200 nM, 500 nM and 5 μM caused a dose-dependent reduction in the EPSP amplitude. (C) DCG-IV 100 nM, 200 nM, 500 nM and 5 μM caused a dose-dependent increase in (normalised) paired-pulse facilitation.

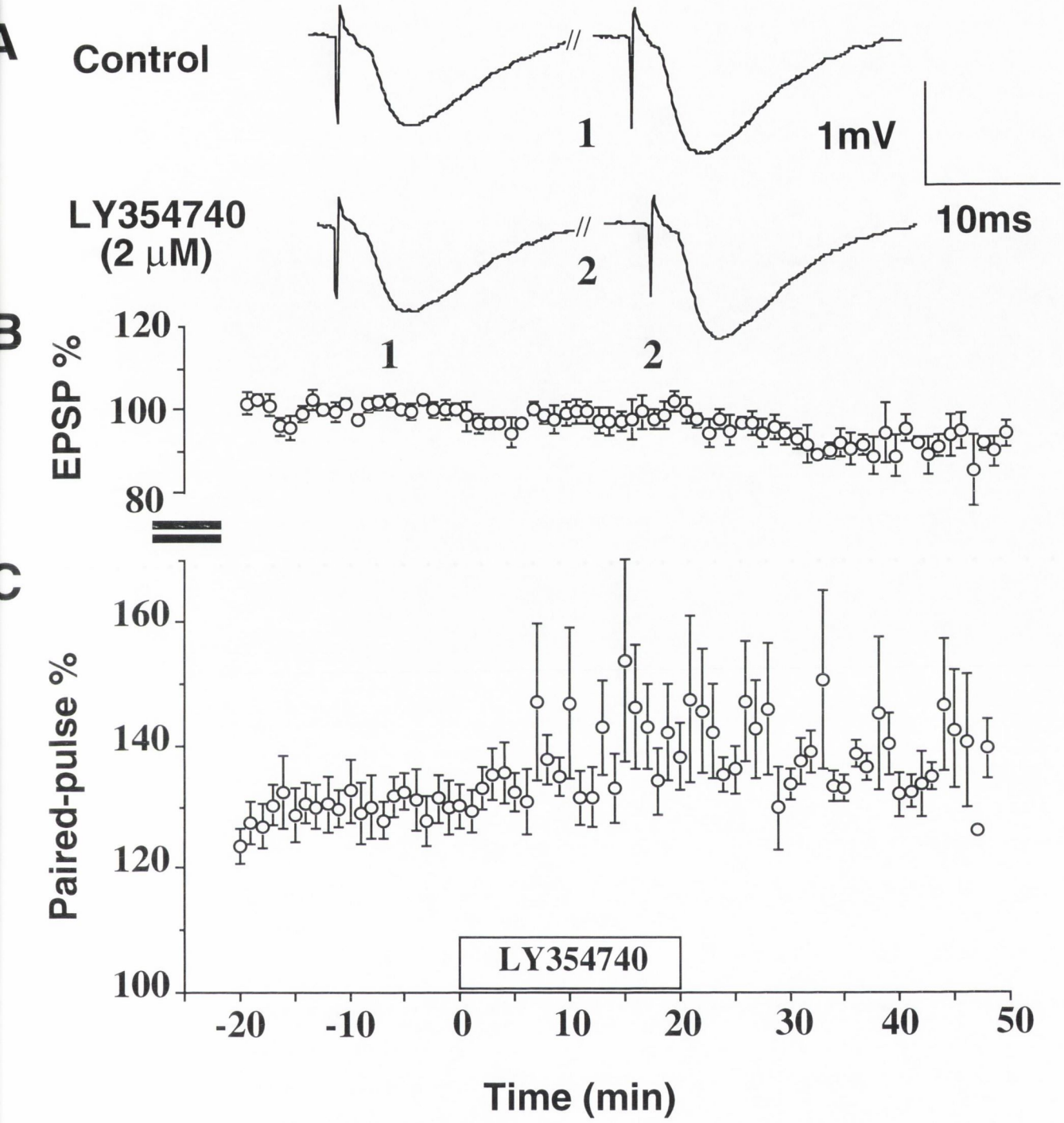


Figure 3.5 LY354740 does not produce an inhibition of excitatory synaptic transmission in the CA1 hippocampus *in vitro*. (A) Typical example of pairs of EPSPs (25 Hz) in control (top) and after perfusion with LY354740 (2 μM) (bottom) (B) LY354740 (2 μM) does not inhibit the EPSP amplitude in CA1. (C) LY354740 does not produce a significant change in paired-pulse facilitation in CA1.

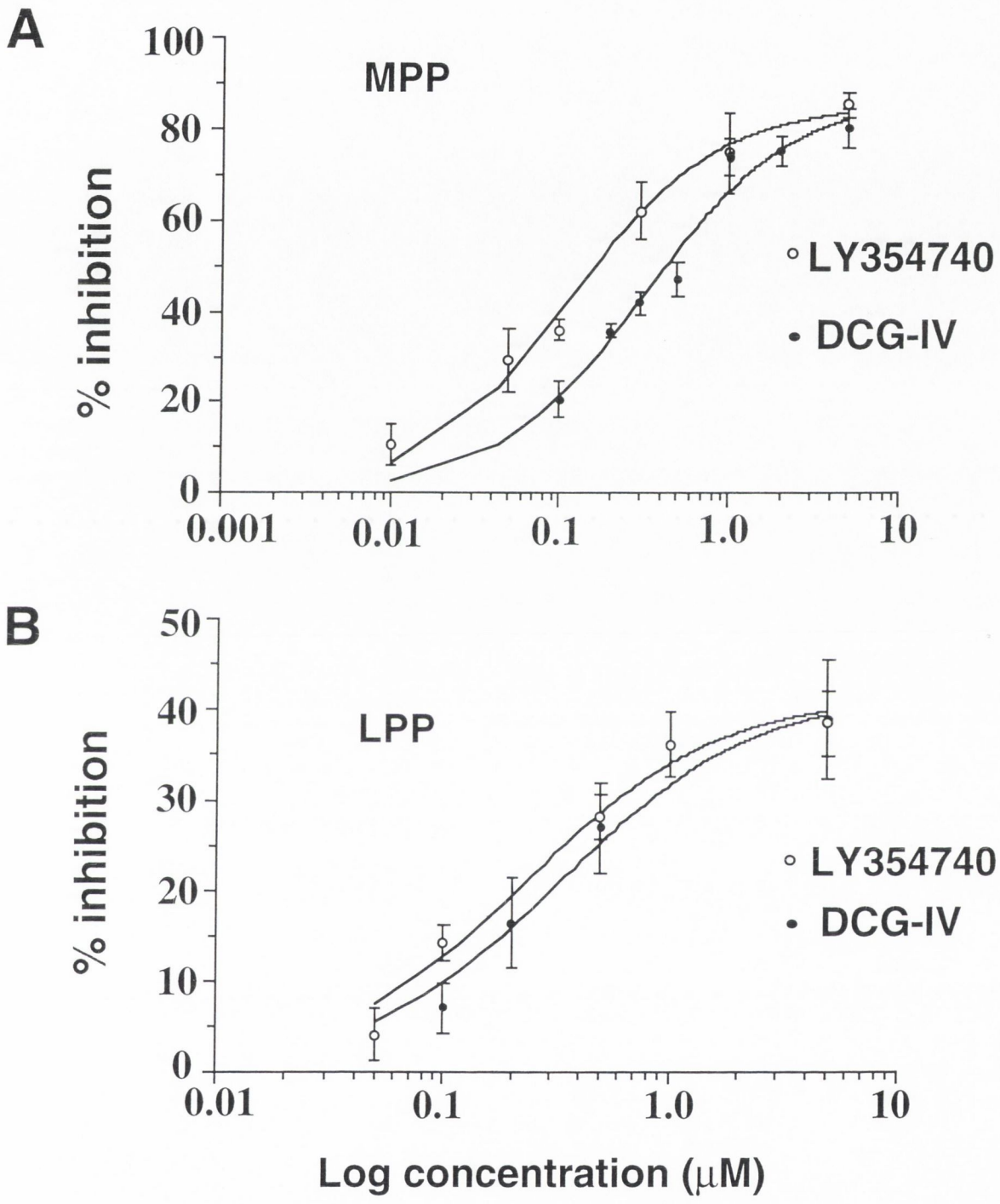


Figure 3.6 Dose - response curves for the action of LY354740 and DCG-IV in the medial and lateral peforant paths. The dose-response curves were best fitted with a one-site binding hyperbola (Graphpad Prism software)Y = Bmax * X / (Kd + X) where B max is the maximal binding, and Kd is the concentration of ligand required to reach half-maximal binding. The EC50 for LY354740 was estimated to be 115±16nM and 230 ±58nM in the medial (A) and lateral (B) perforant paths, respectively. The EC50 for DCG-IV was estimated to be 317±54nM and 334 ± 69nM in the medial (A) and lateral (B) perforant paths, respectively. Each point plots the mean ± SEM values for 4-8 slices.

3.5 Discussion

The results of this electrophysiological study suggest that at present, LY354740 is the most potent agonist at group II mGluR. This subgroup of the mGluR family has been shown to mediate presynaptic inhibition of the EPSP in the medial perforant path *in vitro* (Anwyl, 1999). In this study at the medial perforant path-granule cell synapses the threshold dose at which LY354740 reduced synaptic transmission was about 10 nM and the EC_{50} value was estimated to be 115 ± 16nM. Thus in this pathway LY354740 compares favourably with other established group II mGluR agonists; such as DCG-IV, which has been shown to have an EC_{50} value close to 300nM (present study), while LCCG-1 has an EC_{50} of 30 μM (Ugolini and Bordi, 1995). The electrophysiological data presented here is in agreement with neurochemical studies which have suggested that LY354740 is the most potent group II agonist synthesised, with an EC_{50} value of 5 nM and 24 nM for the inhibition of forskolin-stimulated cAMP at expressed human mGluR 2 and mGluR 3 respectively (Schoepp et al., 1997) and a similar potency for group II mGluR in the rat cerebral cortex and hippocampus (Monn et al., 1997; Schoepp et al., 1997).

In the lateral perforant path, LY354740 and DCG-IV were found to induce a dose-dependent reversible depression of the EPSP amplitude, however this pathway was less sensitive to the agonists. The EC_{50} values for LY354740 and DCG-IV in the lateral perforant pathway were 230 ± 58 nM and 334 ± 69 nM respectively, values higher than in the medial perforant path. Furthermore the maximal inhibition observed in the lateral perforant path was only 40 - 50 %, as opposed to 80 - 90 % in the medial perforant path. The most plausible explanation for the different sensitivity displayed by these two pathways to these agonists, is that there is a lower density of

group II mGluR in the lateral perforant path (Shigemoto et al., 1997). A precedent for this differential mGluR-induced depression in medial and lateral perforant paths has been previously reported in the literature, in 1996 Conn's group showed that in the adult rat, the extent of inhibition by activation of group II mGluR differed between the medial and lateral perforant paths. However they also found a lower sensitivity to DCG-IV in both pathways, with an EC_{50} of 1.6 μM and > 3 μM for the medial and lateral perforant paths respectively (Macek et al., 1996). This discrepancy made be due to the fact that in that study they used much larger rats (100-150g) than in this present study (50-70g), and a developmental decrease in the group II mGluR sensitivity has been previously reported in CA1 (Vignes et al., 1995; Shigemoto et al, 1997). Nevertheless in this laboratory, no developmental decrease in the sensitivity to group II mGluR agonists has been observed in the medial perforant path. Previous electrophysiological studies with DCG-IV have failed to induce any presynaptic depression of excitatory synaptic transmission in the CA1 region of the rat hippocampus (Gereau and Conn, 1995; Breakwell et al, 1997). In this study LY354740 had no effect in CA1 and confirms neurochemical studies which have found that group II mGluRs are not located in this area in any great numbers (Shigemoto et al., 1997).

The inhibition of the EPSP amplitude by LY354740 and DCG-IV was accompanied by a change in paired-pulse depression / facilitation. Such changes demonstrate that the group II mGlu receptor agonists caused a decrease in the presynaptic probability of transmitter release. The most likely receptor mediating the presynaptic inhibition demonstrated in the present study is mGluR 2. Several immunohistochemical studies have shown that mGluR 2 is located at a very high density on perforant path axons in the molecular layer of the dentate gyrus, especially in the medial

perforant path (Ohishi et al., 1993; Neki et al., 1996; Petralia et al., 1996; Shigemoto et al., 1997).

Although mGluR 3 is only present at a much lower density in the hippocampus (Petralia et al., 1993), it is possible that this receptor is responsible for the observed inhibition. The mechanism by which the mGluR-mediated inhibition occurs is the subject of ongoing speculation (Anwyl, 1999). It has been proposed that activation of group II mGluR may lead to the suppression of a presynaptic Ca^{2+} current (Wu and Saggau, 1994) or even a direct inhibition via the transmitter release machinery (Burke and Hablitz, 1994; Gereau and Conn, 1995; Hille, 1994). There is evidence that the mGluR 2 are located preterminally, rather than at the release site and corresponding Ca^{2+} channels, therefore it is possible that they are linked to the opening of a K^+ channel (Sladeczek et al., 1993; Cochilla and Alford, 1998). In theory this might reduce the duration or amplitude of the presynaptic action potential and as a consequence, Ca^{2+} influx would fall, resulting in a decrease in the probability of neurotransmitter release and a consequent presynaptic inhibition of excitatory synaptic transmission. It is my opinion based on these findings and other unpublished observations that the mechanism by which group II mGluR mediated inhibition occurs is most likely via the suppression of a presynaptic Ca^{2+} current.

Chapter 4

Presynaptic group II mGluR modulation of short-term plasticity in the dentate gyrus.

4.1 Abstract

Modulation of short-term plasticity by the activation of groups II and III metabotropic glutamate receptors (mGluR II and III) was investigated in the medial and lateral perforant paths respectively in the hippocampal dentate gyrus *in vitro*. Brief trains of stimulation (10 stimuli at 1-200 Hz) evoked short-term depression of field EPSPs in the medial and lateral perforant paths, with a steady-state depressed level being attained by the end of the train. The steady-state level of depression was much stronger in the medial than the lateral path. The curve relating transmitter release per unit time to frequency increased linearly up to a certain limiting frequency, but flattened at higher frequencies. The limiting frequency was higher in the lateral (20 Hz - 50 Hz) than the medial (10 Hz - 20 Hz), correlating to the much greater steady-state level of short-term depression in the medial perforant path. Activation of groups II and III mGluR in the medial and lateral perforant paths, reduced short-term depression above a certain frequency, 20 Hz and 10 Hz respectively, and shifted the limiting frequency to higher values in the medial and lateral perforant paths following maximal activation of group II and III mGluR.

4.2 Introduction

Synaptic depression is an activity-dependent reduction in synaptic efficacy which has been observed at a wide variety of synapses in the CNS (reviewed by Zucker, 1989); at the Calyx of Held (Von Gersdorff et al., 1997), neocortex (Varela et al., 1997; Abbott et al., 1997; Markram and Tsodyks, 1996; Galerreta and Hestrin, 1998), ventrial striatum (Pennartz and Lopes da Silva, 1994), nucleus magnocellularis (nMAG) (Brenowitz et al., 1998), medial nucleus of the trapezoid body (MNTB) (Wang and Kaczmarek., 1998), cultured hippocampal neurons (Isaacson and Hille., 1997; Maki et al., 1995; Dobrunz and Stevens., 1997) and hippocampus (Scanzani et al., 1995; Wu and Saggau, 1995). This depression is widely believed to result from a reduction in the amount of neurotransmitter released from presynaptic terminals. One theory which has gained widespread support is that the depression results from a depletion of a readily-releasable store of neurotransmitter (Betz, 1970; Kasuno and Landau., 1975; Dobrunz and Stevens, 1997) although other mechanisms which have been proposed include; a calcium-dependent adaptation of neurotransmitter release (Hsu et al., 1996), presynaptic autoreceptor activation (Forsythe and Clements, 1990; Von Gersdorff et al., 1997) and postsynaptic AMPA receptor desensitisation (Otis et al., 1996).

The dentate gyrus provides an excellent experimental model with which to study short-term depression and its modulation by the activation of presynaptically located mGluRs. This is because the unique laminar structure of the hippocampus ensures that the two distinct afferent pathways from layer II of the entorhinal cortex to the granule cell layer can be easily recognised and accessed (Witter, 1989). Experimentally the medial and lateral perforant paths have been differentiated on the basis of

their response to paired stimuli (McNaughton 1980; Kahle and Cotman, 1993; Harris and Cotman, 1983; Kilbride et al., 1998). Under normal physiological conditions or experimentally, when the extracellular calcium / magnesium ratio is one to one, the medial perforant path displays more depression and less facilitation than the lateral perforant path in response to paired-pulse paradigms (Kilbride et al., 1998). This is likely to result from different release probabilities between the two pathways and as such one would expect longer trains of stimuli to further reveal the differential expression of short-term plasticity between the two pathways.

Short-term plasticity may be modulated by experimentally manipulating the release probability from presynaptic terminals (Varela et al., 1997; Tsodyks and Markram, 1997), again the dentate gyrus proves an ideal preparation for this since it has been shown to contain different presynaptic mGluR subtypes. In the medial perforant path, binding studies have revealed a high density of group II mGluRs, in contrast to the lateral perforant path where there is a predominance of group III mGluRs (Shigemoto et al., 1997). Numerous studies have shown that at low stimulation frequencies, activation of group II mGluRs with selective agonists such as LY354740 (Kilbride et al., 1998) or DCG-IV(Brown and Reyman, 1994; Macek et al., 1996; Yokai et al., 1996; Kilbride et al., 1998, Huang et al., 1998); LCCG-1 (Ugolini and Bordi, 1995) or group III agonists such as L-AP4 (Koerner and Cotman, 1981; Bushell et al., 1996) results in a concentration-dependent reversible depression of the excitatory synaptic transmission in the medial and lateral perforant paths respectively. Although the exact mechanism through which group II and III mGluR agonists depress synaptic transmission is a matter of ongoing speculation (Anwyl, 1999) It would appear that they may reduce Ca^{2+} influx to presynaptic terminals, either via an inhibition of Ca^{2+} channels

(Wu and Saggau, 1994) or through an activation of K^+ channels (Sladeczek et al., 1993), alternatively the site of action may lie downstream from Ca^{2+} influx, at the release machinery (Hille, 1994).

Presynaptic receptors are usually classified as being solely inhibitory because their activation at low stimulation rates results in a reduction in transmitter release and as a consequence an inhibition of the postsynaptic response (see above). However these studies are limited in as far as they only examine the effect of presynaptic neuromodulation on single or paired stimuli at low frequencies where no synaptic depression occurs. Recently a number of studies which have employed stimulation paradigms that are more reminiscent of the firing patterns that occur *in vivo*, have demonstrated that synaptic depression is a dynamic, activity-dependent phenomenon (Varela et al., 1997; Abbott et al., 1997). The rate of onset and extent of steady-state depression are shaped by the frequency of stimulation and the presence of neuromodulators. There is strong evidence to suggest that the action of neuromodulators on a number of presynaptic receptors is also context-dependent, that is, the neuromodulator may act in an inhibitory or an excitatory manner depending on the prior activity at the synapse. For instance, the action of baclofen on $GABA_B$ receptors at the nMAG (Brenowitz et al., 1998), cultured hippocampal neurons (Isaacson and Hille, 1997) and visual cortex (Varela et al., 1997) or the action of acetylcholine at neocortical synapses (Tsodyks and Markram, 1997), and ventral striatum (Pennartz and Lopes da Silva, 1994). In fact Trussell's group have shown that presynaptic $GABA_B$ may have either an inhibitory or an excitatory action on synaptic transmission depending on the history of activity at the nMAG synapses (Brenowitz et al., 1998).

Activation of presynaptic receptors by pharmacological agents has been shown to have a profound influence on synaptic transmission (Conn and Pin, 1997). What is far less certain is whether these receptors are activated *in vivo* under normal physiological conditions, or what some see as being a more plausible scenario, during situations of severe pathological stress such as during epileptic activity or hypoxia. In studies where a role for presynaptic autoreceptor activation on short-term depression has been proposed it is usually only possible under experimental conditions which favour the accumulation of glutamate in the synaptic cleft. Such manipulations include recording from preparations maintained at room temperature (Scanzani et al., 1997; Barnes-Davies and Forsythe., 1995; Von Gersdorff et al., 1997; Shen and Horn., 1996) or in the presence of re - uptake inhibitors (Scanzani et al., 1997).

The aims of this study were to characterise the frequency-dependent depression exhibited in both the medial and lateral perforant paths and to determine under which conditions and to what extent presynaptically distinct mGluRs could modulate this short-term depression.

4.3 Materials and Methods

4.3.1 Slice preparation

All experiments were carried out on hippocampal slices obtained from Wistar rats (50-70g) (BioResources Unit, Trinity College, Dublin, Ireland). Slices were obtained as described previously (Kilbride et al., 1998). Briefly, the brain was rapidly removed after decapitation and placed in cold (5°C) oxygenated (95 % O_2, 5 % CO_2) artificial cerebro-spinal fluid (ACSF) containing in mM: NaCl, 120; $NaHCO_3$, 26; NaH_2PO_4, 1.25; KCl, 2.5; Mg_2SO_4, 2; $CaCl_2$, 2; glucose, 10). Hippocampal slices (350 μM) were cut using a Campden vibroslice (Campden Group Instruments, London, U.K.) and transferred immediately to an incubation chamber, maintained at room temperature, for a period of at least 60 minutes. Single slices were then transferred to a submersion type recording chamber at 30 - 31°C unless otherwise stated.

4.3.2 Electrophysiology

Field excitatory postsynaptic potentials (EPSPs) were recorded using standard glass electrodes filled with extracellular ACSF containing in mM: NaCl, 120; $NaHCO_3$, 26; NaH_2PO_4, 1.25; KCl, 2.5; Mg_2SO_4, 2; $CaCl_2$, 2; glucose, 10). This saline was oxygenated (95 % O_2, 5 % CO_2) before being used to perfuse the slices. In certain experiments the concentration of extracellular calcium was reduced to 1.6mM, 1.2mM and 0.8mM using a separately made saline. Field EPSPs were evoked using a Grass S48 stimulator (pulse width 0.1 ms) via a bipolar insulated tungsten wire electrode, adjusted to give about 30 % of the maximal response (~1mV). EPSP amplitudes were measured using Maclab Scope V3.5.6. Test field EPSPs were recorded at a frequency

of 0.0166 Hz. Field EPSPs were recorded from the middle and lateral perforant paths by placing the recording electrodes in the middle third and outer third of the molecular layer of the dentate gyrus respectively. Stimulation and recording from the medial or lateral perforant pathway was verified by the use of paired-pulse stimulation (interstimulus interval 40ms). Paired-pulse depression (~30 %) was indicative of the medial perforant path, and paired-pulse facilitation (~15 %) was indicative of the lateral perforant path (McNaughton, 1980). Further verification was obtained by observing the switch from source to sink upon moving the recording electrode from one pathway to the other, and the pharmacological selectivity of each pathway was used as a post-experimental cue to the identity of the recorded pathway.

4.3.3 Data analysis and statistics

Brief trains of pulses were delivered at frequencies of between 1-200 Hz, with the test EPSP amplitude ~1mV, unless otherwise stated. Summarised results are expressed as EPSP mean amplitude ± S.E.M. The amplitude of each EPSP in a train was expressed as a percentage of the 1st control EPSP. However the "normalised" EPSP (%) was calculated by normalising every value in a particular condition to the 1st EPSP amplitude in that condition. Data was analysed using student's paired t-test, and repeated measures analysis of variance, (ANOVA), at the 5 % level of significance.

The rate of onset of depression in the medial perforant path was calculated using Graphpad Prism software (Apple Macintosh) and verified on Jandel Sigmaplot software (PC). The summary data from 7 experiments at a range of frequencies between 0.5 Hz and 200 Hz were fitted with a one phase exponential decay curve using the following equation:

$$Y = Span * \exp(-K * x) + plateau$$

Starts at span and decays to plateau with a rate constant K. The tau of decay was calculated as 1 / K

The dose-response curve for L-AP4 was constructed using Graphpad Prism software where the curve was best fitted using a one-site binding hyperbola $Y=B_{max}*X / (K_d+X)$ where B_{max} is the maximal binding and K_d is the concentration of ligand required to reach half-maximal binding.

In figures 4.8 and 4.17 the reduction in short-term depression by activation of groups II and III mGluR was estimated as follows. Data points were constructed by expressing, as a percentage, the extent to

which the control steady-state EPSP amplitude was depressed by the application of either LY354740 (100 nM or 5 μM) or L-AP4 (2.4 μM or 10 μM) for a range of frequencies. The arrows show the threshold frequency at which a LY354740 or L-AP4-induced significant attenuation of the short-term depression observed in control conditions occurs. This was established by comparing the % inhibition at each frequency with the % inhibition at 0.0166 Hz (test frequency) using the student's t-test at the 5 % level of significance. Mean and SEM (n = 4-11).

4.3.4 Compounds

Where stated experiments in the dentate gyrus were carried out in the presence of 100 μM picrotoxin to block $GABA_A$ receptor-mediated inhibition. Drugs were added directly to the perfusate after establishing a steady baseline. LY354740 was a generous gift from Eli Lily, USA. L-AP4 and MSOP, MSOPPE and Cyclothiazide were obtained from Tocris Cookson.

4.4 Results

4.4.1 Short-term depression in the medial and lateral perforant path

In the medial and lateral perforant paths, at the test frequency (0.0166 Hz), paired-stimuli (0.1 ms) separated by an interstimulus interval of 40 ms induced either a depression (20 - 30 %) or a facilitation (10 - 20 %) respectively, of the second EPSP amplitude with respect to the first. At this test frequency, stimulation over a 20 minute baseline period was not accompanied by any depression of the EPSP amplitude. In the medial perforant path, short trains of 10 stimuli at a range of frequencies between 1 and 200 Hz induced a reversible depression of the EPSP amplitude. After a rapid onset of depression, the EPSP amplitude stabilised by the 10^{th} stimuli at a level which will be referred to as the steady - state EPSP amplitude from here on in, to distinguish it from the initial (1^{st}) EPSP amplitude.

Figures 4.1 and 4.2 show families of EPSPs elicited in response to 10 stimuli at a range of frequencies between 1 and 200 Hz in the medial and lateral perforant paths respectively. In both pathways the extent of steady-state depression increased with frequency. For example the steady-state EPSP amplitude after 10 stimuli at 1, 20 and 100 Hz was 74.8 $\pm$ 2.9 % (n = 5), 42.3 $\pm$ 4.6 % (n = 4) and 18.5 $\pm$ 2.3 % (n = 4) of the initial EPSP amplitude in the medial perforant path. (Fig. 4.1, 4.3A; ANOVA, $P < 0.05$). However the lateral perforant path, which displays more facilitation, showed less frequency-dependent depression over the same range of stimuli; 86.4 $\pm$ 1.3 % (n = 7), 76.4 $\pm$ 1.0 % (n = 6) and 41.1 $\pm$ 2.2 % (n = 16) (Fig. 4.2, 4.3A, ANOVA, $P < 0.05$). The data points from Fig. 4.1 were well fitted with a one phase exponential decay curve ($0.93 < r^2 > 0.99$), which gave a tau of decay for 1 Hz, 10 Hz and 50 Hz of 813 ms, 210 ms

and 17 ms respectively (n = 7). When the time constants of decay were expressed as a function of stimulation frequency, the relationship was found to be linear (r^2 = 0.935, Figure 4.4), this suggests that at higher frequencies no other mechanisms of depression were recruited (Betz, 1970).

In all experiments the stimulation intensity was adjusted to give a test EPSP of ~1 mV. The rate of onset and extent of short-term depression were found to be almost identical when the stimulation intensity was adjusted to give test EPSPs which varied between 0.4 mV and 2.2 mV (Fig. 4.5)

4.4.2 Short-term depression in the medial perforant path is relieved by activation of group II mGluRs at high stimulation frequencies.

LY354740 is a recently synthesised potent and specific agonist at group II mGlu receptors (Bond et al., 1997; Monn et al., 1997; Schoepp et al., 1997). Previous electrophysiological studies carried out in this laboratory have strengthened this claim, demonstrating that it has a potent presynaptic reversible inhibitory action on EPSPs evoked at the test frequency (0.0166 Hz). The effect is dose-dependent with an EC_{50} of 115 ±16 nM and a maximum inhibition of 80 % at a concentration of 5 μM (Kilbride et al., 1998). In order to examine what effect, if any, activation of group II mGluRs would have on short-term depression, brief-trains of 10 stimuli (1-200 Hz) were applied to the medial perforant path after the inhibition induced by LY354740 had reached equilibrium (~15 mins). LY354740 was usually applied for up to 45 mins, during this time no desensitisation of the induced inhibition was observed.

At frequencies up to 10 Hz, short-term depression was unaffected by either an intermediate (100 nM) or a high (5 μM) dose of LY354740. At 1Hz, the curves describing the absolute EPSP amplitude as a function of pulse number in control and LY354740 were parallel (Fig. 4.6B). Moreover when the data was normalised to the 1st EPSP in each condition, the curves overlapped. After 10 pulses the normalised steady - state EPSP amplitude was 74.8 ± 2.9 % (n = 4), 72.3 ± 2.0 % (n = 4) and 71.3 ± 5.2 % (n = 4) in control, 100 nM and 5 μM LY354740 respectively, values not significantly different. (Fig 4.6C) The response to stimulation at 5 Hz and 10 Hz was very similar (Fig 4.8).

At frequencies greater than 20 Hz, short-term depression was reduced by the activation of group II mGluRs by LY354740. For example at 50 Hz the absolute steady-state EPSP amplitude in control and LY354740 are not significantly different (Fig. 4.7B). The normalised data shows more clearly the reversal of the initial depression to facilitation and the subsequent relief of short- term depression in the presence of LY354740. Thus the normalised steady-state depressed amplitude of the EPSP was 32.4 ± 2.3 % (n = 9), 51.1 ± 1.8 % (n = 7) and 91.6 ± 4.6 % (n = 10) in control, 100 nM and 5 μM LY354740 respectively (ANOVA, $P < 0.05$, Fig 4.7C).

Figure 4. 8 shows the percentage inhibition of the control steady-state EPSP amplitude by 100 nM and 5 μM LY354740 for a range of frequencies between 0.0166Hz and 100Hz. The concentration and frequency dependence of the group II mGluR attenuation of short-term depression is clearly seen. The threshold frequency at which LY354740 reduced short-term depression was 50 Hz for 100 nM and 20 Hz for 5μM. Interestingly at 100 Hz, the steady-state EPSPs were actually

larger in the presence of LY354740 compared to control. (see Brenowitz et al., 1998)

The modulation of short-term depression by activation of group II mGluR is further revealed by the curves relating the normalised EPSP per unit time as a function of frequency. In the presence of LY354740 the curve was shifted upwards in a concentration dependent manner, as the frequency at which the EPSP per unit time became independent of frequency was shifted from 10 Hz in control to 50 Hz in LY354740 (5 μM) (Fig. 4.9).

4.4.3 Short-term depression in the medial perforant path is relieved by lowering extracellular calcium at high stimulation frequencies.

Previous studies have suggested that the inhibitory action of presynaptic mGluRs on EPSPs elicited at low frequencies is a result of a reduced influx of calcium into the presynaptic terminals. The exact mechanism may involve an inhibition of Ca^{2+} channels (Wu and Saggau., 1995; Takahashi et al., 1996), a facilitation of K^{+} channels (Sladeczek et al., 1993) or indeed the action may be at a site downstream from the site of calcium entry (Hille, 1994). In this study the effect of lowering extracellular calcium on short-term depression was very similar to that of LY354740 and as such, both are consistent with a reduction in transmitter release via a reduction in calcium influx (Varela et al., 1997). At the test frequency of 0.0166 Hz, the steady-state EPSP amplitude was reduced as the level of extracellular calcium was lowered. In 1.6 mM, 1.2 mM and 0.8 mM Ca^{2+}, the test (0.0166 Hz) EPSP amplitude was measured as being 63.9 ± 16.8 % (n = 3), 49.8 ± 10.3 % (n = 4) and 29.6 ± 8.6 % (n = 5) of control (2 mM) (ANOVA, $P < 0.05$,). At 1 Hz the curves relating the short-term depression in control

(2 mM), 1.6 mM, 1.2 mM and 0.8 mM extracellular calcium overlap, moreover the normalised steady-state EPSP amplitudes were not significantly different; 70.6 ± 2.4 %, (n = 8), 76.6 ± 2.4 %, (n = 3), 77.9 ± 3.0 % (n = 6) and 69.9 ± 18.1 % (n = 4, Fig 4.10) respectively. As was the case with LY354740, high-frequency stimulation (HFS) in low extracellular calcium was able to partly overcome short-term depression. For example at 50 Hz, the reduction in the level of extracellular calcium was accompanied by an slowing down of the rate of onset of depression and a reduction in the extent of short-term depression. The normalised steady-state EPSP amplitudes were 33.0 ± 2.0 % (n = 11), 43.8 ± 2.3 % (n = 3), 56.1 ± 4.8 % (n = 7) and 126.2 ± 32.1 % (n = 4) in 2.0 mM, 1.6 mM, 1.2 mM and 0.8 mM calcium ($P < 0.05$, Fig 4.11) The curves relating the normalised EPSP per unit time to frequency were shifted upwards as the limiting frequency was extended from 10 - 20 Hz in control to over 50 Hz in 0.8 mM calcium (Fig 4.12).

4.4.4 Short-term depression in the lateral perforant path is relieved by activation of group III mGluRs at high stimulation frequencies.

Binding studies have shown that the lateral perforant path contains a high concentration of group III mGluR (Bradley et al., 1996; Shigemoto et al., 1998). Furthermore electrophysiological studies have revealed that application of group III mGluR agonists like (L(+)-2-Amino-4-phosphonobutyric acid) L-AP4 causes a concentration-dependent and reversible depression of excitatory synaptic transmission (Koerner and Cotman, 1981; Bushell et al., 1996). In this study perfusion of L-AP4 at concentrations of 0.5 μM, 1 μM, 5 μM, and 10 μM reduced the EPSP amplitude to 85.1 ± 3.8 %, 73 ± 4.3 %, 36.7 ± 4.2 % and 27.1 ± 3.6 % of the baseline value. (ANOVA, $P < 0.05$, $n = 9$, Figure 4.13). In figure 4.14 the dose-response curve for the inhibitory action of L-AP4 on EPSP amplitude in the lateral perforant path is shown, the EC_{50} value was estimated to be 2.4 μM and a maximal inhibition of ~80 % was observed with a concentration of 10 μM. In order to ascertain if activation of group III mGluRs would effect short-term depression, brief trains of 10 stimuli (1-200 Hz) were applied to the lateral perforant path after the inhibition induced by L-AP4 had reached equilibrium (~15 mins). L-AP4 was usually applied for periods up to 45 minutes and during this time no desensitisation of the induced inhibition was observed.

At frequencies up to 5 Hz, short-term depression was unaffected by either an intermediate (2.4 μM) or a high (10 μM) dose of L-AP4. For example at 1 Hz, the curves describing the absolute EPSP amplitude as a function of pulse number in control and L-AP4 are parallel (Fig. 4.15B). Furthermore when the data was normalised to the 1st EPSP in each condition, the curves overlapped. After 10 pulses the normalised steady-state EPSP amplitude was 86.5 ± 1.6 % ($n = 7$), 102.5 ± 7.1% ($n = 5$) and 90.5 ± 4.0

% (n = 7) in control, 2.4 μM and 10 μM L-AP4 respectively, values not significantly different (Fig. 4.15C)

At frequencies less than 10 Hz, short-term depression was reduced by the activation of group III mGluR by L-AP4 . At 50 Hz the absolute steady-state EPSP amplitude in control and L-AP4 were very similar (Fig. 4.16B). The normalised data shows more clearly the enhancement of the initial facilitation and the subsequent relief of short-term depression in the presence of L-AP4. Thus the normalised steady-state depressed amplitude of the EPSP was 73.3 ± 2.3 % (n = 10, $P < 0.05$), whereas the steady-state EPSP amplitude in the presence of L-AP4 (2.4 μM and 10 μM) was actually facilitated above the initial value, 130.8 ± 3.8 (n =7, $P < 0.05$) and 167.3 ± 10 % (n = 9, $P < 0.05$) respectively (Fig. 4.16C).

Figure 4.17 shows the percentage inhibition of the control steady-state EPSP by 2.4 μM and 10 μM L-AP4 for a range of frequencies between 0.0166 Hz and 100 Hz. The concentration and frequency dependence of the group II mGluR attenuation of short-term depression is clearly seen. The threshold frequency at which L-AP4 reduced short-term depression was 20 Hz and 10 Hz for 2.4 μM and 10 μM L-AP4 respectively.

The modulation of short-term depression by activation of group III mGluR is further revealed by the curves relating the normalised EPSP per unit time as a function of frequency. In the presence of L-AP4 the curve was shifted upwards in a concentration-dependent manner, as the frequency at which the EPSP per unit time became independent of frequency was shifted from 20 - 50 Hz in control to 50 - 100 Hz in 2.4 μM and 10 μM L-AP4. (Fig. 4.18).

4.4.5 Presynaptic mGluR autoreceptors are not involved in short-term depression in the medial or lateral perforant paths.

Previous studies have suggested that the activation of presynaptic autoreceptors does not make a significant contribution to short-term depression at Calyx type synapses. (Von Gersdorff et al., 1997; Barnes-Davies and Forsythe, 1995). Presynaptic group II mGluRs are located in the extrasynaptic region (Petralia et al., 1996; Yokai et al., 1996; Ottersen and Landsend, 1997; Shigemoto et al., 1998) and might therefore be favourably positioned to be activated by a spillover of glutamate from adjacent terminals (Aztely et al., 1997; Scanzani et al., 1997). In contrast presynaptic mGluR III are located close to the active zones (Shigemoto et al., 1997). Because of their different locations, these receptors might be expected to differ in their response to trains of stimuli delivered at either low or high frequencies.

L - *trans* - pyrrolidine - 2, 4 - dicarboxylic acid (t-PDC) has been used successfully to inhibit the re-uptake of glutamate from the synaptic cleft into the presynaptic terminals of hippocampal CA1 synapses (Isaacson and Nicol, 1993) and CA3 (Scanzani et al., 1997). In this study, in the medial perforant path, t-PDC was applied first, the rationale being that in the presence of t-PDC, the potential for glutamate in and around the synaptic cleft to activate the presynaptically located group II mGluR would be maximised. If this occurred it might manifest itself as a increase in either the rate or extent of short-term depression has been demonstrated at the calyx of held (Von Gersdorff et al., 1997). ((RS)- J - Methylserine-O-phosphate monophenyl ester) MSOPPE is a putative antagonist at group II mGluRs (Thomas et al., 1996). In preliminary experiments, I found it blocked the DCG-IV - induced inhibition in the medial perforant path. In the presence of MSOPPE, the rate of onset

and / or extent of short-term depression might be reduced as the presynaptic autoreceptors are blocked and therefore unable to contribute to synaptic depression. In this study, application of t-PDC on its own or in the presence of MSOPPE, induced no observable effect on short-term depression at either 1 Hz or 50 Hz.

Figure 4.19 shows the effect of t-PDC (100 μM) and MSOPPE (200 μM) on short - term depression at 1 Hz. The curves describing the absolute EPSP amplitude as a function of pulse number in control, t - PDC and t-PDC & MSOPPE are parallel. The lack of effect of both compounds was emphasised when the data was normalised, as the overlapping curves in figure 4.19C shows. After 10 pulses the steady-state EPSP amplitude was 69.4 ± 2.6 %, ($P < 0.05$, $n = 5$), 71.4 ± 2.5 % ($P < 0.05$, $n = 5$), and 71.5 ± 3.9 %, ($P < 0.05$, $n = 5$) in control, t - PDC, and t - PDC and MSOPPE respectively. Values not significantly different from each other. At 50 Hz, no alteration in short-term depression was observed, the normalised steady-state EPSP amplitude after 10 pulses was 38.1 ± 10.9 % ($P < 0.05$, $n = 4$), 34.8 ± 12.1% ($P < 0.05$, $n = 4$), and 31.8 ± 13.0 % ($P < 0.05$, $n = 4$), in control, t-PDC (100 μM) alone or t - PDC (100 μM) with MSOPPE (200 μM) respectively, again values not significantly different from each other, figure 4.20.

In the lateral perforant path, preliminary experiments at 32° C with the putative group III mGluR antagonist MSOP (Jane et al., 1996) did not suggest any contribution from this autoreceptor towards short-term depression. Therefore the subsequent experiments were conducted at room temperature (21° C - 24° C) in order to reduce the re-uptake of glutamate into the presynaptic terminals and enhance the likelihood of

autoreceptor activation as has been shown previously (Scanzani et al., 1995). Perfusion of the putative group III mGluR antagonist (RS)-J-Methylserine-O-phosphate, MSOP (Jane et al., 1996) at concentrations of 25 μM and 50 μM had no significant effect on either the rate of onset or the extent of steady-state depression 1 Hz. After 10 stimuli delivered at 1 Hz the normalised steady-state EPSP amplitude was 81.2 ± 3.2 % ($P < 0.05$, $n = 8$), 83.1 ± 5.3 % ($P < 0.05$, $n = 8$) and 86.3 ± 2.9 % ($P < 0.05$, $n = 8$) in control and MSOP (25 μM and 50 μM). Values not significantly different from each other. Furthermore high-frequency stimulation did not reveal any involvement by group III mGluR autoreceptors in short-term depression. For example at 50 Hz the curves relating the normalised EPSP as a function of pulse number in control, and MSOP (25 μM and 50 μM) overlapped, 34.9 ± 5.5 % ($P < 0.05$, $n = 8$), 33.8 ± 3.6 % ($P < 0.05$, $n = 8$) and 32.8 ± 3.5 % ($P < 0.05$, $n = 8$) respectively. Values not significantly different from each other.

4.4.6 Cyclothiazide reduced high-frequency induced short-term depression in the medial perforant path.

6-chloro-3,4-dihydro-3-(2-norbornen-5-yl)-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide), Cyclothiazide (CTZ) has previously been shown to have effects at both the pre- and postsynaptic receptors at glutamatergic synapses. CTZ blocks the postsynaptic desensitisation of AMPA receptors (Jones and Westbrook, 1996) and the paired-pulse depression observed in the chick brainstem has been attributed in part to this. (Trussell et al., 1993; Otis et al., 1996). In this study, the timecourse of individual EPSPs was visibly lengthened by the application of cyclothiazide, in keeping with its action as a inhibitor of postsynaptic AMPA receptor desensitisation. The curves describing the absolute EPSP amplitude as a function of pulse number in control and cyclothiazide at 1

Hz were parallel (Fig. 4.22B). Moreover when the data was normalised to the 1st EPSP in each condition, the curves overlapped. After 10 pulses the normalised steady-state EPSP amplitude was 72.3 ± 2.2 % in control ($n = 5$, $P < 0.05$) and 77.1 ± 4.8 % ($n = 4$, $P < 0.05$), values not significantly different (Fig. 4.22C). However at 50 Hz, short-term depression was reduced by the application of cyclothiazide. The normalised data shown in figure 4.23C illustrates this; after 10 pulses the EPSP amplitude in control was 32.5 ± 1.4 % ($n = 4$, $P < 0.05$), whereas in the presence of cyclothiazide, the depressed EPSP amplitude was 39.8 ± 1.3 % ($n = 4$, $P < 0.05$), values significantly different ($P < 0.05$).

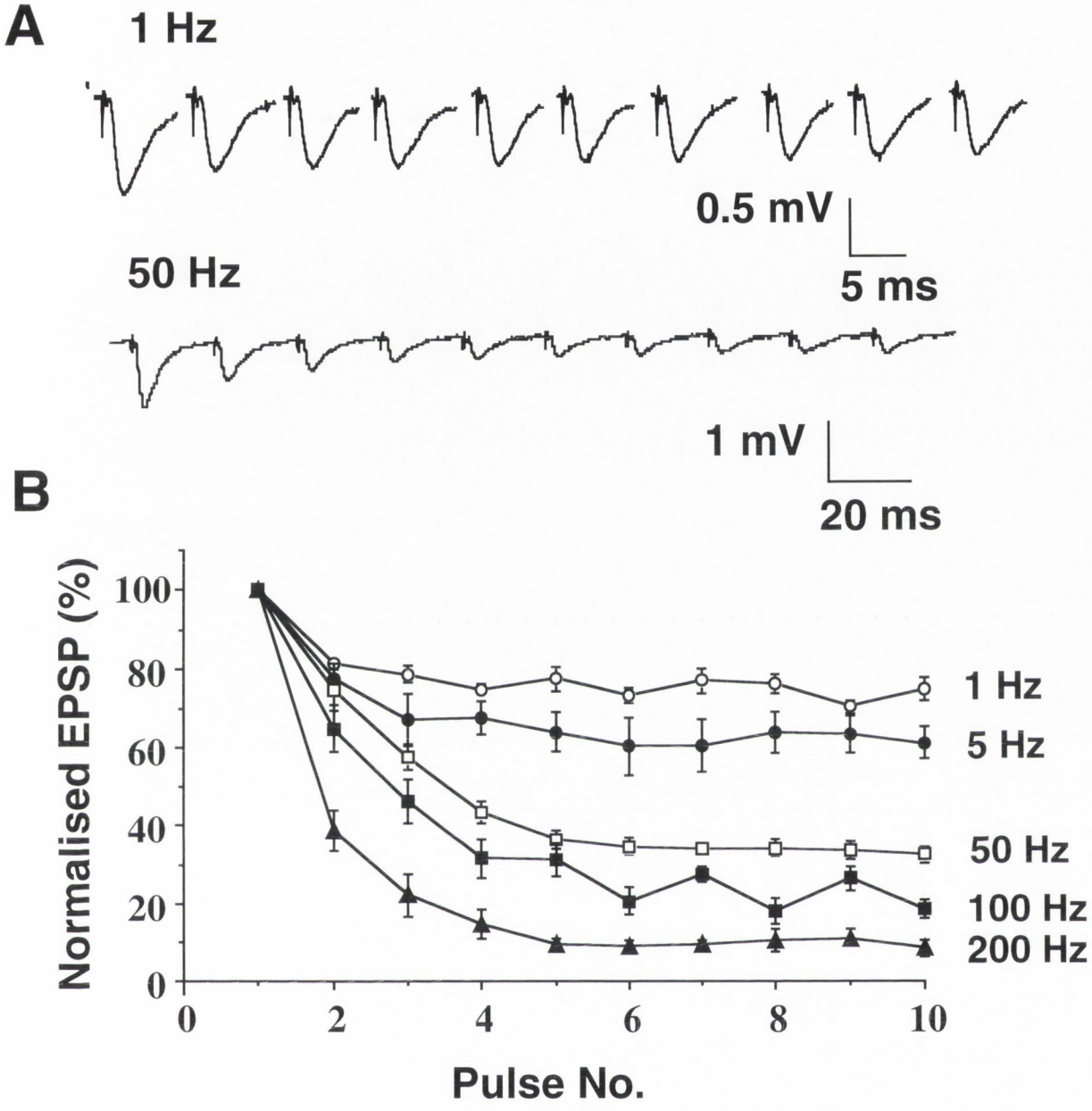


Figure 4.1 Frequency-dependent depression in the medial perforant path. (A)Typical example of trains of EPSPs recorded at 1 Hz (upper) and 50 Hz (lower) in the medial perforant path. (B) Families of normalised EPSPs recorded during trains of 10 stimuli at 1, 5, 50, 100 and 200 Hz ($n = 4 - 9$, ANOVA $P < 0.05$).

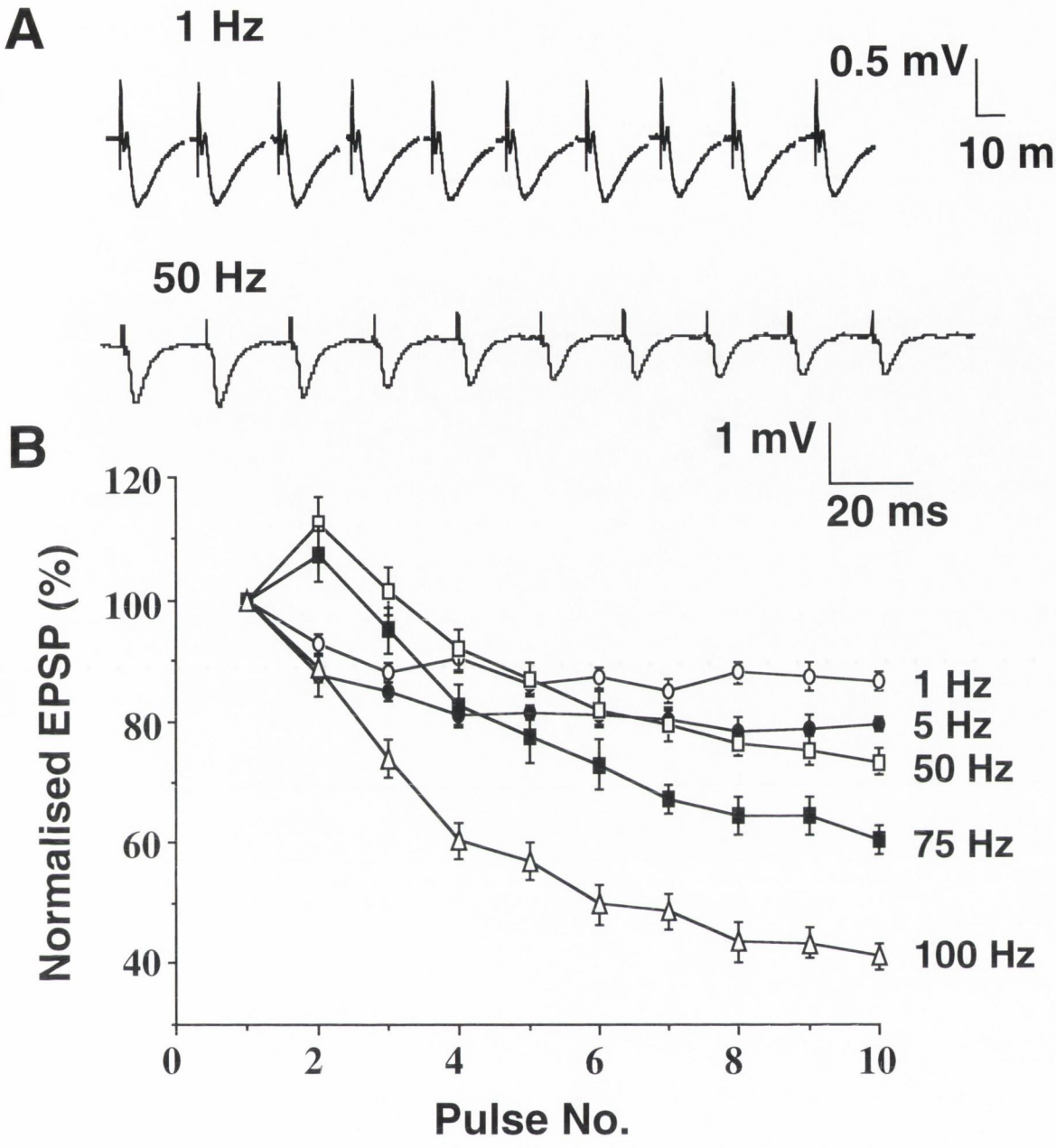


Figure 4.2 Frequency-dependent depression in the lateral perforant path. (A) Typical example of trains of EPSPs recorded at 1 Hz (upper) and 50 Hz (lower) in the lateral perforant path. (B) Families of normalised EPSPs recorded during trains of 10 stimuli at 1, 5, 50, 75 and 100 Hz. ($n = 4$ -16, ANOVA $p < 0.05$).

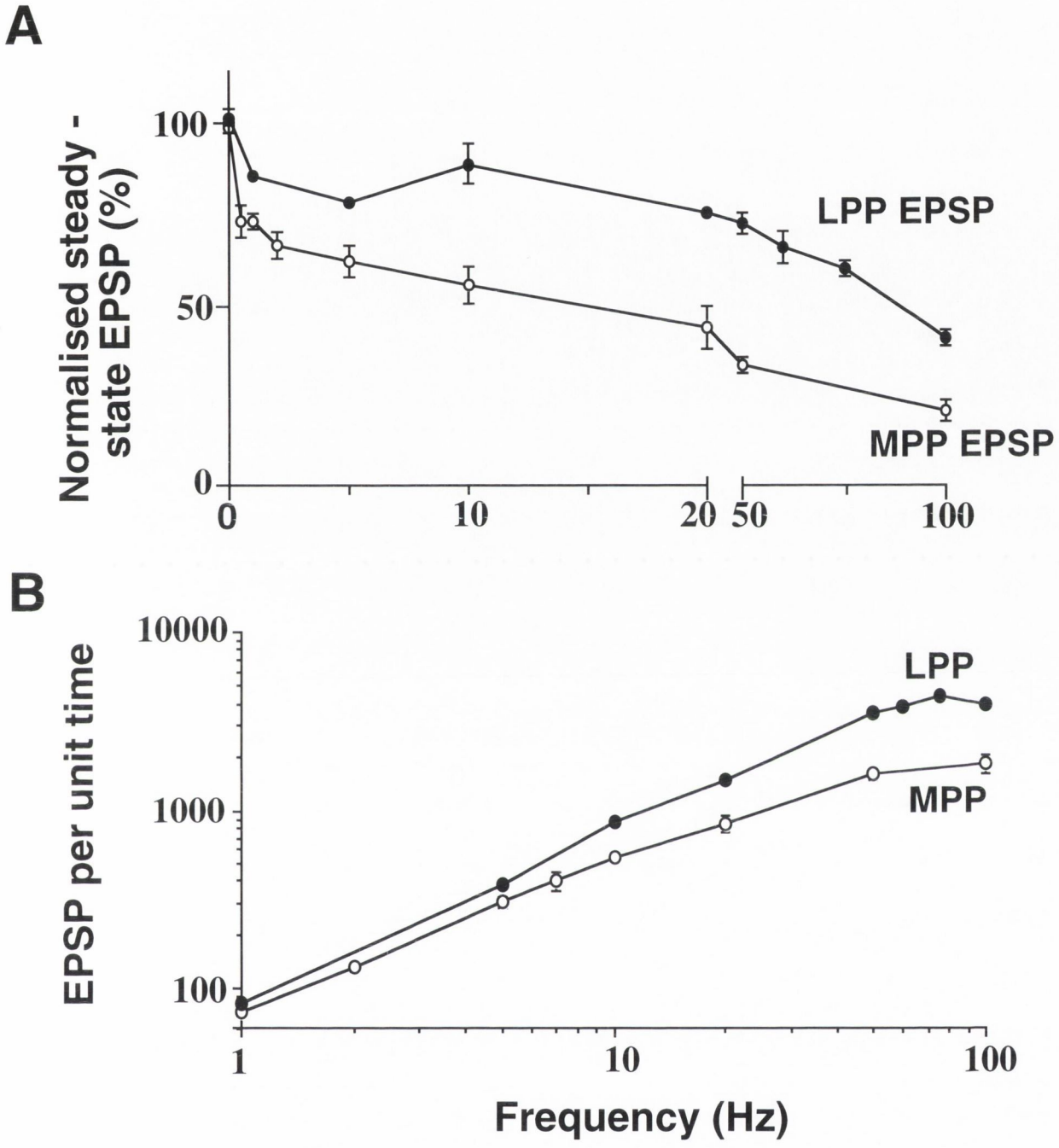


Figure 4.3 Short-term depression increases with frequency.
(A) Steady-state EPSP amplitude, normalised to 0.0166 Hz, plotted as a function of stimulation frequency for field EPSPs in the medial perforant path (open circles), lateral perforant path (filled circles). (B) EPSP per unit time (calculated as steady-state EPSP amplitude (%) x frequency) plotted as a function of frequency in the medial (open circles) and lateral perforant paths (filled circles) and expressed as arbitrary units. Each point plots the mean ± SEM values (n = 4-16).

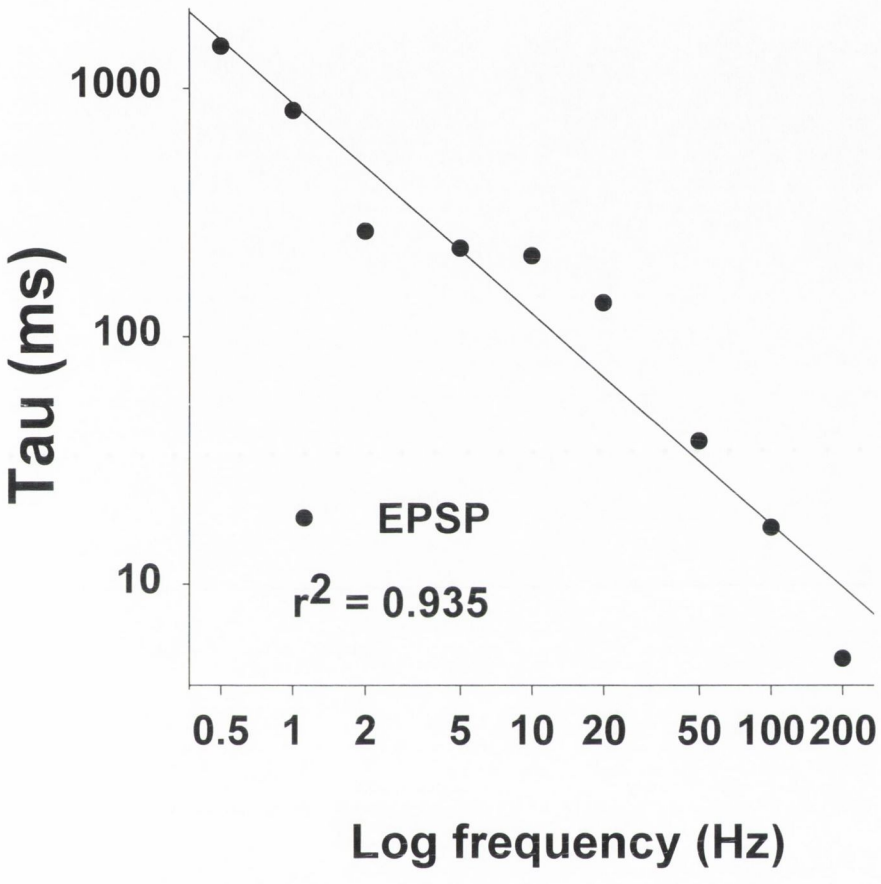


Figure 4.4 The rate of onset of short-term depression in the medial perforant path. Time constant (tau) of rate of onset of depression for EPSPs (filled circles) in the medial perforant path. The data points from figure 4.1 were fitted with an equation representing a single - exponential decay (f = a exp (-bx) + s, starts at f = (0) and decays with a time constant of (1/b) to a steady - state (s). The line is a linear regression line with $r_2 = 0.935$ (n= 4-9).

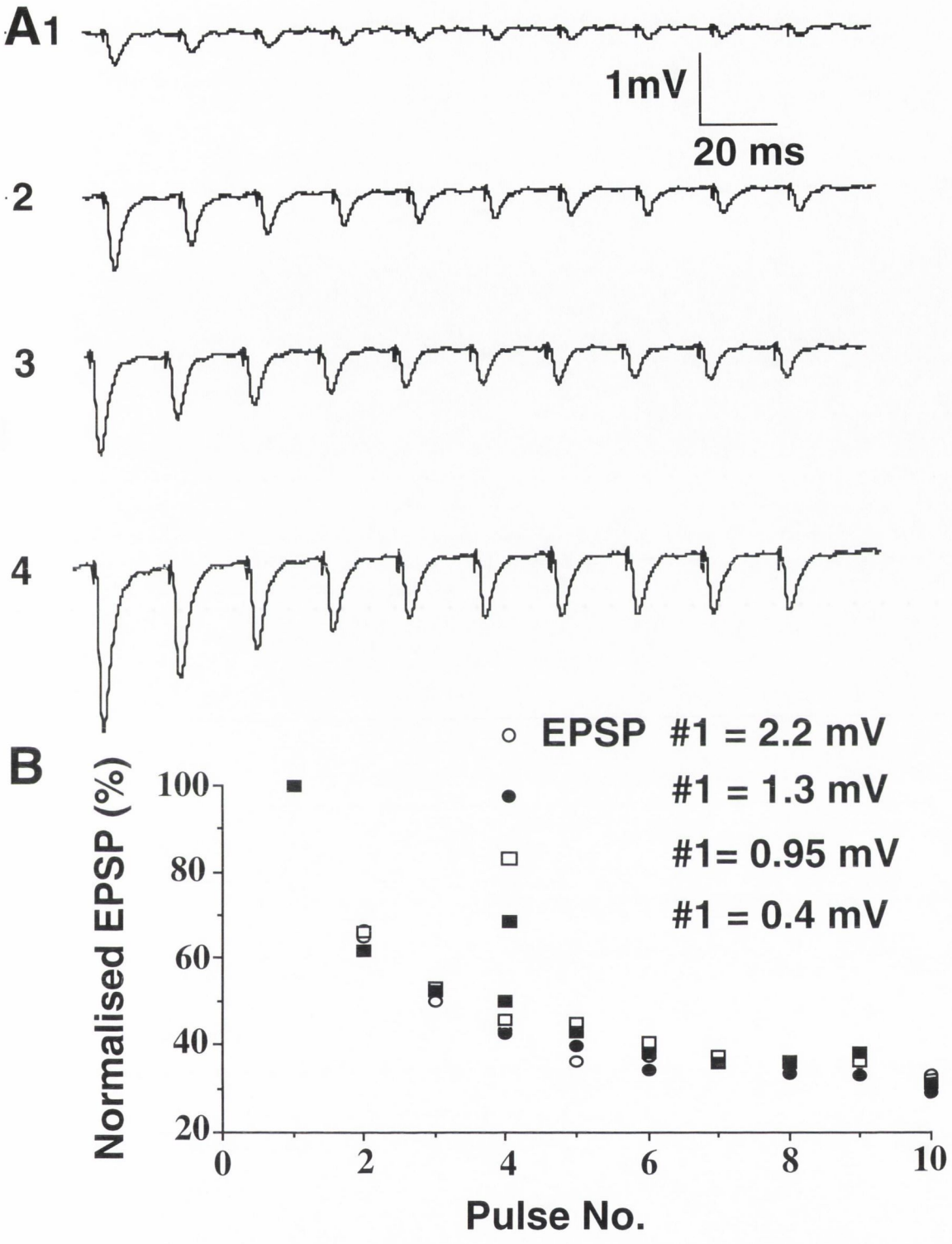


Figure 4.5 Stimulation intensity does not affect short-term depression in the medial perforant path. (A) Example of trains of EPSPs recorded in the medial perforant path in response to 10 stimuli delivered at 50 Hz at a stimulation intensity which gave an initial EPSP amplitude of 0.4 mV (1), 0.95 mV (2), 1.3 mV (3) and 2.2 mV (4). (B) Normalised EPSP amplitude plotted as a function of pulse number for each train shows that the rate of onset and extent of steady-state depression are equal regardless of the initial EPSP amplitude.

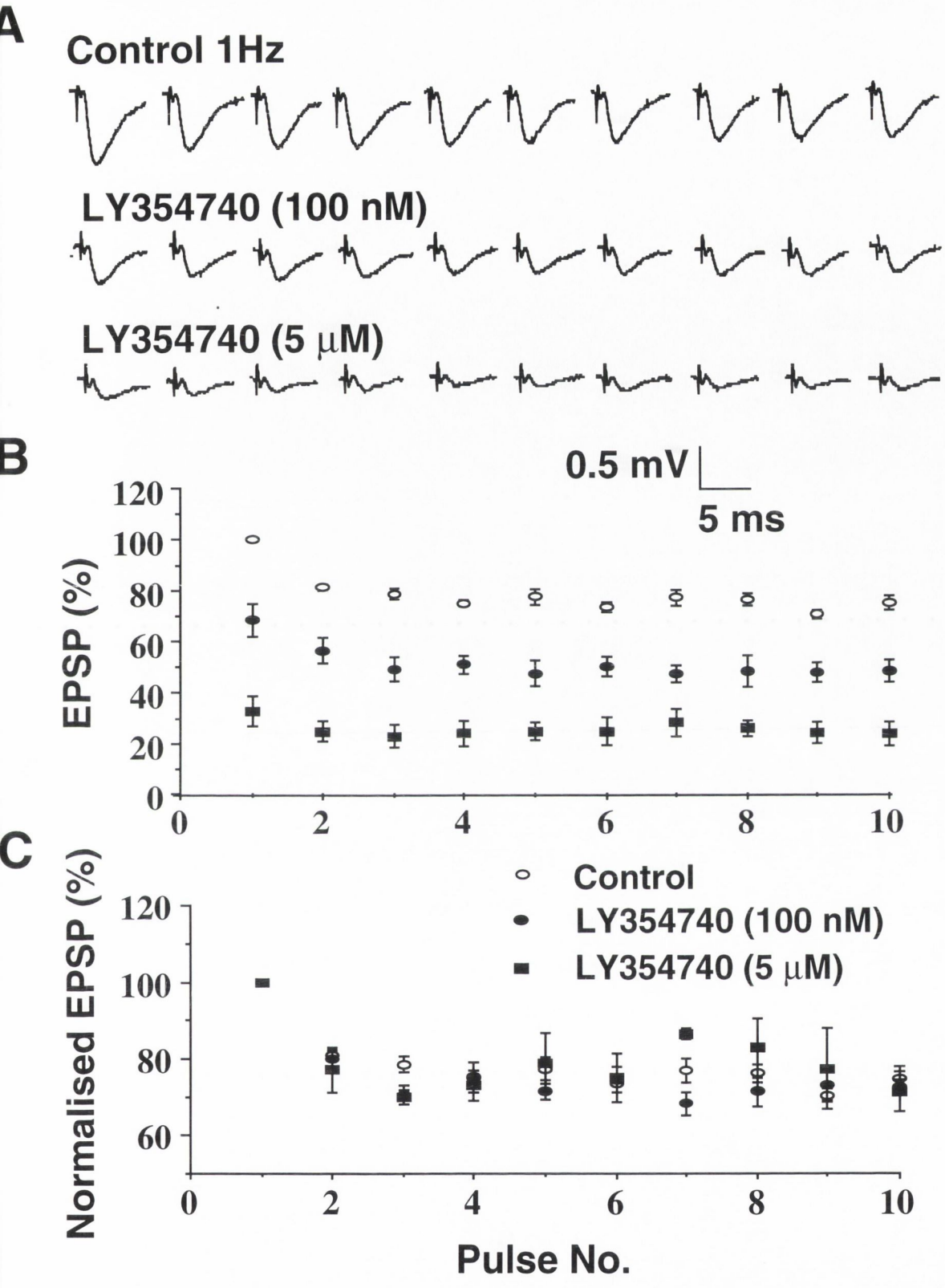


Figure 4.6 The group II mGluR agonist LY354740 has no effect on short-term depression evoked at 1 Hz in the medial perforant path. (A) Typical traces showing trains of EPSPs elicited with 1Hz stimulation in the medial perforant path, in control (upper), LY354740 (100 nM) (middle) and LY354740 (5 μM) (lower). Absolute (B) and normalised (C) EPSP amplitude plotted as a function of pulse number in control (open circles), LY354740 (100 nM) (filled circles) and LY354740 (5 μM) (filled squares). Each point is the mean ± SEM of 4 slices.

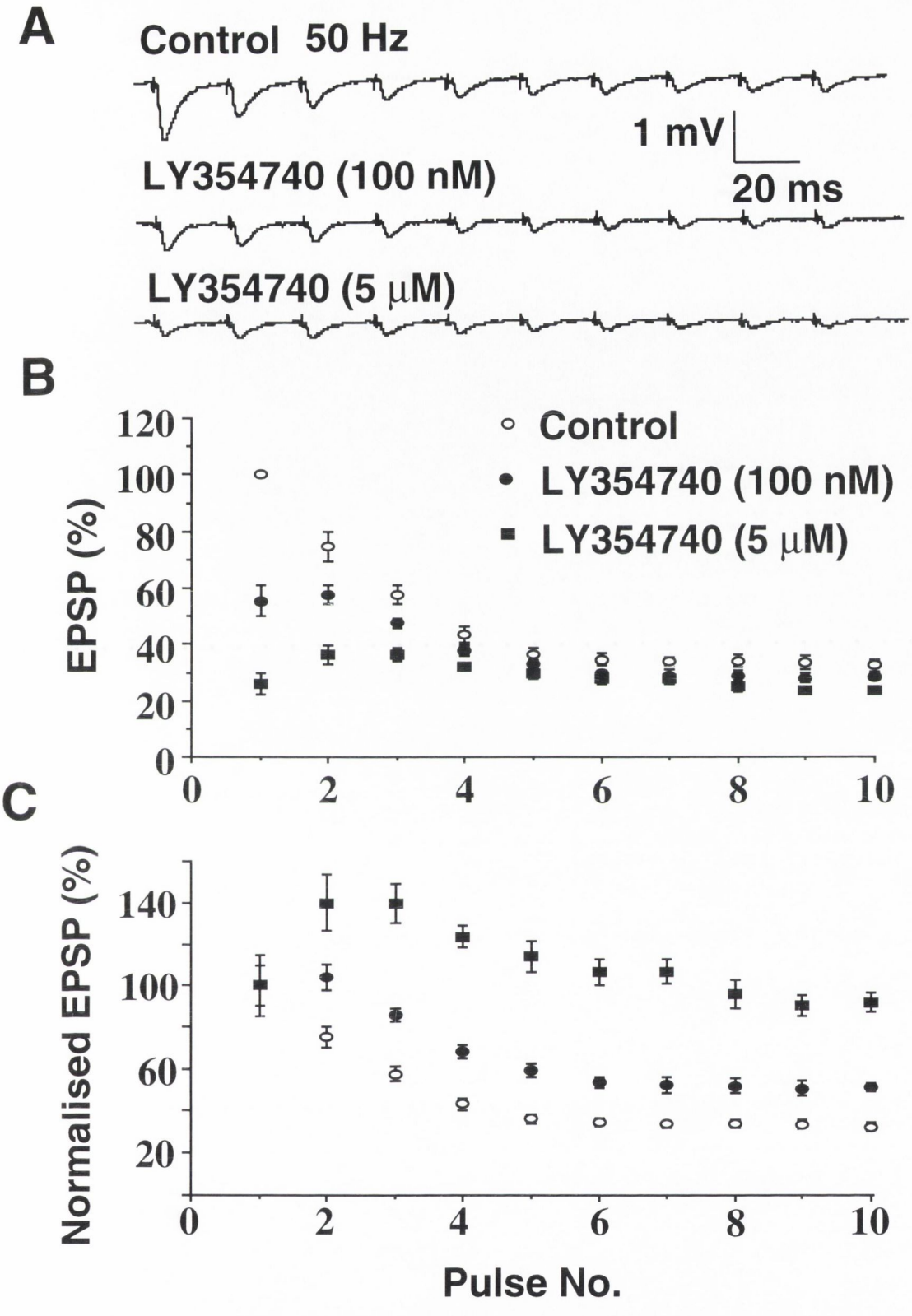


Figure 4.7 The group II mGluR agonist LY354740 attenuates short-term depression evoked at 50 Hz in the medial perforant path. (A) Typical traces showing trains of EPSPs elicited with 50 Hz stimulation in the medial perforant path, in control (upper), LY354740 (100 nM) (middle) and LY354740 (5 μM) (lower). Absolute (B) and normalised (C) EPSP amplitude plotted as a function of pulse number in control (open circles), LY354740 (100 nM) (filled circles) and LY354740 (5 μM) (filled squares) Each point plots the mean ± SEM (n = 7,9,11).

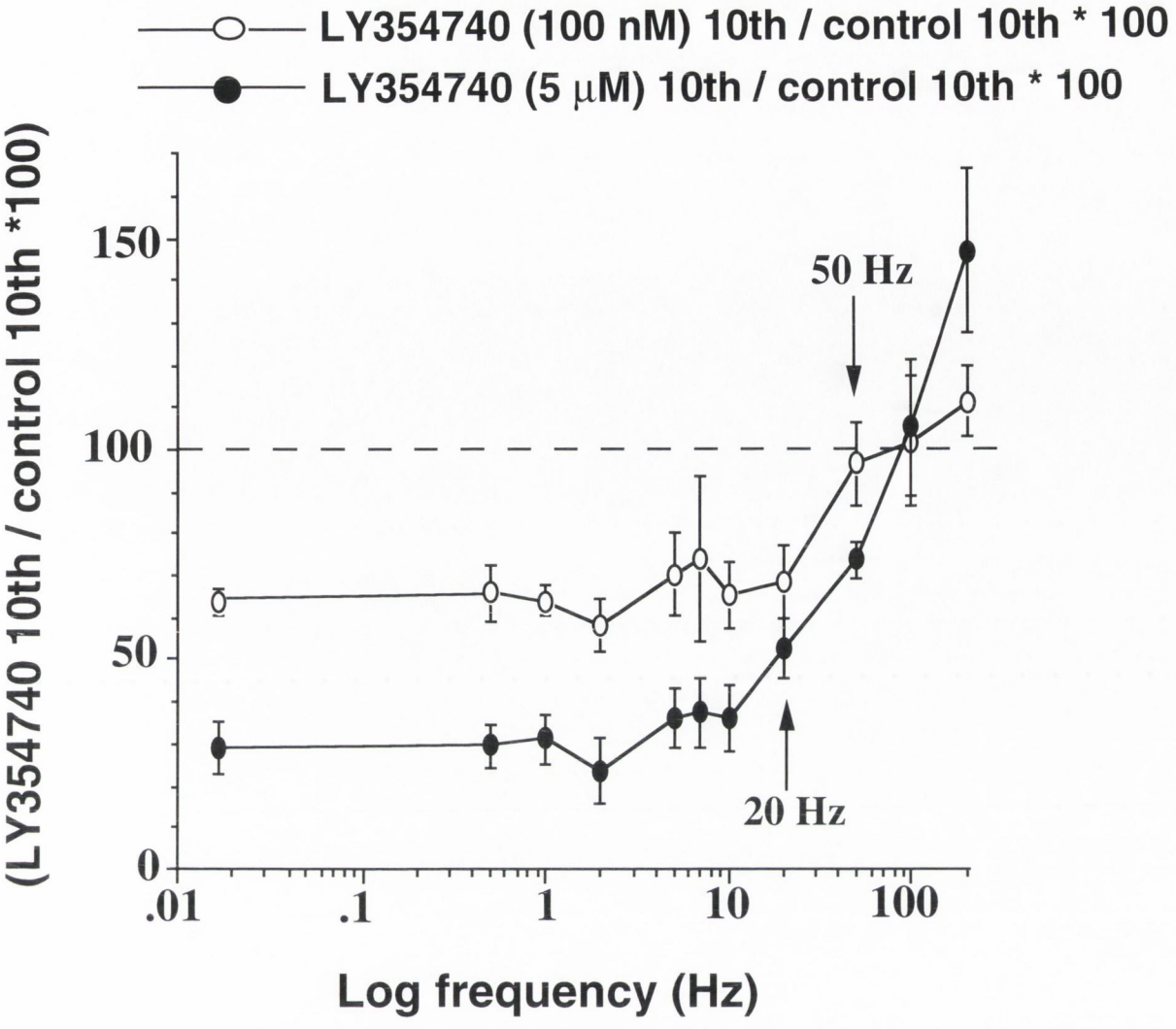


Figure 4.8 Frequency dependence of group II mGluR modulation of short-term depression in the medial perforant path. Steady-state EPSP amplitude in 100 nM (open circles) and 5 μM LY354740 (filled circles) expressed as a percentage of the control steady-state EPSP amplitude for a range of frequencies between 0.0166 Hz and 200 Hz. The concentration and frequency-dependence of the group II mGluR attenuation of short-term depression is clearly seen. At low frequencies (0.0166 Hz) the epsp amplitude in LY354740 is ~30 % (5 μM) and ~65 % (100 nM) of control, however at higher frequencies the attenuation of short-term depression is revealed by the convergence of the steady-state EPSP amplitudes in LY354740 and control. The threshold frequency at which LY354740 reduced short - term depression was 50 Hz for 100 nM and 20 Hz for 5 μM. At 200 Hz, the steady-state EPSPs were actually larger in the presence of LY354740 compared to control. Data points for each given frequency were constructed by expressing the amplitude of the 10 th EPSP evoked in the presence of LY354740 as a percentage of the 10 th EPSP evoked under control conditions, for each frequency shown on the x-axis. The arrows show the threshold frequency at which attenuation of short-term depression occurs. This was established by comparing each frequency with 0.0166Hz (test frequency) using the student's t-test at the 5% level of significance. Each point plots the mean ± SEM (n = 4 -11)

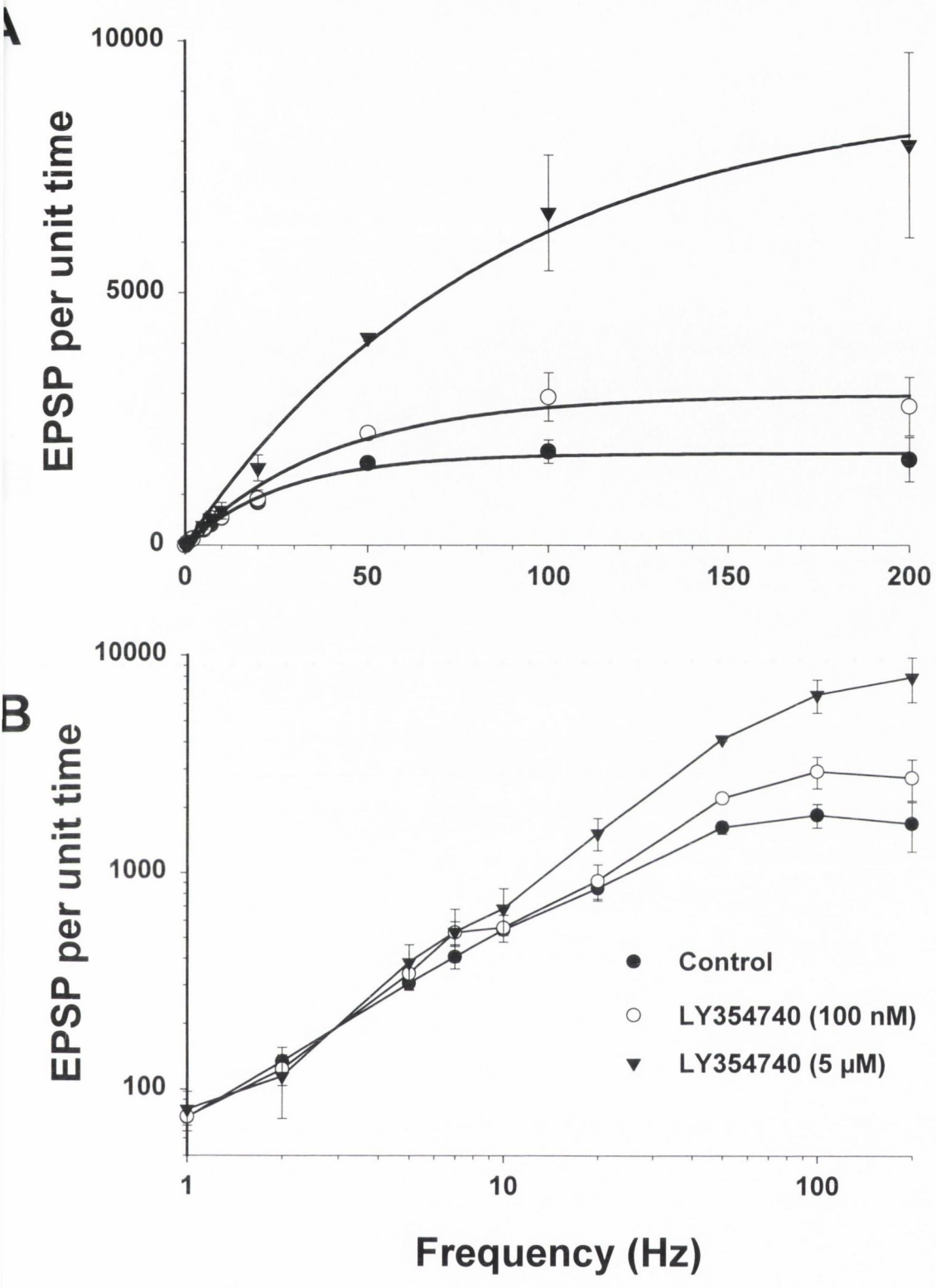


Figure 4.9 Transmitter mobilisation is extended by activation of group II mGluR. The EPSP per unit time (EPSP amplitude x frequency, expressed as arbitrary units) for control (filled circles), LY354740 (100 nM) (open circles) and LY354740 (5 µM) (filled triangles) is plotted as a function of frequency on a linear (A) and a log scale (B). Each point plots the mean and SEM (n = 4-10).

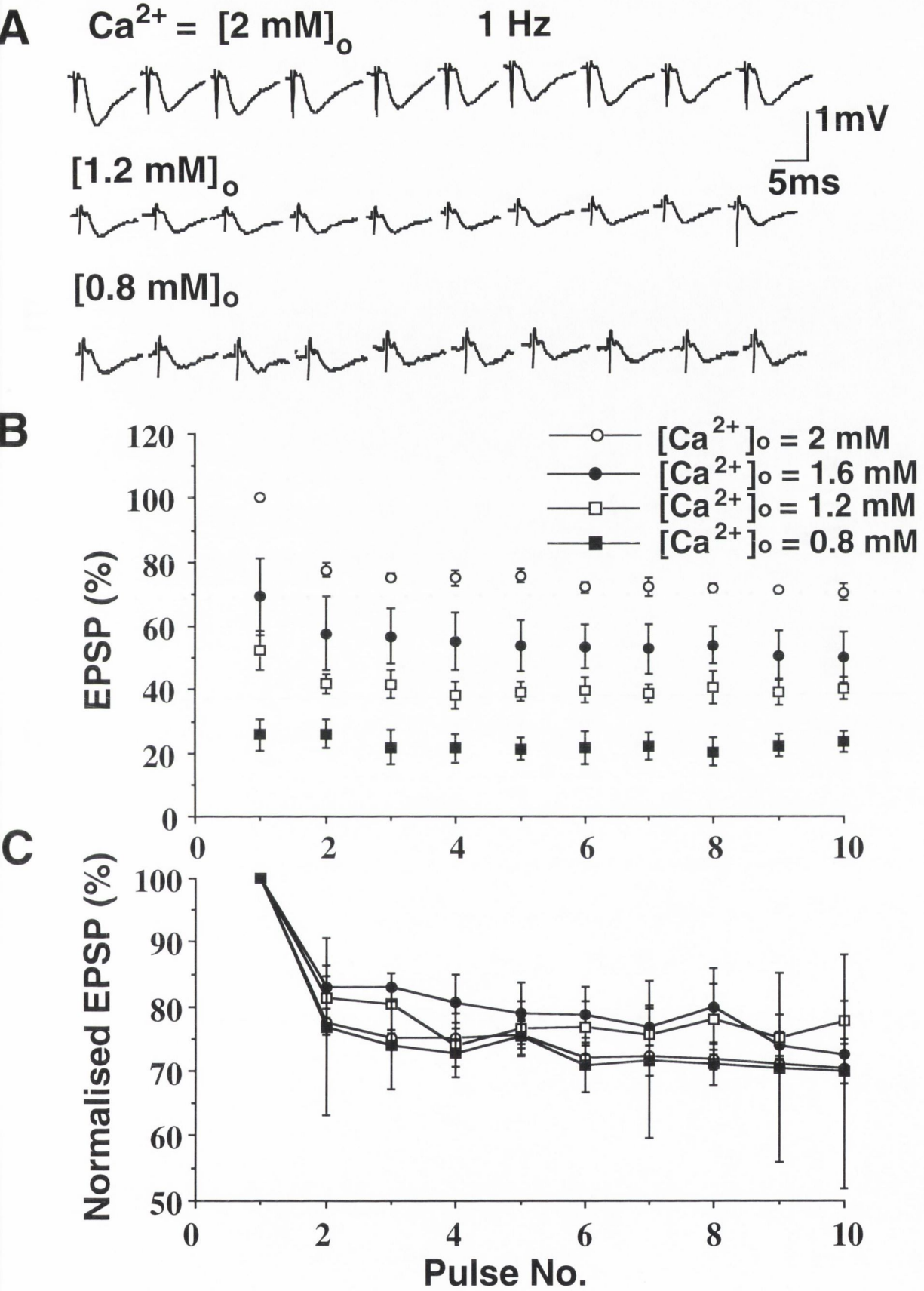


Figure 4.10 Lowering extracellular calcium has no effect on short-term depression evoked at 1 Hz in the medial perforant path. (A) Typical traces showing trains of EPSPs elicited with 1 Hz stimulation in the medial perforant path, in 2 mM (upper), 1.2 mM (middle) and 0.8 mM extracellular calcium (lower). Absolute (B) and normalised (C) EPSP amplitude plotted as a function of pulse number in 2 mM (open circles), 1.6 mM (filled circles), 1.2 mM (open squares) and 0.8 mM extracellular calcium (filled squares) Each point plots the mean ± SEM (n = 8, 3, 6, 4).

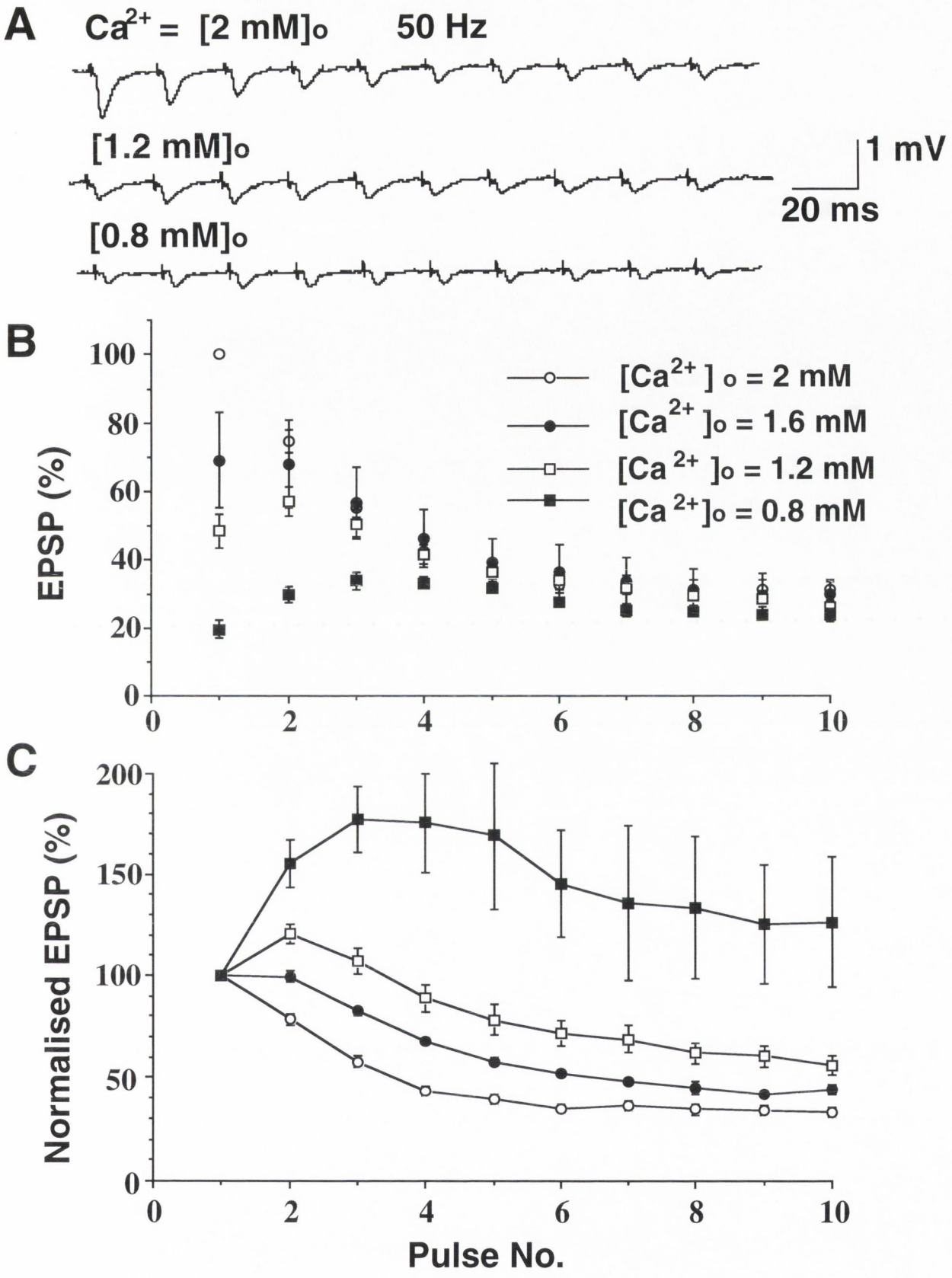


Figure 4.11 Lowering extracellular calcium attenuates short-term depression evoked at 50 Hz in the medial perforant path. (A) Typical example showing trains of EPSPs elicited with 50 Hz stimulation in the medial perforant path, in 2 mM (upper), 1.2 mM (middle) and 0.8 mM extracellular calcium (lower). Absolute (B) and normalised (C) EPSP amplitude plotted as a function of pulse number in 2 mM (open circles), 1.6 mM (filled circles), 1.2 mM (open squares) and 0.8 mM extracellular calcium (filled squares). Each point plots the mean ± SEM (n = 11, 3, 7, 4).

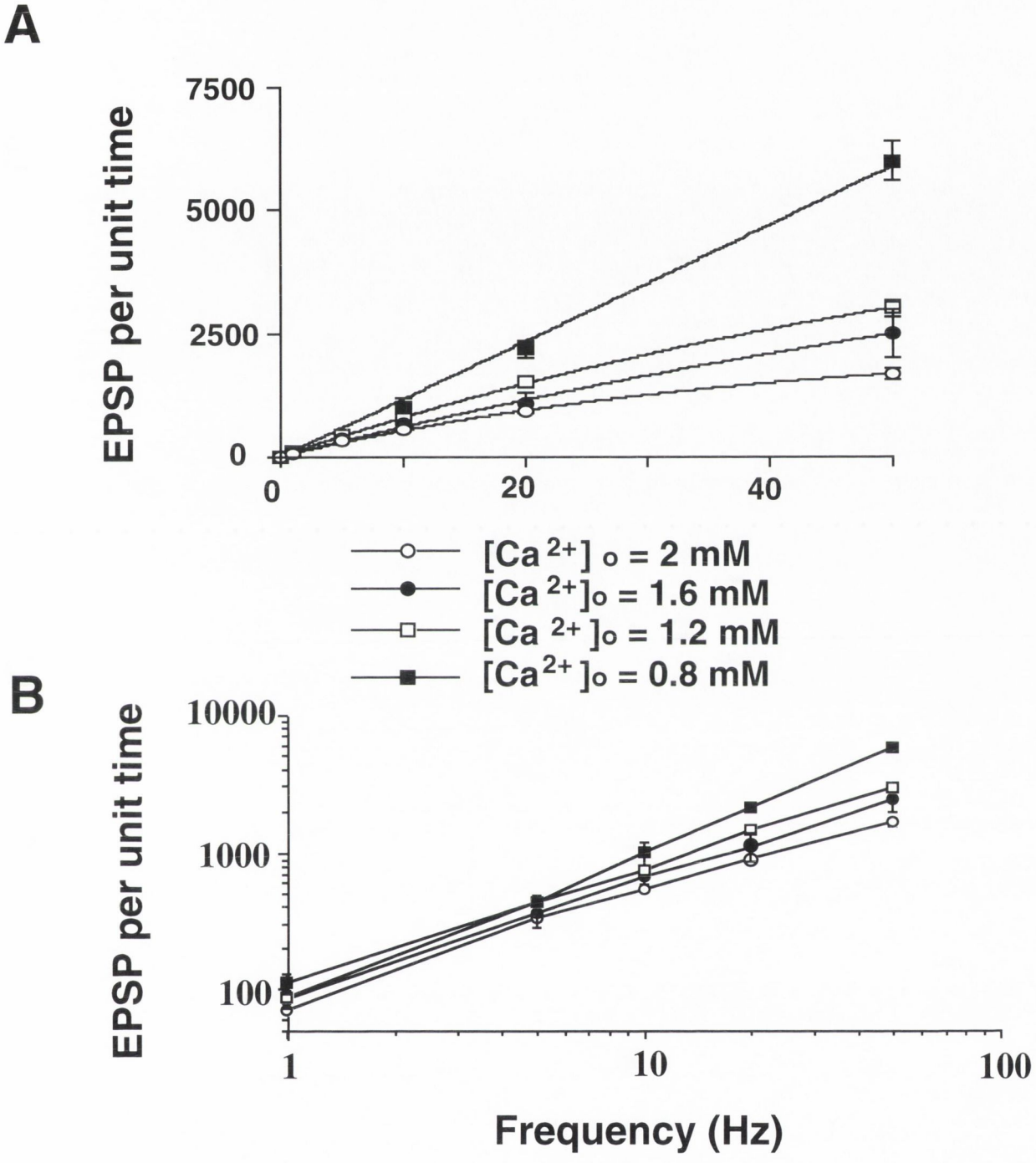


Figure 4.12 Transmitter mobilisation is extended by lowering extracellular calcium. The EPSP per unit time (steady-state EPSP amplitude (%) x frequency, expressed in arbitrary units) for control (2 mM, open circles), 1.6 mM (filled circles), 1.2 mM (open squares), 0.8 mM (filled squares) is plotted as a function of frequency on a linear (A) and on a log scale (B). Each point plots the mean ± SEM values for (n = 4 - 11).

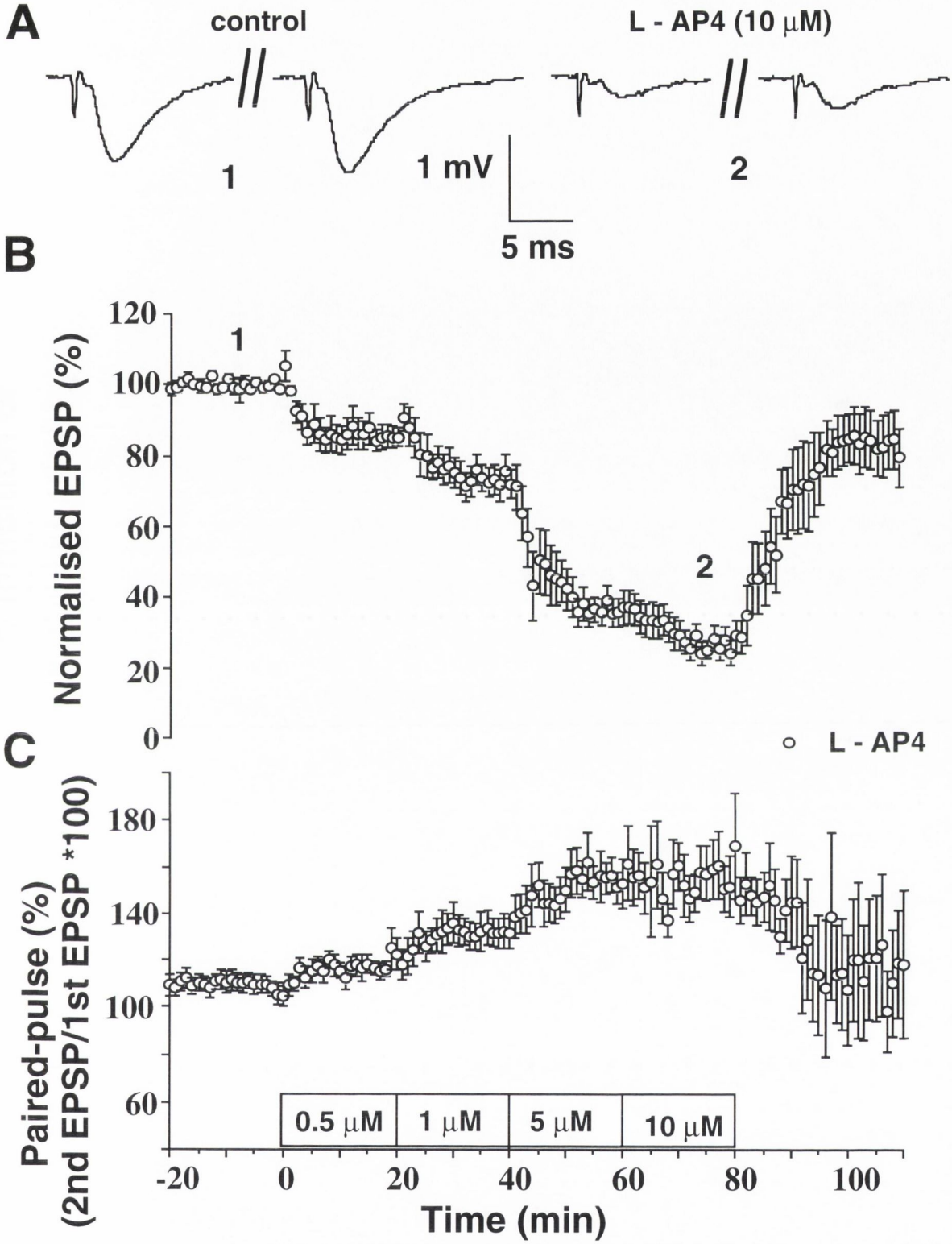


Figure 4.13 Activation of group III mGluR by L-AP4 reduces the EPSP amplitude in a dose-dependent manner in the lateral perforant path. (A)Typical example of pairs of EPSPs recorded at the test frequency (0.0166 Hz), (interstimulus interval of 40 ms) in control (left) and in the presence of L-AP4 (10 μM) (right). (B) Normalised data from 9 cells showing the dose-dependent reversible depression of the EPSP amplitude which accompanies the application of L-AP4 in the lateral perforant path. (C) Paired-pulse facilitation is increased by L-AP4, suggesting that the agonist is acting at presynaptically located mGluR III. Each point is mean ± SEM (n = 9).

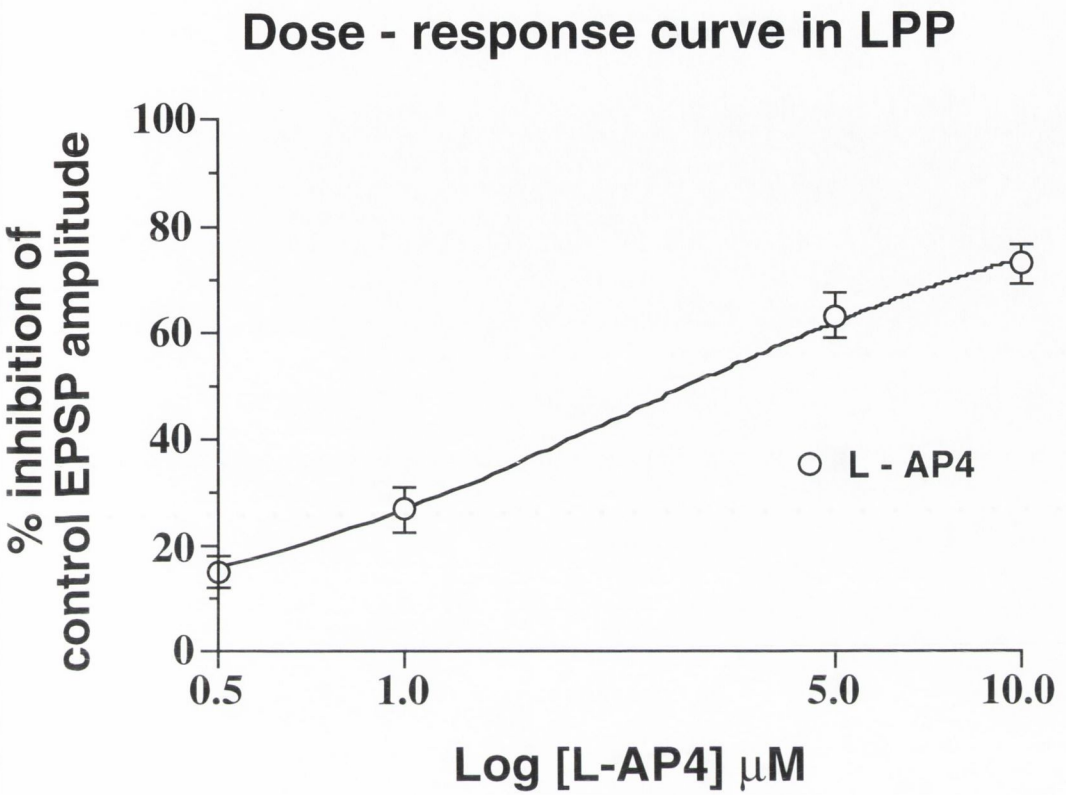


Figure 4.14 Dose-response curve for the action of L-AP4 in the lateral perforant path. The dose-response curve was best fitted with a one-site binding hyperbola (Graphpad Prism software). Y = Bmax * x / (Kd + X) where B max is the maximal binding, and Kd is the concentration of ligand required to reach half-maximal binding. The EC50 for L-AP4 was estimated to be 2.4 μM in the lateral perforant path. Each point plots the mean ± SEM values for between 4 and 8 slices.

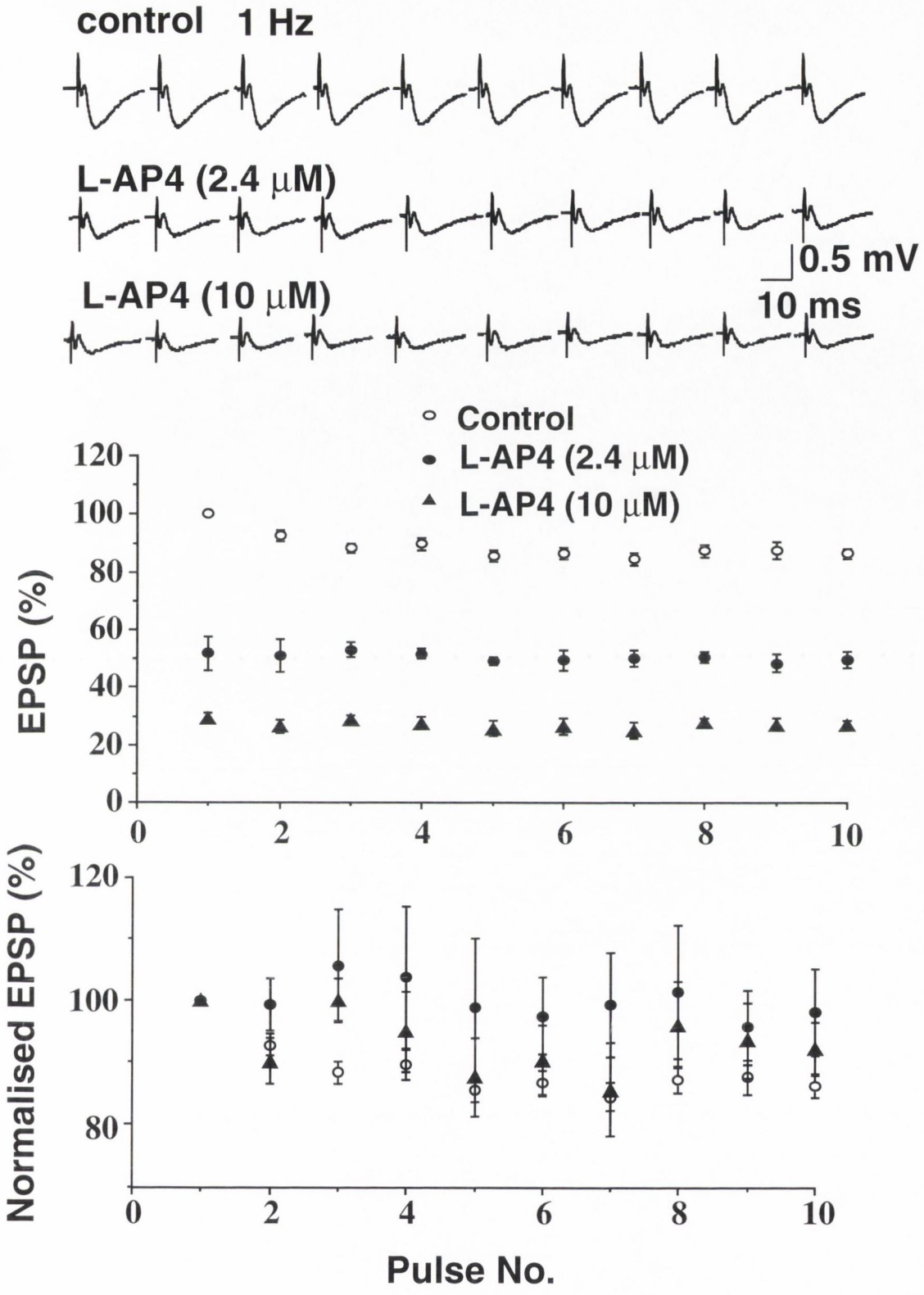


Figure 4.15 The group III mGluR agonist L-AP4 has no effect on short-term depression evoked at 1 Hz in the lateral perforant path. (A) Typical traces showing trains of EPSPs elicited with 1 Hz stimulation in the lateral perforant path, in control (upper), L-AP4 (2.4 μM) (middle) and L-AP4 (10 μM) (lower). Absolute (B) and normalised (C) EPSP amplitudes plotted as a function of pulse number in control (open circles), L-AP4 (2.4 μM) (filled circles,) and L-AP4 (10 μM) (filled triangles). Each point plots the mean ± SEM (n = 7, 5, 7).

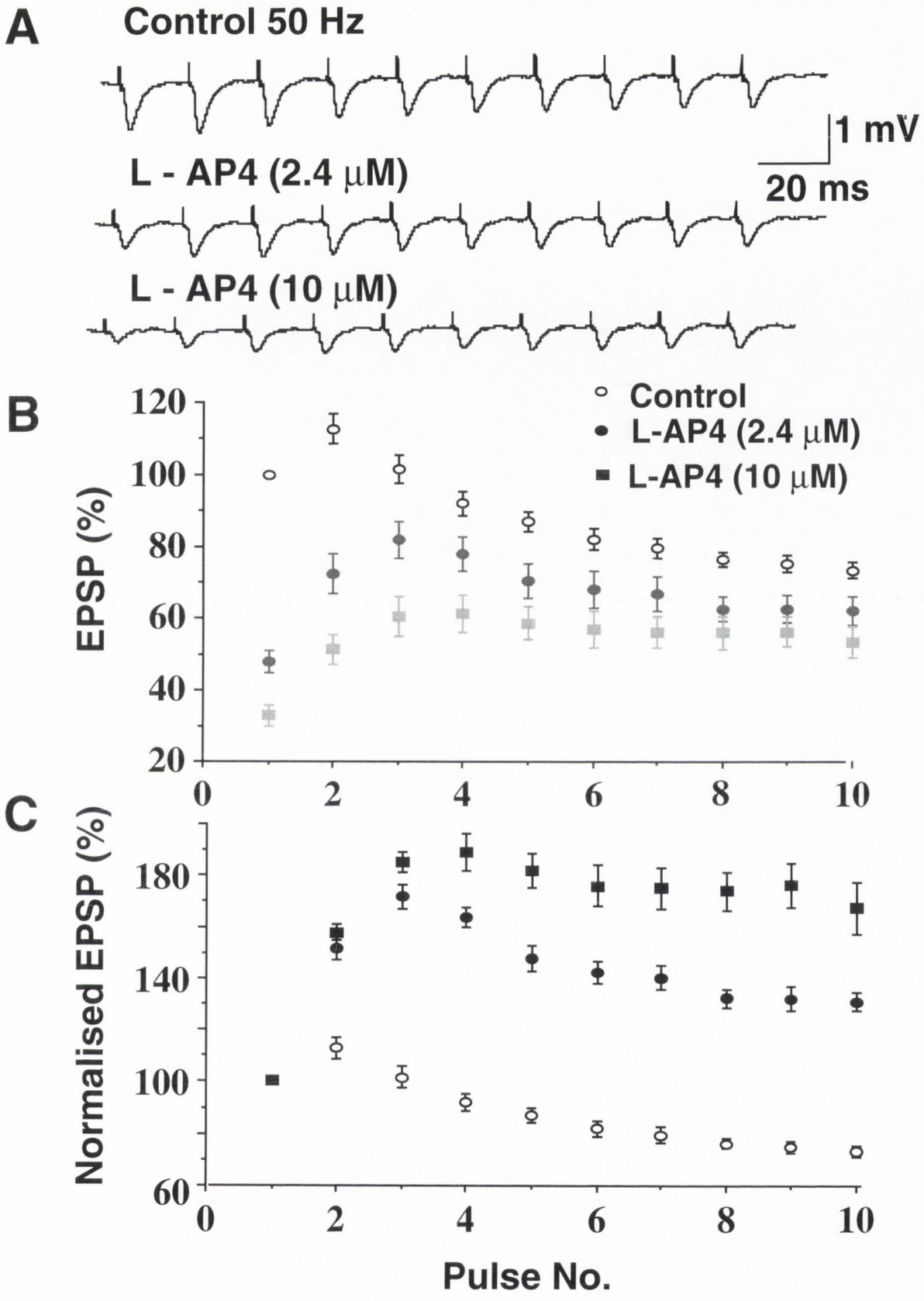


Figure 4.16 The group III mGluR agonist L-AP4 attenuates short-term depression evoked at 50 Hz in the lateral perforant path. (A) Typical traces showing trains of EPSPs elicited with 50 Hz stimulation in the lateral perforant path, in control (upper), L-AP4 (2.4 μM) (middle) and L-AP4 (10 μM) (lower). Absolute (B) and normalised (C) EPSP amplitude plotted as a function of pulse number in control (open circles), L-AP4 (2.4 μM, closed circles) and L-AP4 (10 μM, filled squares). Each point plots the mean ± SEM (n = 10, 7, 9).

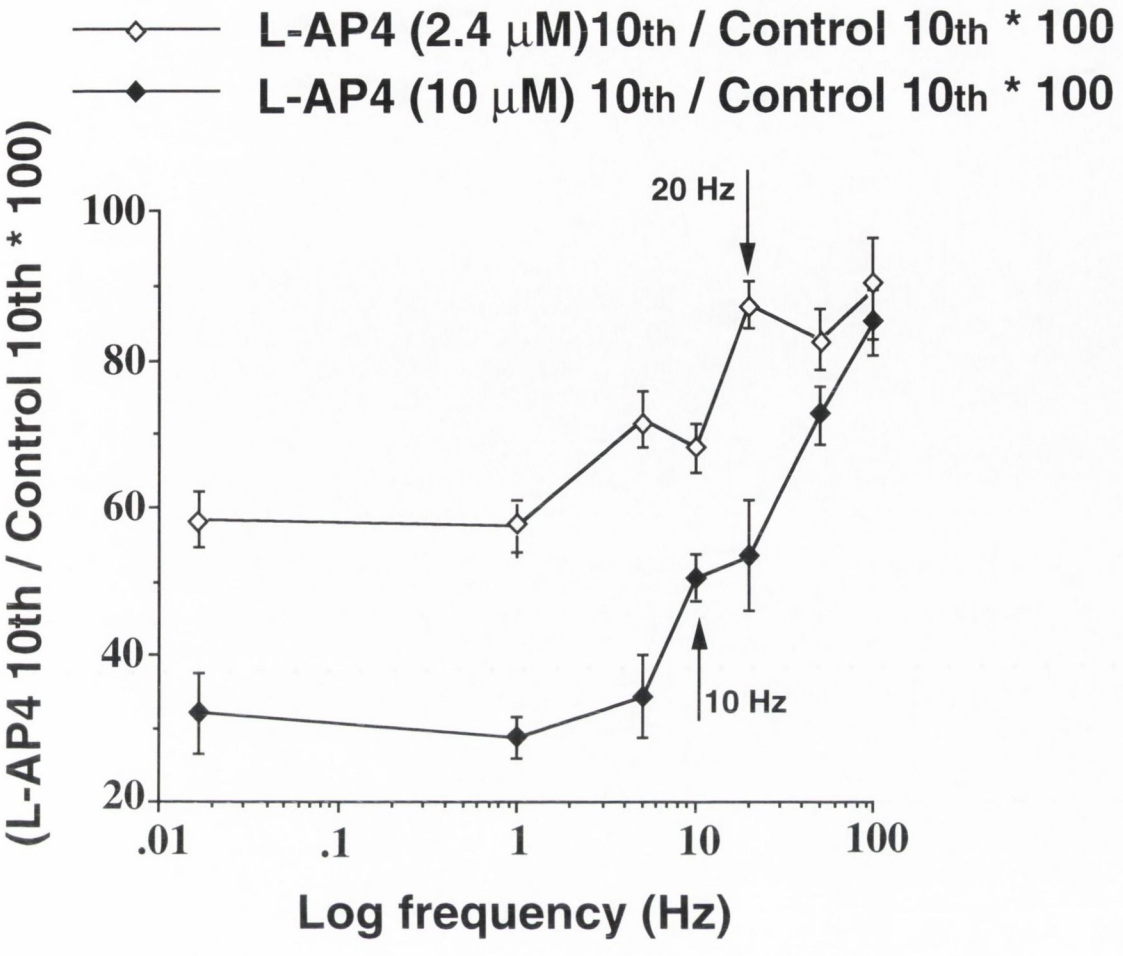


Figure 4. 17 Frequency dependence of group III mGluR modulation of short-term depression in the lateral perforant path. The percentage inhibition of the control steady-state EPSP by 2.4 μM (open diamonds) and 10 μM L-AP4 (closed diamonds) for a range of frequencies between 0.0166 Hz and 100 Hz in the lateral perforant path. The concentration and frequency dependence of the group III mGluR attenuation of short - term depression is clearly seen. The threshold frequency at which L-AP4 reduced short - term depression was 20 Hz and 10 Hz for 2.4 μM and 10 μM L-AP4 respectively. Data points were constructed by expressing the amplitude of the 10th EPSP evoked in the presence of L-AP4 as a percentage of the 10th EPSP evoked under control conditions, for a range of frequencies. The arrows show the threshold frequency at which inhibition of the short-term depressed EPSP occurs. This was established by comparing the % inhibition at each frequency with the % inhibition at 0.0166Hz (test frequency) using the student's t-test at the 5 % level of significance. Each point plots the mean ± SEM (n = 4 -16)

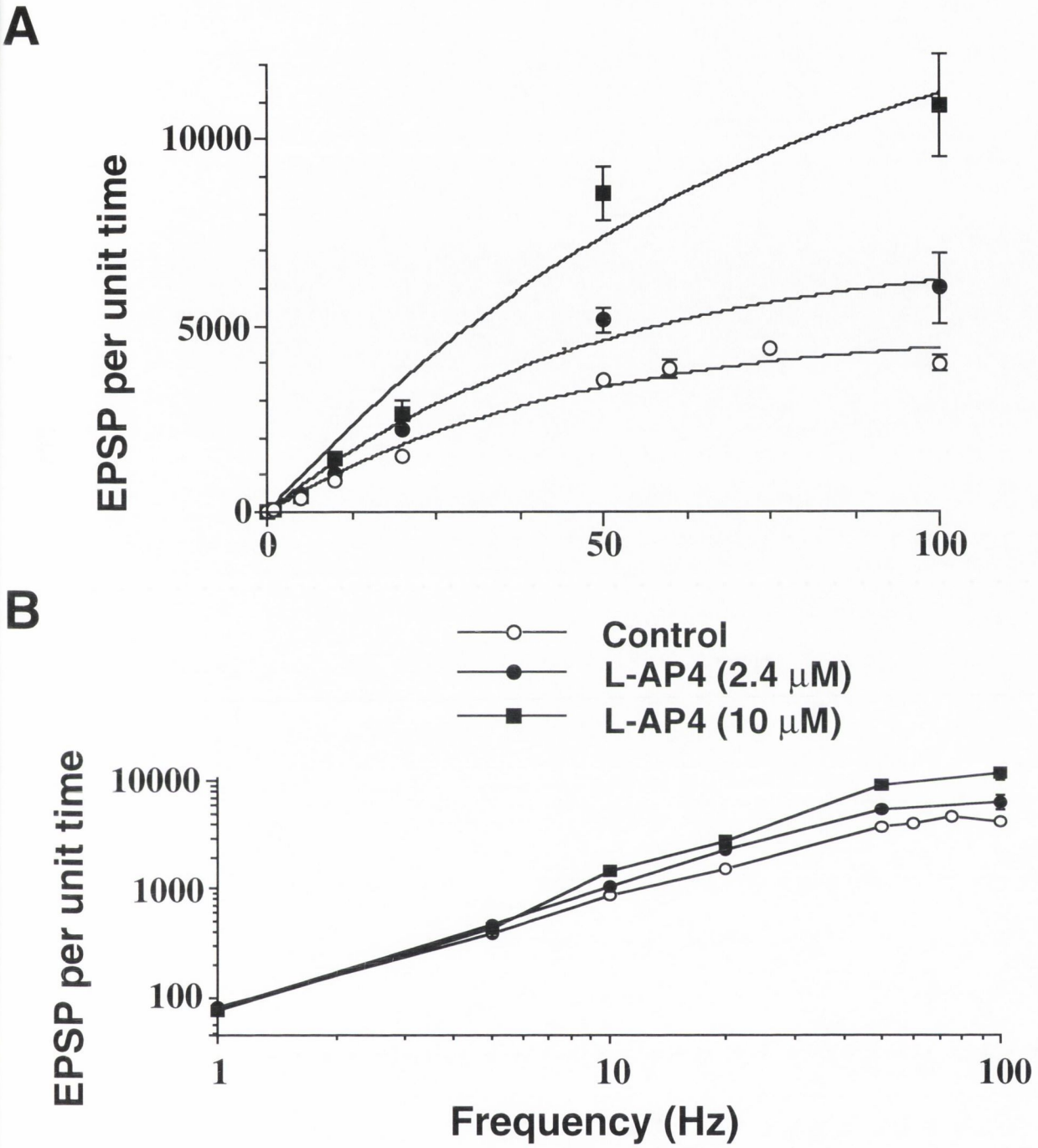


Figure 4.18 Transmitter mobilisation is extended by activation of group III mGluR. The EPSP per unit time (steady-state EPSP amplitude (%) x frequency; arbitrary units) for control (open circles), L-AP4 (2.4 μM), (filled circles) and LAP-4 (5 μM), (filled squares) is plotted as a function of frequency on a linear (A) and on a log scale (B). Each point plots the mean ± SEM (n = 4 - 10)

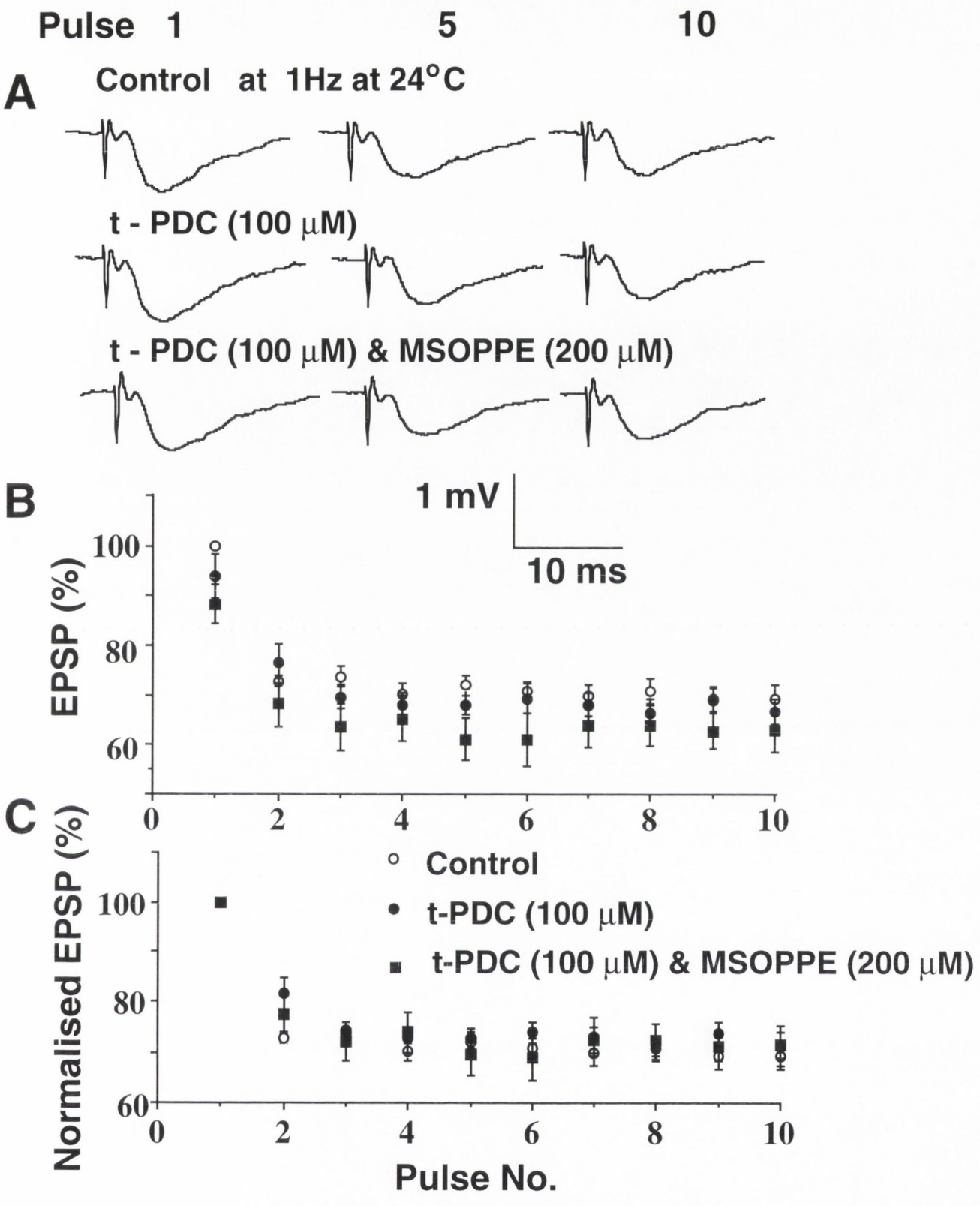


Figure 4.19 t-PDC and MSOPPE have no effect on short-term depression elicited at 1 Hz in the medial perforant path. (A) Typical example of the 1st, 5th and 10th EPSPs in a train of 10 stimuli delivered at 1 Hz at room temperature in control (top), in the presence of t-PDC (100 μM) alone (middle), and in t-PDC (100 μM) and MSOPPE (200 μM) (bottom). Absolute (B) and normalised (C) EPSP amplitudes plotted as a function of pulse number in control (open circles), t-PDC (100 μM) (filled circles) and t-PDC (100 μM) & MSOPPE (200 μM) (filled squares). In the normalised graph, the curve overlap, illustrating the lack of effect of either compound on short-term depression. (Each point is the mean ± SEM of 5 slices).

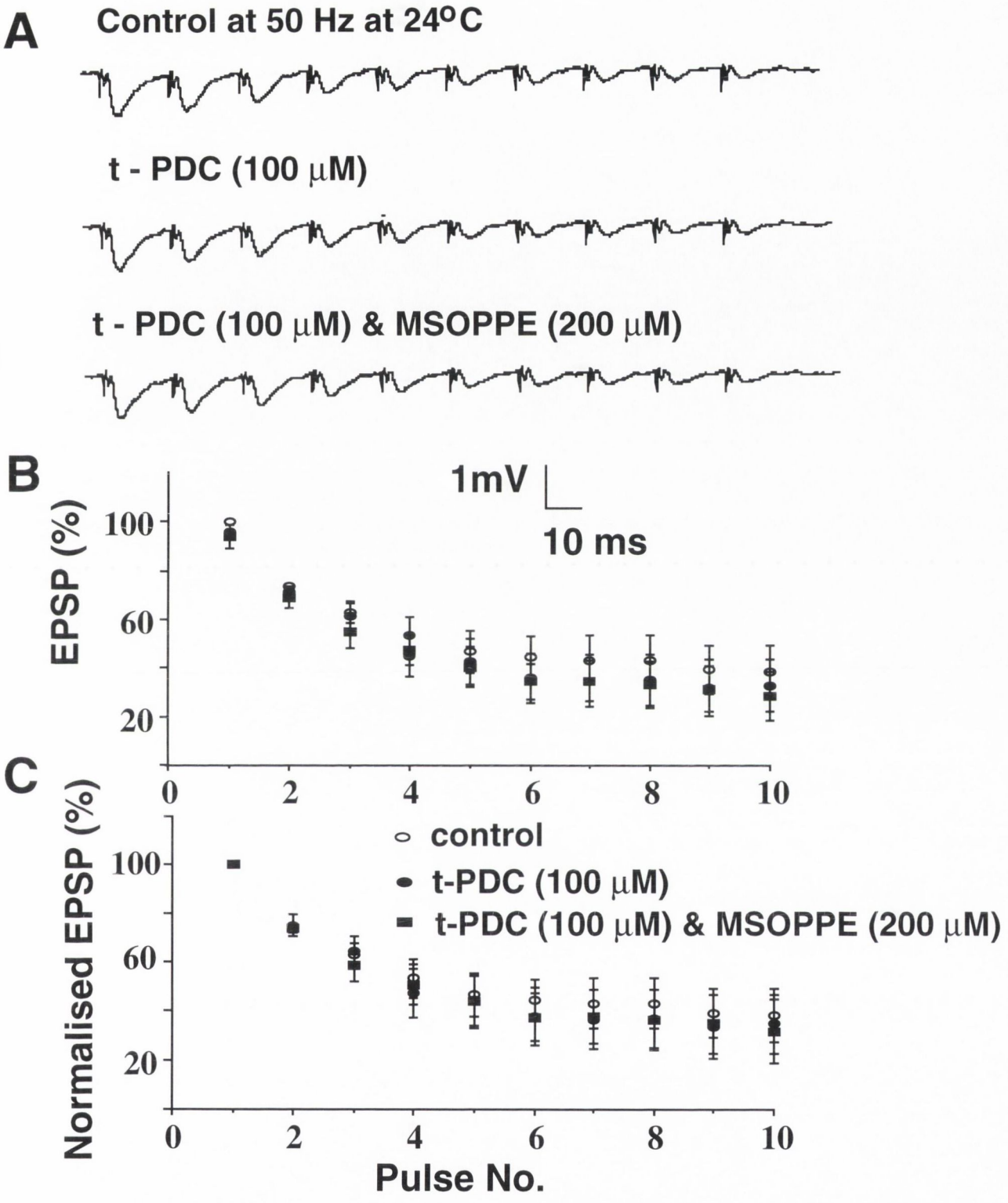


Figure 4.20 t-PDC and MSOPPE have no effect on short-term depression elicited at 50 Hz in the medial perforant path. (A) Typical example showing the 1st, 5th and 10th EPSPs elicited in a train of 10 stimuli delivered at 50 Hz at room temperature in control (top), in the presence of t-PDC (100 μM) alone (middle), and after the addition of MSOPPE (200 μM) (bottom). Absolute (B) and normalised (C) EPSP amplitude plotted as a function of pulse number in control (open circles), t-PDC (100 μM) (closed circles) and t-PDC (100 μM) & MSOPPE (200 μM) (filled circles). In the normalised graph, the curves overlap, illustrating the lack of effect of either compound on short-term depression. (Each point is the mean ± SEM of 5 slices).

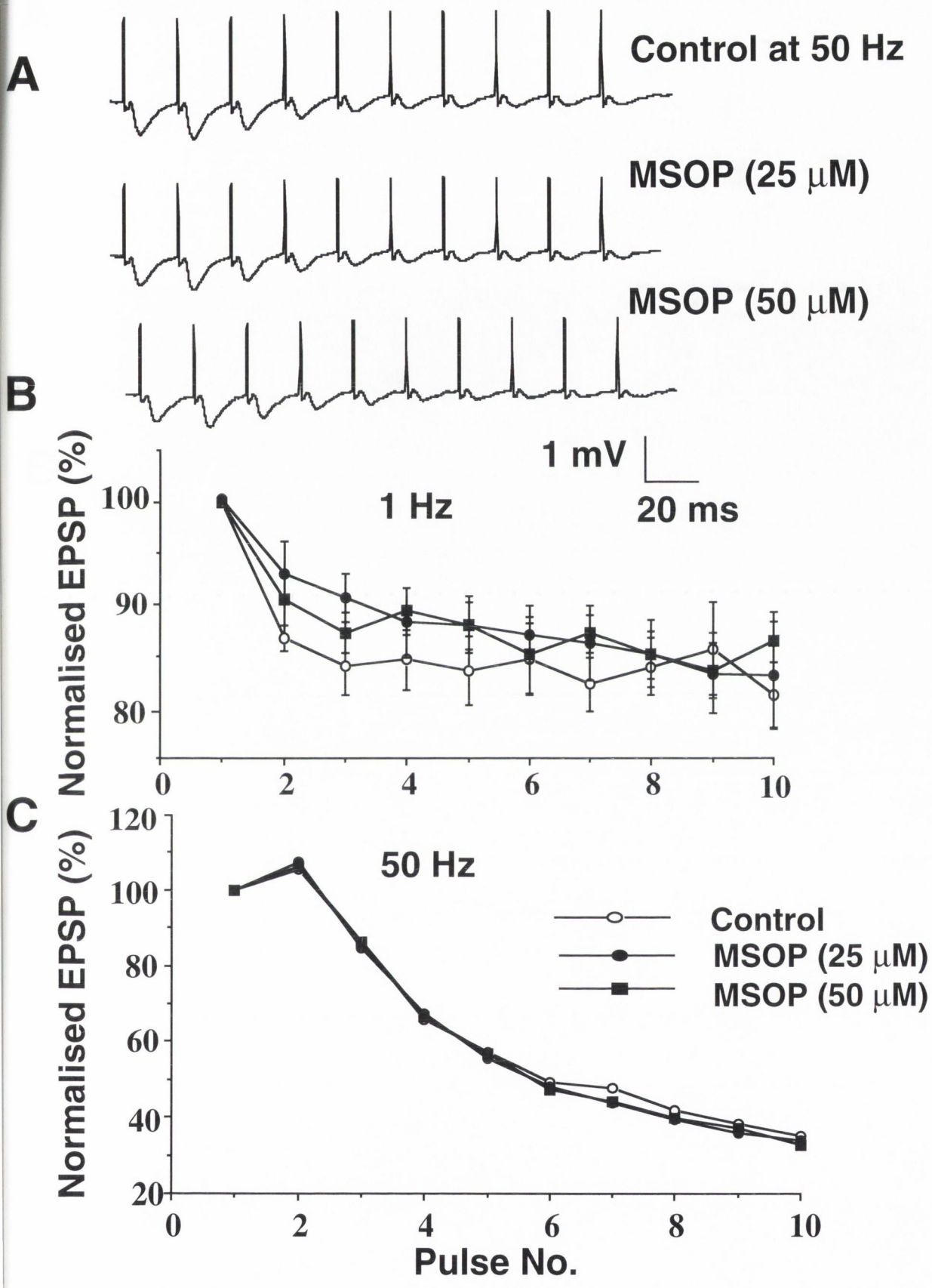


Figure 4.21 MSOP has no effect on short-term depression at 1 Hz or 50 Hz in the lateral perforant path. (A) Typical example of trains of stimuli elicited with 50 Hz stimulation in the lateral perforant path in control (top), and in the presence of 25 μM (middle) and 50 μM MSOP (bottom). Normalised data showing EPSP amplitude as a function of pulse number for 1 Hz (B) and 50 Hz (C) in control (open circles), and 25 μM (closed circles) and 50 μM (filled squares) MSOP. The curves overlap at both low and high frequencies indicating the lack of effect of MSOP on short-term depression at these frequencies. Each point is mean ± SEM from 8 slices.

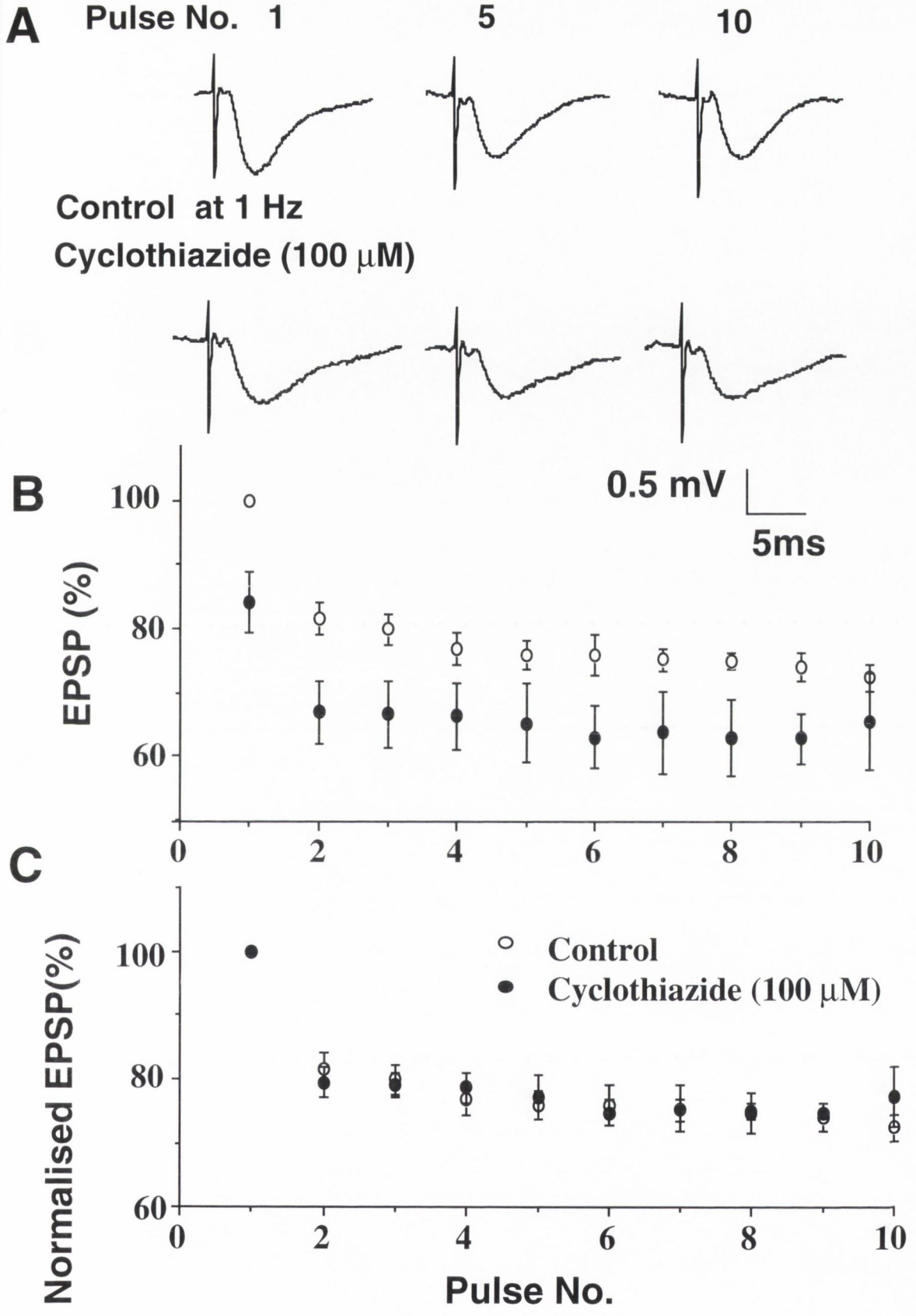


Figure 4.22 Cyclothiazide has no effect on short-term depression evoked at 1 Hz in the medial perforant path. (A) Typical traces showing the 1st, 5th and 10th EPSPs recorded at 1 Hz in control (top) and in the presence of 100 μM Cyclothiazide (bottom). Absolute (B) and normalised (C) EPSP amplitude plotted as a function of pulse number in control (open circles) and in the presence of cyclothiazide (100 μM) (filled circles). Each point plots mean ± SEM for 5 slices.

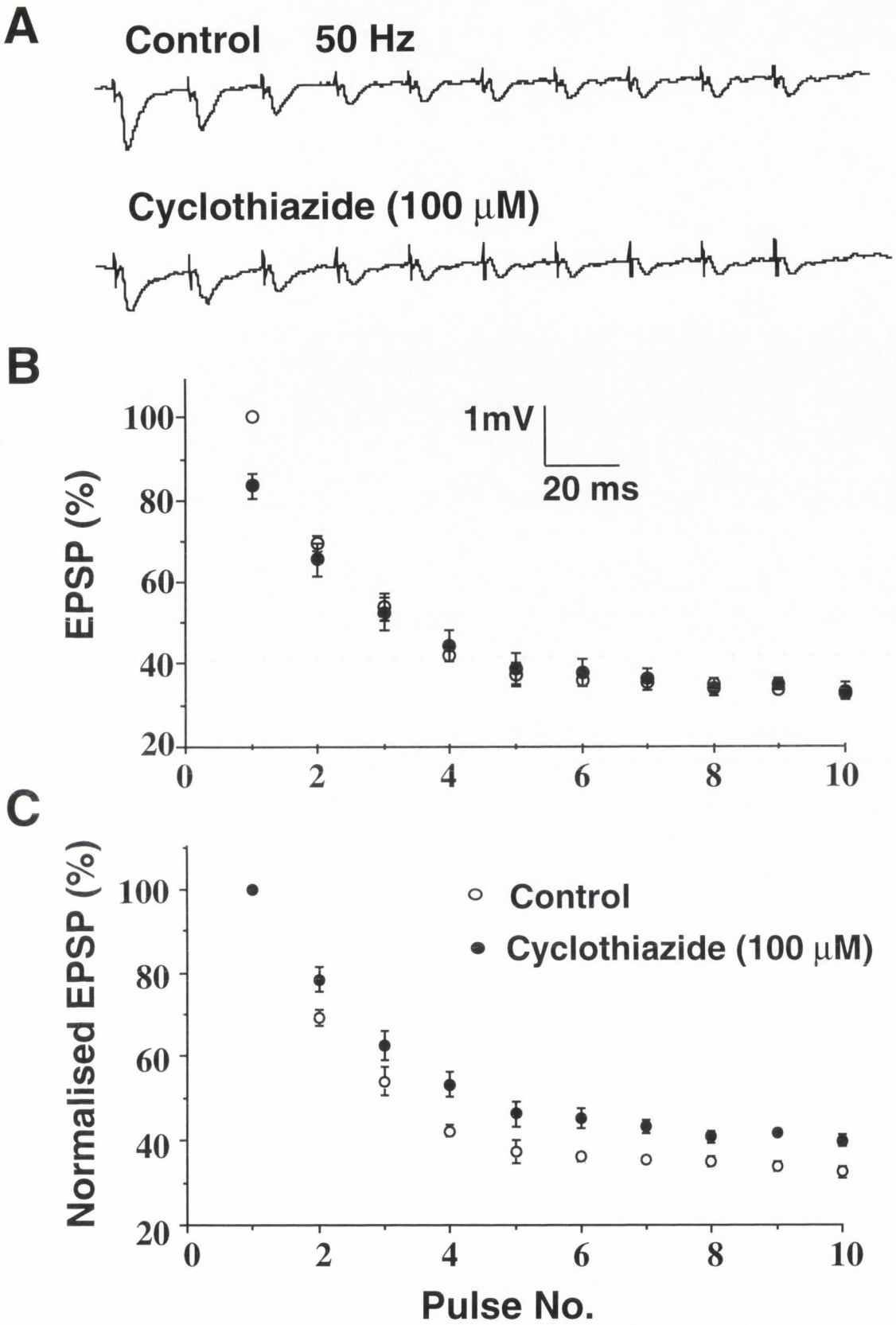


Figure 4.23 Cyclothiazide attenuates short-term depression evoked at 50 Hz in the medial perforant path. (A) Typical traces showing trains of EPSPs recorded at 50 Hz in control (top) and in the presence of 100 μM Cyclothiazide (bottom). Absolute (B) and normalised (C) EPSP amplitude plotted as a function of pulse number in control (open circles) and in the presence of cyclothiazide (filled circles). Each point plots mean ± SEM for 5 slices.

4.5 Discussion

The results of this study have shown that short-term plasticity in the medial and lateral perforant paths of the dentate gyrus is a context-dependent and dynamic phenomenon. In the medial perforant path, under normal conditions, depression predominates, whereas when the probability of release is lowered, such as occurs in low extracellular calcium or in the presence of the group II mGluR agonist LY354740, then facilitation becomes more prominent at high firing rates. In the lateral perforant path, the initial probability of release is lower and as a consequence there is more facilitation under control conditions.

In the medial perforant path the rate of onset and extent of short-term depression were found to increase with frequency. The decrease in the amplitude of the EPSP in response to a train of stimuli was found to be exponential over a range of frequencies between 1 Hz and 200 Hz (Kasuno and Landau, 1975; Varela et al., 1997). The relationship between the time constant of the onset of depression at different frequencies was linear which suggests that only one process was responsible for the short-term depression (Betz, 1970). The observed depression in both the medial and lateral perforant paths is consistent with the depletion hypothesis of synaptic depression, as has been suggested at other synapses (Betz 1970; Dobrunz and Stevens., 1997; Varela et al., 1997).

The exact mechanisms by which presynaptic group II mGluR activation depresses the EPSP / EPSC amplitude is the subject of much debate. Some evidence suggests that their activation leads to a block of presynaptic calcium channels and as a consequence the influx of calcium

into the terminals is reduced (Wu and Saggau, 1994; Takahashi et al., 1996). Other groups have suggested that the site of action is downstream from Ca^{2+} influx, possibly on the machinery involved in vesicle filling, mobilisation and release (Hille, 1994). An alternate viewpoint holds that these receptors are linked to presynaptic K^+ channels (Sladeczek et al., 1993) Although the techniques employed in this study were not sophisticated enough to resolve the issue, the results presented here, (specifically that the reduction in extracellular calcium mirrored the effect of LY354740 on frequency-dependent depression) are consistent with the hypothesis that activation of group II and III mGluR leads to a reduction in Ca^{2+} influx.

These results show that activation of presynaptic mGluRs results in a concentration and frequency dependent reduction in the synaptic depression observed in both the medial and lateral perforant paths. Although this effect of presynaptic mGluRs has not been fully investigated (Canepari and Cherubini., 1998), there is a precedent for other presynaptic receptors in the nervous system performing this role. (Lev-Tov and Pinco, 1992; Pennartz and Lopes da Silva, 1994; Barnes-Davies and Forsythe, 1995; Markram and Tsodyks, 1996; Shen and Horn, 1996; Isaacson and Hille, 1996; Varela et al., 1997; Brenowitz et al., 1998). At visual cortical synapses (Varela et al., 1997) and at cultured hippocampal synapses (Isaacson et al., 1997), researchers have demonstrated that the presence of baclofen (acting at $GABA_B$ receptors) could overcome presynaptic depression during high-frequency stimulation. Hille's group proposed that the co-operative action of intracellular calcium on exocytosis, could at high frequencies, negate the dependence of transmitter release on calcium influx. Alternatively if vesicle recycling is a rate limiting factor during high-frequency stimulation (which it has been

shown to be, see Curtis and Eccles, 1968; Richards 1972), then in the presence of baclofen the rate of transmitter release falls and is less sensitive to calcium influx (Isaacson et al., 1997).

Trussell's group went a step further by saying that the modulatory action of GABA on presynaptic receptors at the nucleus magnocellularis (nMAG) could be either inhibitory or excitatory depending on the level of activity at the synaptic target. They argued that the reduction in release probability induced by baclofen, reduces vesicle depletion and postsynaptic receptor desensitisation and allows an enhancement of synaptic efficacy to be revealed (Brenowitz et al., 1998).

At Bullfrog sympathetic fibres, the modulation of nicotinic transmission by presynaptic muscarinic receptors was examined by Shen and Horn in 1996. Their findings indicated that inhibition was a biphasic function, with inhibition increasing between 1 and 5 Hz and then disappearing at 20 Hz. This they explained as resulting from an accumulation of intracellular calcium which saturates the release process and makes it less sensitive to calcium entry. (Shen and Horn, 1996). In contrast Pennartz and Lopes da Silva found that the muscarinic modulation of the steady-state synaptic response was significantly stronger in the low frequency range (0.25 - 4 Hz) than at the higher frequencies (8 - 12 Hz) (Pennartz and Lopes da Silva, 1994).

In this study the relief of short-term depression only occurred at frequencies greater than 10 - 20 Hz. In the medial perforant path, LY354740 at concentrations of 100 nM and 5 μM reduced the test (0.0166 Hz) steady-state EPSP amplitude by ~50 % and ~80 % respectively. The removal of short-term depression by group II mGluR activation, only

became significant above 50 Hz and 20 Hz for concentrations of 100nM and 5 μM respectively. In fact at 100 Hz the steady- state EPSP amplitude was slightly larger in the presence of both concentrations of LY354740 than in control. In the lateral perforant path, which displays less depression and greater facilitation under control conditions, the removal of short- term depression via group III mGluR activation occurred at 10Hz and 5 Hz for concentrations of 2.4 μM and 10 μM L-AP4 respectively. These results are in broad agreement with previous studies which have found a relief of short-term depression by presynaptic receptor activation only when the frequency was higher than 5-10 Hz. It appears that the "cut-off" frequency above which there is a relief of short- term depression is inversely related to the probability of release (see below).

In 1960 Curtis and Eccles showed that in motorneurons of the cat spinal cord there existed a relationship of direct proportionality between the amplitude of EPSPs and the stimulus interval for firing rates above 250 Hz (Curtis and Eccles, 1960). Effectively this meant that the mobilisation and liberation of neurotransmitter became rate limiting at this frequency. In the olfactory cortex, Richards (1972) plotted the EPSP per unit time (steady-state amplitude x rate) as a function of the firing rate; at rates greater than 50 Hz the curve levelled off which again indicates that the release processes have become rate limiting (Richards, 1972).

In more recent times this idea has been elaborated by Abbott's and Markrams' groups who have separately come to the conclusion that synaptic depression can impart a gain control over a synapse, equalising tonic input from afferents firing over a certain "limiting" frequency. As a consequence, within this range the sensitivity to sudden transient rate changes is independent of the firing rate of the afferents (Abbott et al.,

1997, Tsodyks and Markram, 1997; Markram et al., 1998). In the present study, the limiting frequency for transmitter mobilisation was in the region of 20 Hz in the medial perforant path, as compared with about 50 Hz in the lateral perforant path, which displays less short-term depression. These results are consistent with previous results at cortical synapses, which display strong short-term depression, where the limiting frequency was found to be about 10Hz in the visual cortex (Abbott et al., 1997) and 10 - 25 Hz in the somatosensory cortex (Tsodyks and Markram, 1997). The lower limiting frequencies found in their studies may be explained by the fact that both these groups use a high Ca^{2+} to Mg^{2+} ratio in their extracellular saline which has the effect of increasing release probability and as a consequence increasing synaptic depression. In this study, experimental manipulations which reduced Ca^{2+} entry; such as the lowering of extracellular Ca^{2+} or perfusion of the specific groups II and III mGluR agonists, LY354740 and L-AP4 in the medial and lateral perforant paths respectively, extended the linear range of the curves relating the EPSP per unit time to frequency. The attenuation of short-term depression and the extension of the limiting frequency by activation of groups II and III mGluRs may be interpreted in the following terms. A mGluR-induced reduction in calcium influx causes transmitter release to fall to a lower level that in control, and as such the effect of depletion is lessened. Furthermore the co-operative action of intraterminal calcium influx on exocytosis reduces the dependence of transmitter release on calcium influx (Isaacson et al., 1997). Therefore the range over which transmitter mobilisation and release were seen to increase with increases in frequency was extended and also HFS can overcome short-term depression. (Katz and Miledi, 1968; Isaacson et al., 1997).

Possible mechanisms which have been proposed to explain frequency-dependent depression include vesicle depletion (Elmquist and Quastel, 1965; Betz 1970, Dobrunz and Stevens, 1997), presynaptic autoreceptor activation (Von Gersdorff et al., 1997; Forsythe and Clements, 1990; Scanzani et al., 1997); postsynaptic receptor densensitisation (Trussel et al, 1993; Otis et al., 1996) and a adaptation of the calcium sensor for exocytosis (Hsu et al., 1996). Even at synapses which show robust short - term depression such as the calyx of Held in the mammalian brainstem, presynaptic autoreceptor activation has been shown to account for only a small fraction (~10 %) of the observed activity-dependent depression (Von Gersdorff et al., 1997). Moreover this usually requires that within the experimental paradigm, there exists a predisposition for the accumulation of neurotransmitter within and around the synaptic cleft, such as occurs with high-frequency stimulation. Furthermore this may be used in combination with measures which slow the removal of neurotransmitter from the synaptic cleft such as conducting the experiments at room temperature (Scanzani et al., 1997; Asztely et al., 1997; Barnes-Davies and Forsythe, 1995; Von Gersdorff et al., 1997; Shen and Horn 1996) or employing re-uptake blockers like trans-PDC (Isaacson and Nicol, 1993; Scanzani et al., 1997).

Our experiments in both the medial and lateral perforant paths, have failed to provide any evidence to support the belief that presynaptic autoreceptor activation contributes significantly to frequency-dependent depression under physiological conditions (Gluam and Miller, 1994; Scanzani et al., 1997; Takahashi et al., 1996). In the medial perforant path and in area CA3 of the hippocampus, the group II mGluRs are located some distance from the release sites in the extrasynaptic domain (Petralia et al., 1996; Yokai et al., 1996; Ottersen and Landsend, 1997; Shigemoto et al., 1998)

and might therefore be favourably positioned to be activated by a spillover of glutamate from adjacent terminals (Asztely et al., 1996; Scanzani et al., 1997). However the response to both low (1 Hz) and high (50 Hz) frequency stimulation was unaffected by the presence of the glutamate re-uptake blocker (t-PDC) alone (see Isaacson and Nicol, 1993) or in addition to the putative group II mGluR antagonist MSOPPE. At the lateral perforant path, the frequency dependent depression at 1 Hz and 50 Hz was unaffected by the presence of the putative group III mGluR antagonist MSOP at either 32° C or at room temperature. Nevertheless the duration of the trains employed here (10 pulses) may not have been sufficiently long to allow glutamate to accumulate and diffuse towards the autoreceptors. Alternatively, it may be that these receptors are only employed during pathological conditions where glutamate release is greatly enhanced above and beyond the normal physiological levels. Such a scenario may occur during the high- frequency ripple activity (Chrobak and Buzsaki, 1996) or during the bursting associated with epileptic activity. The results presented here suggest that at high-frequencies activation of presynaptically located mGlu receptors reduces synaptic depression in a similar manner to that which has been described elsewhere in the CNS (Hille, 1997; Brenowitz et al., 1997). This would contradict the argument that the receptors are employed to somehow reduce the hyper - excitability associated with an elevation in glutamate release. Therefore it may be more likely that an as yet unidentified role under physiological conditions is attributable to these autoreceptors.

Cyclothiazide, which prevents AMPA receptor desensitisation reduced the initial EPSP amplitude and slowed the decay phase of individual EPSPs. However its presence did not alter the rate or extent of depression at 1 Hz which strongly suggests that under these

conditions, no role for postsynaptic AMPA receptor desensitisation can be attributed towards the low frequency depression observed in the medial perforant path. However the depression observed at 50 Hz was reduced by cyclothiazide however it is unclear if this was a pre- or postsynaptic action. Previous studies in hippocampal CA1 (Debanne et al., 1996; Dobrunz and Stevens, 1997), MNTB (Wang and Kaczmarek, 1998), Visual cortex (Varela et al., 1997) have discounted a role for postsynaptic receptor desensitisation, whereas others have made suggestions to the contrary (Trussel et al., 1993; Otis et al., 1996; Jones and Westbrook, 1996). In addition, the similarity of the depression observed during extracellular field EPSP recordings (Fig 4.3A) and intracellular whole-cell EPSCs (Kilbride et al., 1999) suggests that the degree of depression is determined by the amount of presynaptic transmitter release and not by the magnitude of postsynaptic depolarisation, as is the case at cortical synapses (Varela et al., 1998). The presence or absence of picrotoxin made no apparent difference to the observed depression, which suggests that $GABA_A$ inhibitory synapses are not recruited to any significant degree by these stimulation protocols. (Pennartz and Lopes da Silva, 1994).

In summary these results re-affirm the conviction that autoreceptors cannot simply be regarded as inhibitory, but rather their influence is context-dependent and dynamic. Although during low frequency stimulation an autoreceptor may be inhibitory, during high frequency stimulation, facilitatory processes are unmasked and become increasingly significant. (Brenowitz et al, 1998).

Chapter 5

Neural coding in the medial perforant path is modulated by presynaptic group II mGluR

5.1 Abstract

The contribution made by rate and temporal coding to synaptic transmission and the modulation by presynaptic group II mGluR was investigated in the medial perforant path of the dentate gyrus. The postsynaptic response during trains of 10 stimuli at constant frequencies between 0.0166 Hz and 50 Hz was measured. After the 10^{th} pulse, an additional 10 stimuli were delivered at a higher frequency. This switch in frequency was instantaneous and was of the order of (a) 50 % (b) 100 % of the initial frequency or (c) a fixed increment increase of 5 Hz.

The time-averaged postsynaptic response (EPSP per unit time) was found to increase linearly with frequency up to ~25 Hz. At frequencies higher than this the response plateaued, in this range the steady-state time-averaged postsynaptic response was independent of frequency. However the transient response (observed during the switch) was roughly proportional to the magnitude of the change in rate and also showed an equal sensitivity to fixed percentage rate changes for input rates higher than ~10Hz - 20Hz. The sensitivity of the transient response to fixed increment changes in rate was greatest at low frequencies. Activation of group II mGluR extended the limiting frequency and as a result increased the range of frequencies over which rate coding was possible. In addition the responsiveness to temporal changes in rate was reduced by mGluR activation and as such may point towards a possible role for these autoreceptors.

5.2 Introduction

In the CNS the basic unit of information is the action potential. Analogous to a mosaic, individually the pieces can impart little knowledge, it is only after they have been combined that any meaningful information can be extracted. In processing information the brain is faced with a problem; namely how do you integrate these individual action potentials to construct a reliable and efficient code for translating internal and external stimuli into electrical signals. The exact nature of the neural code for information processing in the CNS remains a matter for debate (Konig et al., 1996; Gertstner et al., 1997; Selig et al., 1997). During the 1980s the general consensus amongst neuroscientists was that unreliable linear-threshold neurons encoded information exclusively by assimilating all the inputs that arrived within a certain period of time. This averaged firing rate code could overcome the unreliability associated with single synapses such as release failures and postsynaptic desensitisation. Furthermore the average output from many neurons (population coding) could smooth over any imperfections associated with single neurons and ensure a reliable and precise neural code (Koch, 1997; Sakurai, 1996; Zador, 1998).

Firing rate coding (temporal integration) appears a simple and effective model for neural coding but it is not without its limitations. These arise because by averaging the number of action potentials over a given period of time, the exact choreography of the input is lost, and with it, the ability to encode information in the (changing) pattern of this input (Zador, 1998; Koch, 1997).

Today, rate coding is still widely accepted as being important but it is no longer viewed as the sole mechanism of neural coding. The temporal

arrangement of spikes (temporal coding), (Jefferys et al., 1996; Buonomano and Merzenich, 1995): coincidence detection (Shelig et al., 1997), synchronisity (Lisman, 1997; Whittington et al., 1995), and synfire chains (Abeles, 1991) and in particular the changes in the interspike interval (Tsodyks and Markram, 1997; Abbott et al., 1996; O'Donovan and Rinzel, 1997; Gerstner et al., 1996) are now believed to provide a valuable and complementary coding mechanism. As such the interval between spikes is though to be as important as the spikes themselves.

Undoubtedly our increased knowledge of neural coding has arisen in part from the advances in understanding synaptic transmission more comprehensively. Synaptic transmission is governed by dynamic processes of variable strength (Zucker, 1989). Long-term alterations in synaptic strength such as long-term potentiation (LTP) and long-term depression (LTD), (Bliss and Collingridge, 1989, Malenka, 1994) are linear scaling mechanisms, which enable the strength or "weight" of a synapse to be reset at a new level. As such they have been embraced as the most likely candidates to represent the cellular basis of learning and memory (reviewed by Bliss and Collingridge, 1989).

In contrast short-term plasticity enables synapses to make rapid, activity-driven changes in their efficacy (Zucker, 1989). Although an action potential (spike) may be an all or nothing, binary event, the response it elicits in the post-synaptic cell will vary as a function of the prior activity at that synapse (O'Donovan and Rinzel, 1997 and Nelson and Turrigiano, 1998).

Compared to long-term plasticity, devising an all encompassing explanation for the functional significance of short-term plasticity has

proved more problematic. Abbott's and Tsodyks and Markrams' groups, working on cortical synapses have independently uncovered results which suggest that synaptic depression increases the computational capacity and processing speed of neural coding. (Zador and Dobrunz, 1997; O'Donovan and Rinzel, 1997; Liaw and Berger, 1996).

Essentially they have found that synaptic depression operates as a sort of "automatic gain control mechanism" which unlike inhibitory or adaptive mechanisms has the advantage of being input specific. (Abbott et al., 1997, Tsodyks and Markram, 1997). Therefore afferents that are very active can be handled at a lower gain. This is analogous to the quieter afferents being heard amidst the noise created by the louder afferents. Crucially the overall strength of the synapse, determined postsynaptically, remains untouched.

Specifically both groups have found that when cortical synapses, which at moderate stimulation frequencies display pronounced short-term depression, are driven beyond a certain "limiting frequency", the postsynaptic response per unit time becomes inversely proportional to the presynaptic firing rate (Curtis and Eccles, 1960; Richards, 1972; Tsodyks and Markram, 1997; Abbott et al, 1997). This ensures that above the limiting frequency (found to be between 10 and 25 Hz at cortical synapses) the total synaptic drive becomes saturated. The existence of a limiting frequency has important functional ramifications for the rate coding potential of a neuron. Essentially at rates above this limiting frequency, the only information carried in the presynaptic firing rate that is decoded by the postsynaptic cell is a binary "on or off" signal. (Markram and Tsodyks, 1996; Tsodyks and Markram, 1997).

The constraints that synaptic depression exert on rate coding are compensated for by an increased sensitivity to abrupt changes in firing rate. The steady-state time averaged postsynaptic response before and after a change in firing rate above the limiting frequency will be the same. However there exists a window of opportunity, before synaptic depression occurs, where a transient analogue signal can encode the change in firing rate (Tsodyks and Markram, 1997; Abbott et al., 1997). Specifically the postsynaptic cell can respond to fractional rather than absolute changes in the firing rate (Abbott et al., 1997; Gerstner et al., 1997). In the cortex neurons can respond to a doubling of the firing rate from 10 Hz to 20 Hz and 50 Hz to 100 Hz with equal sensitivity. In contrast, at the same neuron a small increment in the firing rate of slowly firing afferents will be handled with greater sensitivity than if the same increment were to occur at a more rapidly firing afferent. As Abbott pointed out without synaptic depression rapidly firing afferents would dominate the postsynaptic cell to the detriment of more slowly firing afferents. (Abbott et al., 1997).

Zador and Dobrunz suggested that synaptic depression ensures that successful propagation of an input will occur when that input arrives from a population of neurons as opposed to a single neuron (Zador and Dobrunz, 1997). This might ensure that the metabolic and biochemical cost of synaptic transmission is shared across many release sites on different neurons. Furthermore by increasing the signal to noise ratio it might serve to improve the chances of an authentic signal being faithfully transmitted.

Tsodyks and Markram went a step further by suggesting that it is the probability of neurotransmitter release which determines the rate of synaptic depression and it is this in turn which determines the relative contributions made by rate and temporal coding. Specifically they found

that when the rate of synaptic depression was high, for example in high extracellular calcium and / or at high stimulation rates, or after pairing pre- and postsynaptic activity, which resulted in a presynaptically mediated redistribution of synaptic plasticity, the limiting frequency was reduced and as a consequence, rate coding was attenuated and temporal coding became more prominent (Tsodyks and Markram, 1997). One functional consequence of this might be in conditions of excitotoxicity, the increased rate of synaptic depression might serve as a safety valve "dampening" the activity at already hyperexcitable synapses.

The converse also holds true, reducing the probability of release as occurs in the presence of acetylcholine, reduces the rate of synaptic depression without altering the steady-state EPSP amplitude and this is accompanied by a shift from temporal to rate coding (Tsodyks and Markram, 1997).

Others have extended these novel ideas, Galeratta and Hestrin (1998), proposed that this fast short-term depression operates against a much slower background synaptic depression, similar to the type observed during LFS. Furthermore it appears that activity dependent depression is more pronounced at excitatory than inhibitory synapses (Nelson and Turrigano, 1998; Varela et al., 1999).

The hippocampus is believed to play a role in memory formation and information processing. It probably operates as a relay station for the neocortex filtering incoming information before it is consolidated within specific cortical regions. One might expect that if the cortex is in constant contact with the hippocampus then they might use the same language or share a common code. Furthermore the distinct spatial organisation of the inputs to the dentate gyrus coupled to the differential release probability

associated with the medial and lateral perforant paths, makes the comparison between the coding capability of the two inputs to the granule cells very relevant. In addition the presence of presynaptic mGluRs in the dentate gyrus offers a possible candidate for the role of "functional regulator" of the release probability and by extension the coding capacity of these synapses. In this study we have tried to assess the rate and temporal coding capabilities of the medial perforant path-granule cell synapses and the potential for a modulation of neural coding either by activation of presynaptically located mGluR group II or through LFS-induced long-term depression.

5.3 Materials and Methods

5.3.1 Slice preparation

All experiments were carried out on hippocampal slices obtained from Wistar rats (50-70g) (BioResources Unit, Trinity College, Dublin, Ireland). Slices were obtained as described previously (Kilbride et al., 1998). Briefly, the brain was rapidly removed after decapitation and placed in cold (5°C) oxygenated (95 % O_2, 5 % CO_2) artificial cerebro-spinal fluid (ACSF) containing in mM: NaCl, 120; $NaHCO_3$, 26; NaH_2PO_4, 1.25; KCl, 2.5; Mg_2SO_4, 2; $CaCl_2$, 2; glucose, 10). Hippocampal slices (350μM) were cut using a Campden vibroslice (Campden Group Instruments, London, U.K.) and transferred immediately to an incubation chamber, maintained at room temperature, for a period of at least 60 minutes. Single slices were then transferred to a submersion type recording chamber at 30 - 31° C unless otherwise stated.

5.3.2 Electrophysiology

Field excitatory postsynaptic potentials (EPSPs) were recorded using standard glass electrodes filled with extracellular ACSF containing in mM: NaCl, 120; $NaHCO_3$, 26; NaH_2PO_4, 1.25; KCl, 2.5; Mg_2SO_4, 2; $CaCl_2$, 2; glucose, 10). This saline was oxygenated (95 % O_2, 5 % CO_2) before being used to perfuse the slices. The bipolar stimulating electrodes were made from insulated tungsten wire The recording electrodes were low resistance (1 MΩ) glass microelectrodes which were pulled using a Flaming / Brown micropipette puller P - 87 (Sutter Instrument Corp. CA, USA). The recording electrodes were filled with normal ACSF. These stimulation pulses were generated by a Master - 8, eight channel,

programmable pulse generator (A.M.P.I., Jerusalem, Israel) driven by pCLAMP 6 software (Axon instruments Inc. Foster city, CA, USA) on a IBM compatible PC. The EPSPs were amplified by a Grass P16 microelectrode DC amplifier (Grass Instruments, Quincy, Mass., U.S.A.), converted from analogue to digital form (Axon instruments, Digidata 1200) before being stored on a Dell dimension 466 personal computer for subsequent off-line data processing. Trains of 20 stimuli were delivered to the medial perforant path, after 10 stimuli the frequency at which the stimulation was being delivered was instantaneously increased either by a fixed percentage (50 % or 100 %) or by a fixed increment (5 Hz) (see Abbott et al., 1997).

5.3.3 Data analysis and statistics

Brief trains of pulses were delivered at frequencies of 1 - 200 Hz, with the test EPSP ~1mV. Summarised results are expressed as EPSP mean amplitude ± S.E.M. The amplitude of each EPSP in a train was expressed as a percentage of the 1^{st} control EPSP. The EPSP per unit time was calculated as follows; the steady-state epsp amplitude x frequency, in arbitrarty units or (mV / s). Data was analysed using student's paired t-test, and repeated measures analysis of variance. The statistical tests were carried out at the 5 % level of significance, and a p value when given refers to: 10^{th} EPSP (r_1) vs the 1^{st} EPSP (r_1); 11^{th} EPSP (r_2) vs 10^{th} EPSP (r_1); 20^{th} EPSP (r_1) (vs 11^{th} EPSP (r_2). Where r_1 and r_2 refer to the initial and new stimulation rates, (see results text). The analysis was performed on Microsoft Excel and Jandel Sigmaplot as well as Graphpad Prism software applications.

5.3.4 Compounds

Drugs were added directly to the perfusate after the control baseline period. LY354740 was a generous gift from Eli Lily, USA.

5.4 Results

Our primary interest was to observe the transient changes in the evoked synaptic response which occur as a result of instantaneous increases in the presynaptic stimulation rate. Therefore we employed the following protocol; firstly we delivered short trains of 10 stimuli at a range of frequencies (0.5 Hz $\leq r_1 \leq$ 50 Hz) to the medial perforant path. Then we instantaneously increased the stimulation rate by either a fixed increment (5 Hz) or by a fixed percentage (50 % or 100 %) of the initial firing rate. At this new firing rate (r_2), we delivered 10 more stimuli, to give a total train length of 20 stimuli.

The steady-state depressed EPSP amplitude at the initial firing rate (r_1) after 10 stimuli was compared statistically to the 1^{st} response at the initial, (r_1) and new (11^{th}) firing rate (r_2). Furthermore the steady-state depressed EPSP amplitude at the new rate, after the final (20^{th}) stimuli was compared statistically to the 1^{st} response at r_2 (11^{th} stimuli).

5.4.1 The effect on synaptic transmission of an instantaneous increase in the presynaptic firing rate of 50 %.

Figure 5.1 shows the EPSP amplitudes elicited with trains of 20 stimuli normalised to the first EPSP at each frequency and plotted against the stimulus number in the train. The first 10 stimuli were delivered at a range of frequencies between 0.5 Hz and 50 Hz. Subsequently the firing rate was increased by 50 % and a further 10 stimuli were delivered at the new rate.

Following the short-term depression induced by 10 stimuli at a range of frequencies between 0.5 Hz and 50 Hz, an instantaneous increase in the

frequency of stimulation resulted in further depression of the EPSP amplitude, with the extent of the depression being dependent on the firing rate. The change in frequency tested were 0.5 Hz to 0.75 Hz, 10 Hz to 15 Hz, 20 Hz to 30 Hz and 50 Hz to 75 Hz.

The steady-state EPSP amplitude after 10 stimuli delivered at a frequency of 0.5 Hz was 71.4 ± 2.3 % ($P < 0.05$, $n = 4$) of the 1st EPSP . The switch to 0.75 Hz produced an immediate decrease in the EPSP amplitude to 68.4 ± 1.2 % ($P > 0.05$, $n = 4$) of control. The steady-state depressed EPSP amplitude at 0.75 Hz was 65.8 ± 2.4 % ($P > 0.05$, $n = 4$, figure 5.1B).

After delivering 10 stimuli to the medial perforant path at a frequency of 10 Hz, the EPSP amplitude was reduced to 63.8 ± 3.6 % ($p < 0.05$, $n = 7$) of the control 1st EPSP amplitude. Subsequent stimulation at 15 Hz elicited mean EPSP amplitudes of 64.1 ± 3.7 % ($p > 0.05$, $n = 7$) and 54.5 ± 4.0 % ($p < 0.05$, $n = 7$) at the 11th and 20th stimuli respectively (Figure 5.1B).

Stimulating first at 20 Hz before switching to 30 Hz, elicited mean EPSP amplitudes of 43.1 ± 1.8 % ($P < 0.05$, $n = 7$), 40.5 ± 2.4 % ($p < 0.05$, $n = 7$) and 30.7 ± 1.5 % ($P < 0.05$, $n = 7$) of control, measured after the 10th, 11th and 20th stimuli in the train (Figure 5.1B).

The 10th EPSP in the 50 Hz train elicited a steady-state depressed EPSP amplitude of 28.9 ± 3.3 % ($P < 0.05$, $n = 5$) compared to control. At 75 Hz, the 1st and 10th EPSP amplitudes were 25.4 ± 2.8 ($p > 0.05$, $n = 5$) and 17.2 ± 2.1% ($P < 0.05$, $n = 5$) respectively (Figure 5.1B).

5.4.2 The effect on the EPSP per unit time of an fixed percentage, instantaneous increase in the presynaptic firing rate of 50 %.

Measuring EPSPs before, during and after a 50 % increase in the presynaptic firing rate does not reveal the extent to which the transient postsynaptic response is affected by the preceding change in the presynaptic firing rate. Calculating the postsynaptic response per unit time (mV / s) is one way around this problem. However the specific reason for plotting this measure of the postsynaptic response was to illustrate the transient change in the synaptic response that accompanies an instantaneous increase in firing rate. It is for this reason that from here on in the analysis is restricted to the absolute and percentage change in EPSP per unit time that occurred between the 1^{st} stimuli at the elevated firing rate (r_2) and the steady-state level achieved at the initial firing rate (r_1), averaged over the 8^{th}, 9^{th} and 10^{th} stimuli.

When the firing rate was increased from 0.5 Hz to 0.75 Hz the absolute increase in the EPSP per unit time was 15.3 $\pm$ 1.3 mV / s, and this corresponded to a percentage increase of 42.9 $\pm$ 4.5 %, ($P < 0.05$, $n = 4$, figure 5.1C). The absolute increases in the EPSP per unit time associated with instantaneous increases in the firing rate from 10 Hz to 15 Hz, 20 Hz to 30 Hz and 50 Hz to 75 Hz were 314.9 $\pm$ 25.4 mV / s, 369.6 $\pm$ 47.7 mV / s and 440 $\pm$ 137.5 mV / s respectively. This corresponded to percentage increases of 48.5 $\pm$ 4.3 % ($P < 0.05$, $n = 7$), 36.0 $\pm$ 4.3 % ($P < 0.05$, $n = 7$) and 31.3 $\pm$ 10.7 % ($P < 0.05$, $n = 5$, figures 5.1C, 5.12)

5.4.3 The effect on synaptic transmission of an instantaneous increase in the presynaptic firing rate of 100 %.

The graph in figure 5.2 shows the EPSP amplitudes elicited with trains of 20 stimuli normalised to the first EPSP at each frequency and plotted against the stimulus number in the train. The first 10 stimuli were delivered at a range of frequencies between 0.5 Hz and 50 Hz. Subsequently the firing rate was increased by 100 % and a further 10 stimuli were delivered at the new rate.

The steady-state EPSP amplitude after 10 stimuli at 0.5 Hz was 75.5 ± 3.9 % ($P < 0.05$, $n = 7$) of the 1st EPSP. Doubling the firing rate to 1 Hz resulted in further depression of the EPSP amplitude. Measured as 70.3 ± 5.9 % ($P > 0.05$, $n = 7$) and 63.2 ± 5.6 % ($P < 0.05$, $n = 7$) of the first EPSP at the 11th and 20th stimuli respectively (Figure 5.2B, 5.3B).

When the initial 10 stimuli were delivered at 10 Hz, the steady-state EPSP amplitude was 47.6 ± 2.6 % ($P < 0.05$, $n = 7$). The amplitude of the first EPSP delivered at 20 Hz was slightly, if not significantly potentiated (48.5 ± 2.4 %, $P > 0.05$, $n = 7$), however the subsequent stimuli at 20 Hz declined before reaching a steady-state amplitude of 34.7 ± 2.6 % ($P < 0.05$, $n = 7$, figures 5.2B, 5.4B).

Changing the firing rate from 50 Hz to 100 Hz was accompanied by EPSP amplitudes of 28.9 ± 1.8 % ($P < 0.05$, $n = 14$), 20.9 ± 2.2 % ($P < 0.05$, $n = 14$) and 13.1 ± 1.2 % ($P < 0.05$, $n = 14$, figures 5.2B, 5.5B) of control, measured after the 10th, 11th and 20th stimuli respectively.

5.4.4 The effect on the EPSP per unit time of a fixed percentage, instantaneous increase in the presynaptic firing rate of 100 %.

As before we have plotted the EPSP per unit time as a function of every stimulus elicited over all frequencies tested before and after a 100 % increase in presynaptic firing rate.

The absolute increase in the EPSP per unit time associated with an instantaneous increase in the presynaptic firing rate from 0.5 Hz to 1 Hz was 32.8 ± 4.3 mV / s and this was equivalent to a percentage increase of 96.8 ± 2.3 % ($P < 0.05$, $n = 9$, figures 5.2C, 5.13).

The increase in firing rate from 10 Hz to 20 Hz and from 50 Hz to 100 Hz was associated with absolute increases in the EPSP per unit time of 490.4 ± 45.6 mV / s and 563.5 ± 162 mV / s. This corresponded to percentage increases of 104.3 ± 11.6 % ($P < 0.05$, $n = 8$, Figure 2,13) and 35.3 ± 9.1 % ($P < 0.05$, $n = 11$, figures 5.2C, 5.13). The absolute increase in the time averaged postsynaptic response to a 100 % increase in the afferent firing rate did not differ for firing rates above 10 Hz.

5.4.5 The effect on synaptic transmission of an instantaneous increase in the presynaptic firing rate of 5 Hz.

Synapses that do not display short-term depression would be expected to respond to fixed amplitude changes in their presynaptic firing rates with about equal sensitivity over a broad range of frequencies (Abbott et al., 1997). Therefore a 5 Hz increase in firing rate would be expected to produce the same transient increase in the postsynaptic response to inputs arriving at either 1 Hz or 100 Hz. However synapses that display short-term depression do not behave like this. Instead synaptic depression exerts a type of gain control, reducing the response to small fluctuations in

rapidly firing afferents and increasing the sensitivity to the same fluctuations on slowly firing afferents.

The graph in figure 5.7B shows the EPSP amplitudes elicited with trains of 20 stimuli normalised to the first EPSP at each frequency and plotted against the stimulus number in the train. The first 10 stimuli were delivered at a range of frequencies between 0.0166 Hz and 50 Hz. Subsequently the firing rate was increased by 5 Hz and a further 10 stimuli were delivered at the new rate.

Stimulating at 0.0166 Hz did not produce any evidence of short-term depression (99.5 ± 2.5 %, $P > 0.05$, n = 6,). However increasing the firing rate by 5 Hz, induced a dramatic decrease in the EPSP amplitude, both transiently 83.3 ± 2.3 % ($P < 0.05$, n = 6, at stimuli 11) and in the steady-state (68.8 ± 6.1 %, $P < 0.05$, n = 6, figure 5.7B, 5.8B)

The steady-state EPSP amplitude at 10 Hz was 63.8 ± 3.6 % ($P < 0.05$, n = 7). Even though the first EPSP at 15 Hz was slightly facilitated, 64.1 ± 3.7 % ($P > 0.05$, n = 7), the steady-state EPSP amplitude decreased to 54.5 ± 4.0 % ($P < 0.05$, n = 7, figure 5.7B, 5.9B) of control.

At a firing rate of 50 Hz the steady-state EPSP amplitude was 35.9 ± 3.8% ($P < 0.05$, n = 7). Initially the switch to 55 Hz did nothing to the EPSP amplitude (35.7 ± 3.6%, $P > 0.05$, n = 7). However by the final stimuli the EPSP amplitude had plateaued at a more depressed level than before (29.2 ± 3.5% $P < 0.05$, n = 7, figure 5.7B, 5.10B).

5.4.6 The effect on the EPSP per unit time of an fixed increment, instantaneous increase in the presynaptic firing rate of 5Hz.

Unlike the fixed amplitude changes, increasing the afferent firing rate by a fixed amplitude (5Hz) had a greater effect on the EPSP per unit time at low frequencies. Increasing the firing rate from 0.0166 Hz to 5Hz induced an increase of 414.8 ± 11.5 mV / s this represented a quite incredible percentage change of 25185.9 ± 770 % ($P < 0.05$, $n = 6$, Figure 5.7, 5.14).

The 5Hz increment from 10 Hz to 15 Hz brought with it an absolute increase of EPSP per unit time of 314.9 ± 25.4 mV / s. This was equivalent to a more modest percentage change of 48.5 ± 2.6 % ($P < 0.05$, $n = 7$, Figure 5.7, 5.14).

Changing the presynaptic firing rate from 50 Hz to 55 Hz was associated with absolute and percentage increases in the EPSP per unit time of 115.6 ± 75.4 mV / s and 4.8 ± 4.3 % ($P > 0.05$, $n = 7$, Figure 5.7, 5.14).

5.4.7 The effect of the group II mGluR agonist LY354740 on the transient postsynaptic response induced by fixed percentage rate change (100 %).

As was demonstrated in the previous chapter, short-term depression is unaffected by the group II mGluR agonist LY354740 (5 μM) at low frequencies of stimulation, up to about 5 Hz. At higher frequencies both the rate of onset and the steady-state level of depression are altered by the action of this agonist at presynaptic group II mGluR. With this in mind what effect, if any, would LY354740 would have on the rate and temporal coding properties of the medial perforant path-granule cell synapses. Specifically if at higher frequencies synaptic depression is reduced by the presence of LY354740, than one might expect to find not only an increase

in the range of frequencies over which firing rates can be coded, but also an reduced sensitivity to changes in the temporal pattern of input rates. Perfusing the slice with LY354740 (5 μM) for 15-20 minutes reduced the test EPSP amplitude in a manner which was very similar to previous experiments. The initial EPSP amplitude in LY354740 (5 μM) at a frequency of 0.5 Hz was 24.1 ± 3.5 % ($P < 0.05$, $n = 5$) of the control test EPSP amplitude. After 10 stimuli at 0.5 Hz, the steady - state EPSP amplitude was reduced to 21.9 ± 2.9 % ($P > 0.05$, $n = 5$). Doubling the firing rate to 1 Hz reduced the EPSP amplitude, the 11^{th} and 20^{th} EPSP amplitudes measured 18.5 ± 2.6 % ($P < 0.05$, $n = 5$) and 19.9 ± 2.2 % ($P > 0.05$, $n = 5$, figure 5.3B, 5.6B) of the control 1^{st} stimuli. This effect was similar to control and suggests that at low frequencies, the activation of presynaptic group II mGluR does not greatly affect rate or temporal coding.

In LY354740 (5 μM), at a stimulation frequency of 10 Hz, the 1^{st} and 10^{th} EPSP amplitudes measures 29.5 ± 2.9 ($P < 0.05$, $n = 5$) and 23.8 ± 0.6 % ($P > 0.05$, $n = 5$) of control respectively. After the switch to 20 Hz, the 11^{th} and 20^{th} EPSP amplitudes were 29.3 ± 1.8 % ($P > 0.05$, $n = 5$) and 23.7 ± 1.2 % ($P < 0.05$, $n = 5$, figure 5.4B, 5.6B).

At 50 Hz the 1^{st} and 10^{th} EPSPs were 34.3 ± 3.4 % ($P < 0.05$, $n = 4$) and 22.0 ± 2.6 % ($P > 0.05$, $n = 4$) of control. The amplitude of the first EPSP at 100 Hz was 22.8 ± 2.8 % ($P < 0.05$, $n = 4$) and the steady-state EPSP amplitude was 17.6 ± 2.9% ($P > 0.05$, $n = 4$, Figure 5.5B,5.6B).

5.4.8 The effect of the group II mGluR agonist LY354740 on the changes in the EPSP per unit time induced by fixed percentage rate change (100 %).

In LY354740 (5 μM) the absolute increase in the EPSP per unit time associated with a transient change in firing rate from 0.5 Hz to 1 Hz was 7.6 ± 1.4 mV / s . This corresponded to a percentage change of 130 ± 9.2 % ($P < 0.05$, n = 5, Figures 5.6C, 5.13B). A change in the firing rate from 10 Hz to 20 Hz was associated with absolute increases of 348.9 ± 34.4 mV / s. This was equivalent to a percentage change of 146.8 ± 14.9 % ($P < 0.05$, n = 5, Figure 5.6C, 5.13B).

When the stimulation rate was increased from 50 Hz to 100 Hz. The absolute increase was 545.6 ± 134.9 mV / s in LY354740 (5 μM). This related to a percentage increase of 53.0 ± 10.7 % ($P < 0.05$, n = 4, Figure 5.6C, 5.13B).

5.4.9 The effect of the group II mGluR agonist LY354740 on the transient postsynaptic response induced by fixed increment rate change of 5 Hz.

Perfusion of LY354740 (5 μM) for 15-20 minutes reduced the test (0.0166 Hz) EPSP amplitude significantly (36.8 ± 3.7 %, $P < 0.05$, n = 4). Increasing the firing rate by 5 Hz depressed the EPSP amplitude to 35. 6 ± 3.4 % ($P > 0.05$, n = 6) and 32.2 ± 3.5 % ($P > 0.05$, n = 6) at the 11^{th} and 20^{th} stimuli respectively (Figure 5.8B, 5.11B).

In LY354740 (5 μM) the 1^{st} and 10^{th} EPSP amplitudes recorded at 10 Hz were 35.4 ± 1.9 % ($P < 0.05$, n = 7) and 32.6 ± 1.8 % ($P > 0.05$, n = 7,). Increasing the frequency of stimulation, from 10 Hz to 15 Hz was accompanied by little change in the EPSP amplitude. At the 11^{th} and 20^{th}

stimuli the mean EPSP amplitudes were 33.0 ± 1.9 % ($P > 0.05$, $n = 7$) and 28.0 ± 1.0 % ($P < 0.05$, $n = 7$, Figure 5.9B, 5.11B). This reflects the reduction in short-term depression by group II mGluR at frequencies over and above ~10 Hz.

Stimulating at a rate of 50 Hz in the presence of LY354740 (5 μM) induced mean EPSP amplitudes at the 1st and 10th stimuli of 34.3 ± 3.0 % ($P < 0.05$ $n = 7$) and 26.2 ± 1.8 % ($P < 0.05$, $n = 7$). The LY354740 induced attenuation of short-term depression is more prominent at higher frequencies. After changing over to stimulate at 55 Hz, the EPSP amplitude did not decline to any significantly level. At the 11th and 20th stimuli, the EPSP amplitudes were 26.0 ± 2.3 % ($P < 0.05$, $n = 7$) and 22.8 ± 1.8 % ($P < 0.05$, $n = 7$, Figure 5.10B, 5.11B).

5.4.10 The effect of the group II mGluR agonist LY354740 on the changes in the EPSP per unit time induced by fixed increment rate change of 5 Hz.

Unlike the fixed percentage rate changes, increasing the afferent firing rate by a fixed amplitude (5 Hz) had a greater effect on the EPSP per unit time at low frequencies. In addition LY354740 (5 μM) reduced the absolute but not the relative increases in the EPSP per unit time over all frequencies tested. Again its effect was frequency dependent, with the greatest changes occurring at lower frequencies (< 25 Hz)

Increasing the firing rate from 0.0166 Hz to 5 Hz induced an increase of 246.9 ± 58.8 mV / s in LY354740 (5 μM), like the control condition this related to a very large percentage change of 28692.8 ± 1028.0 % ($P < 0.05$, $n = 6$, figure 5.11C, 5.14).

The 5 Hz increment from 10 Hz to 15 Hz brought with it an absolute increase of EPSP per unit time of 175.1 ± 22.6 mV / s in LY354740 (5 μM). This was equivalent to a more modest percentage increase of 54.8 ± 6.5 % ($P < 0.05$, $n = 7$, figure 5.11C, 5. 14).

At higher frequencies, 50 Hz to 55 Hz the increase in the absolute and relative EPSP per unit time values were very not significantly different from control 82.7 ± 48.8 mV/s and 5.6 ± 3.9 % ($P < 0.05$, $n = 7$, figure 5.11C ,5.14).

5.4.11 LTD induction does not affect short-term depression in the medial perforant path.

Having observed a stable baseline for 30 minutes, LTD was induced by low frequency stimuation (900 pulses of 0.2ms duration at a frequency of 1 Hz). The increased stimulas duration aided the induction of a reliable LTD (unpublished observation). After 25 minutes, the mean amplitude LTD was 75.9 ± 9 % ($P < 0.05$, $n = 5$,) of the pre-LFS level. Paired-pulse depression (40 ms interstimulas interval) was unaffected by the induction of LTD, 15.2 ± 23 % and 13.4 ± 4.1% $P > 0.05$, $n = 5$, figure 5.15)

It is possible that paired-pulse depression and activity-dependent depression are mediated by different processes (Bellingham and Walmsley, 1999). With this in mind the response to short trains of stimuli before and after LTD induction were compared. Figure 5.16 shows how the rate of onset and extent of the short-term depression evoked with 10 stimuli delivered at a frequency of 1 Hz, in the medial perforant path, was unaffected by the induction of LTD. The 5^{th} and 10^{th} normalised EPSP

amplitudes were 73.5 ± 0.9 % and 68.9 ± 1.9 % in control and 72.8 ± 2 % and 66.9 ± 5.1 % after LTD induction. ($P > 0.05$, $n = 5$, figure 5.16).

Of course it is possible that any modulation of synaptic depression by LTD induction would only be revealed at higher frequencies of stimulation. Figure 5.17 shows the EPSPs elicited by short trains of 10 stimuli at 50 Hz before and after LTD induction. The normalised data illustrates clearly how the rate of onset and the steady-state degree of synaptic depression at 50 Hz is unaffected by LTD induction. The mean EPSP amplitude recorded at the 5^{th} and 10^{th} stimuli was 38.2 ± 2.1 % and 33.4 ± 2.4 % in control conditions and 30.11 ± 2.1 % and 29.5 ± 2.3 % (Figure 5.17) after LTD induction, values not significanty different.

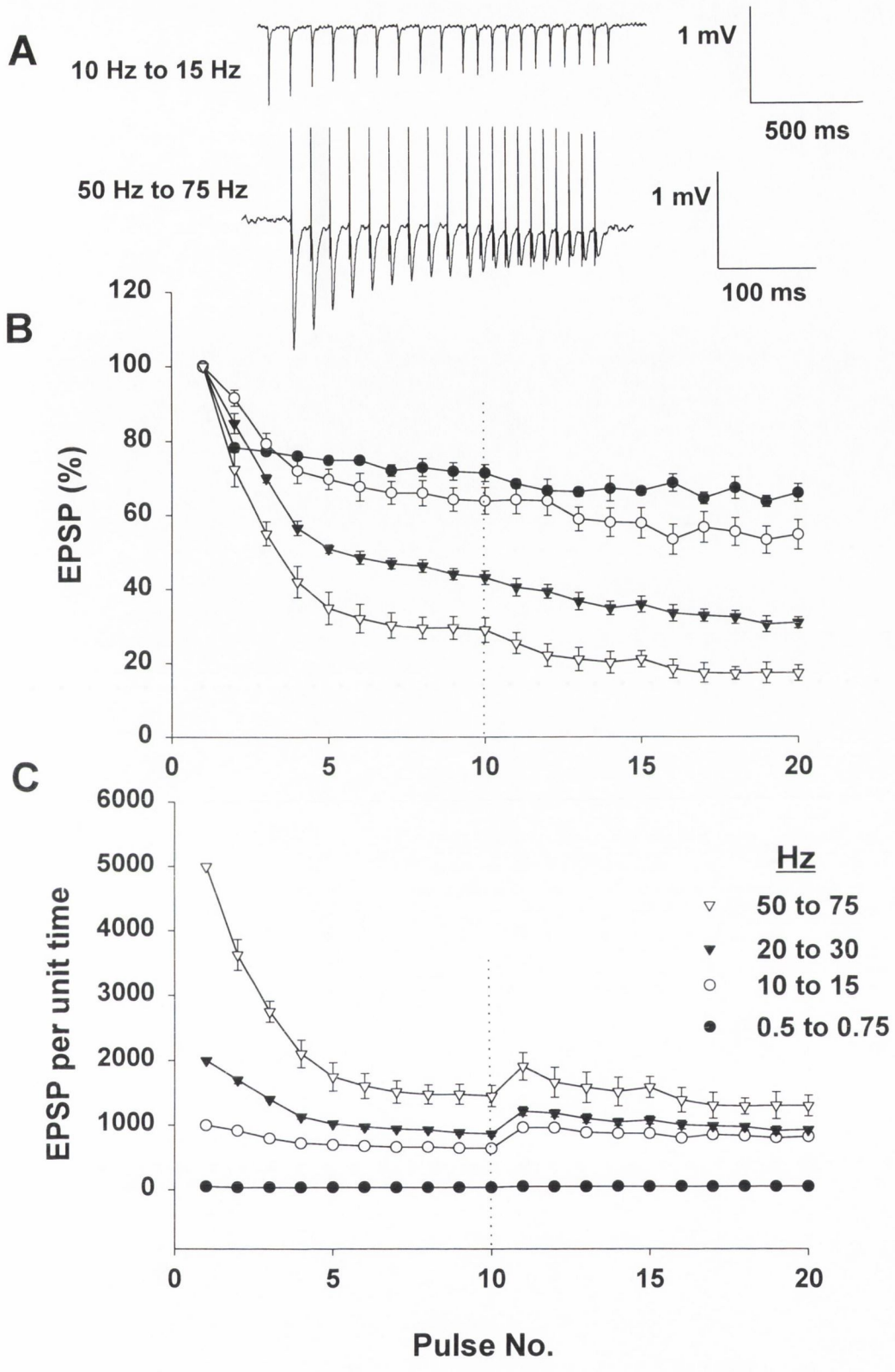


Figure 5.1. The effect on synaptic transmission of an instantaneous increase in the presynaptic firing rate of 50 %. (A) Typical example of trains of EPSPs recorded before and after a 50 % increase in the firing rate. Upper, from 10 Hz to 15 Hz, lower, from 50 Hz to 75 Hz. (B) EPSP amplitudes normalised to the first EPSP in each train and plotted against stimulus number in the train. (C) Normalised EPSP per unit time (EPSP amplitude (%) x frequency) for the data shown in B. Dotted line represents the change in firing rate. Symbols indicate frequencies. Mean and SEM for 4 -7 slices.

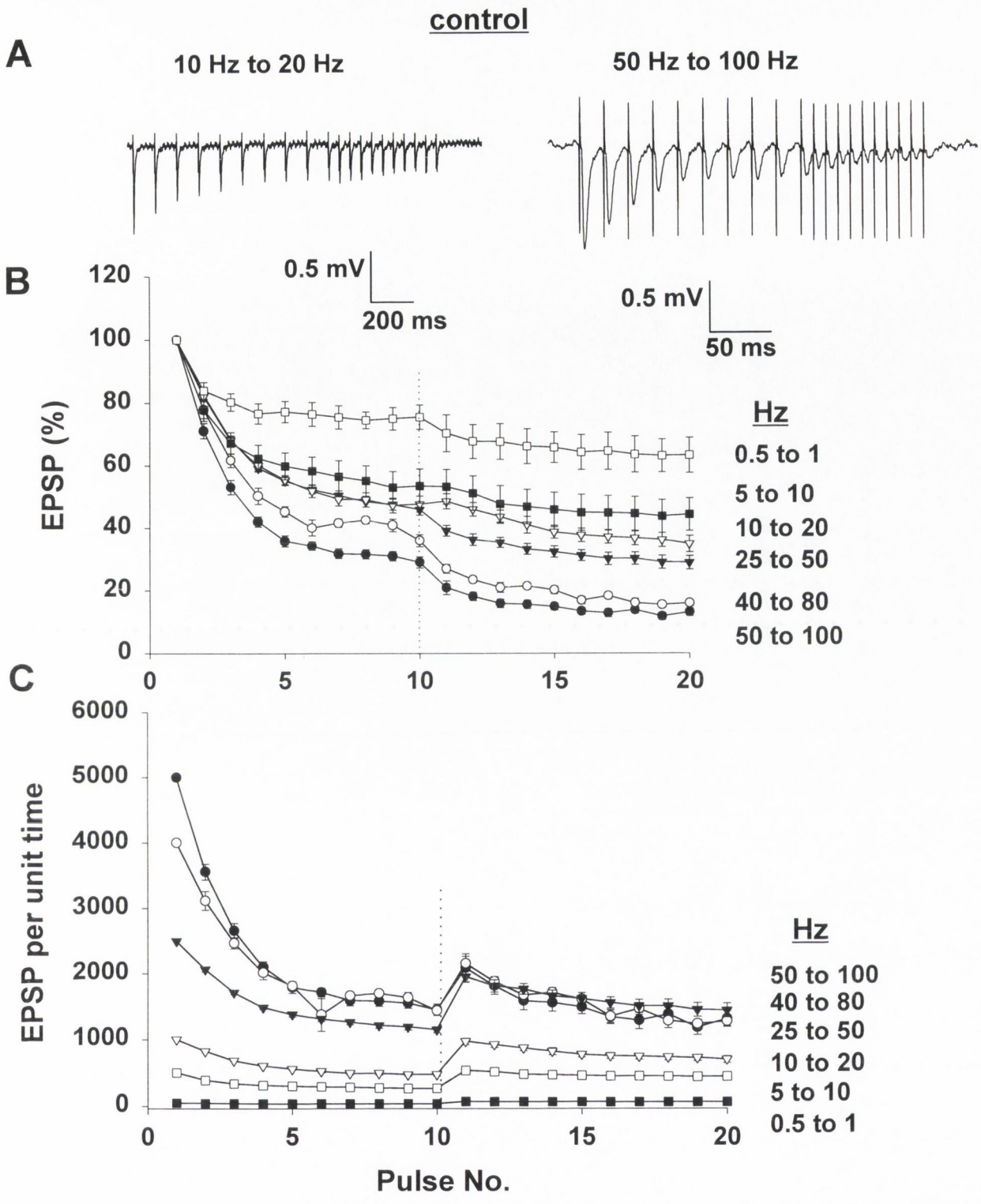


Figure 5.2 The effect on synaptic transmission of an instantaneous increase in the presynaptic firing rate of 100 %. (A) Typical example of trains of EPSPs recorded before and after a 100 % increase in the firing rate. Left, from 10 Hz to 20 Hz and right, from 50 Hz to 100 Hz. (B) EPSP amplitudes normalised to the first EPSP in each train and plotted against stimulus number in the train. (C) Normalised EPSP per unit time EPSP amplitude (%) x frequency, expressed in arbitrary units) for the data shown in B. Dotted line represents the change in firing rate. Symbols indicate frequencies. Mean and SEM for 7-14 slices.

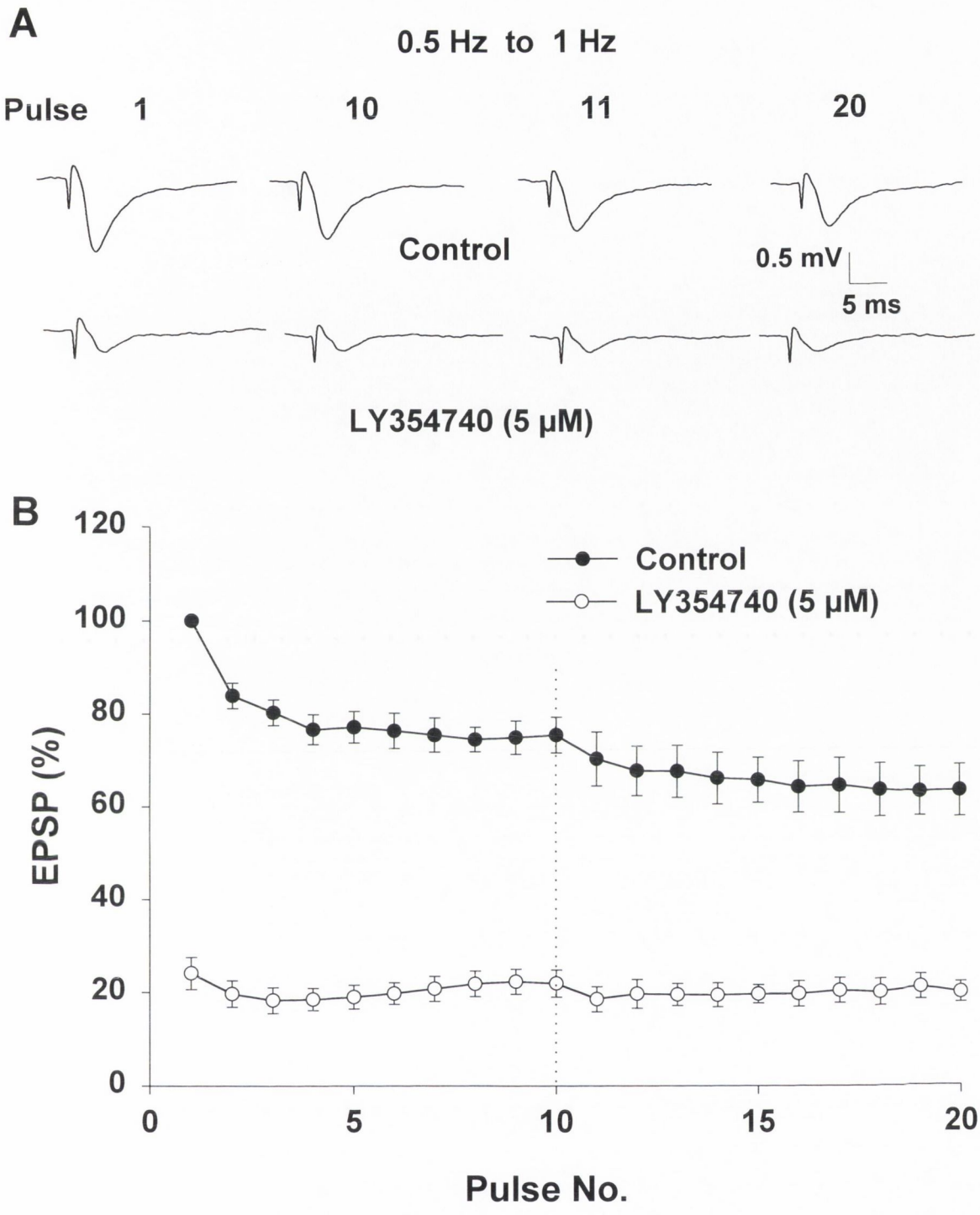


Figure 5.3 LY354740 modulation of synaptic depression is frequency dependent. (A) Typical example of EPSPs recorded during a train delivered at 0.5 Hz (1st and 10th pulses) and after the firing rate was increased to 1 Hz (11th and 20th) in control (upper) and in the presence of LY354740 (5 µM), lower. (B) Summary data (n=7) showing EPSP amplitudes normalised to the first EPSP in control conditions, before and after the change in rate from 0.5 Hz to 1 Hz. Control (filled circles) and LY354740 (5 µM) (open circles).

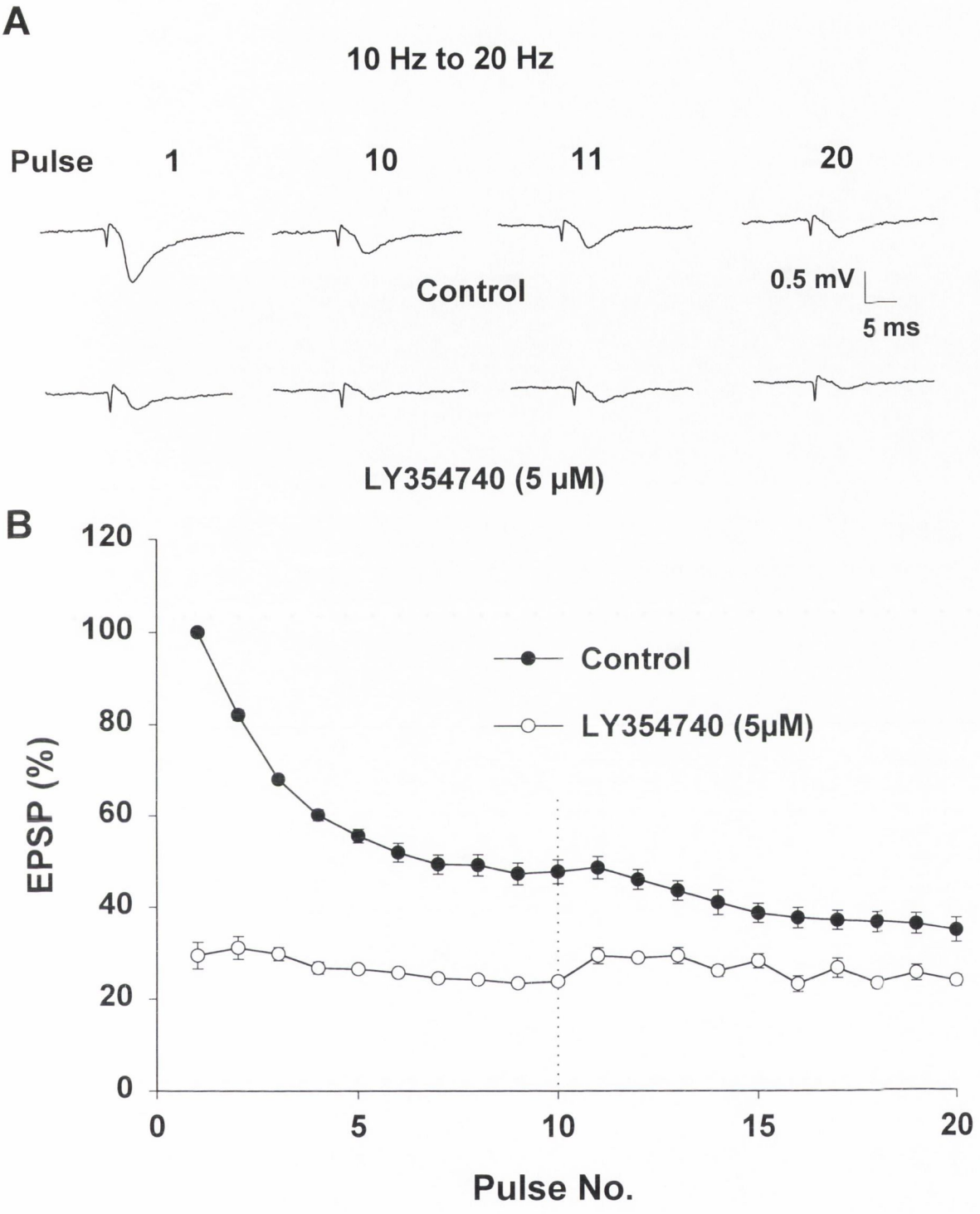


Figure 5.4 Modulation of synaptic depression by LY354740 is frequency dependent. (A) Typical example of EPSPs recorded during a train delivered at 10 Hz (1st and 10th pulses) and after the firing rate was increased to 20Hz (11th and 20th) in control (upper) and in the presence of LY354740 (5 µM), lower. (B) Summary data (n=7) showing EPSP amplitudes normalised to the first EPSP in control conditions, before and after the change in rate from 10 Hz to 20 Hz. Control (filled circles) and LY354740 (5 µM) (open circles).

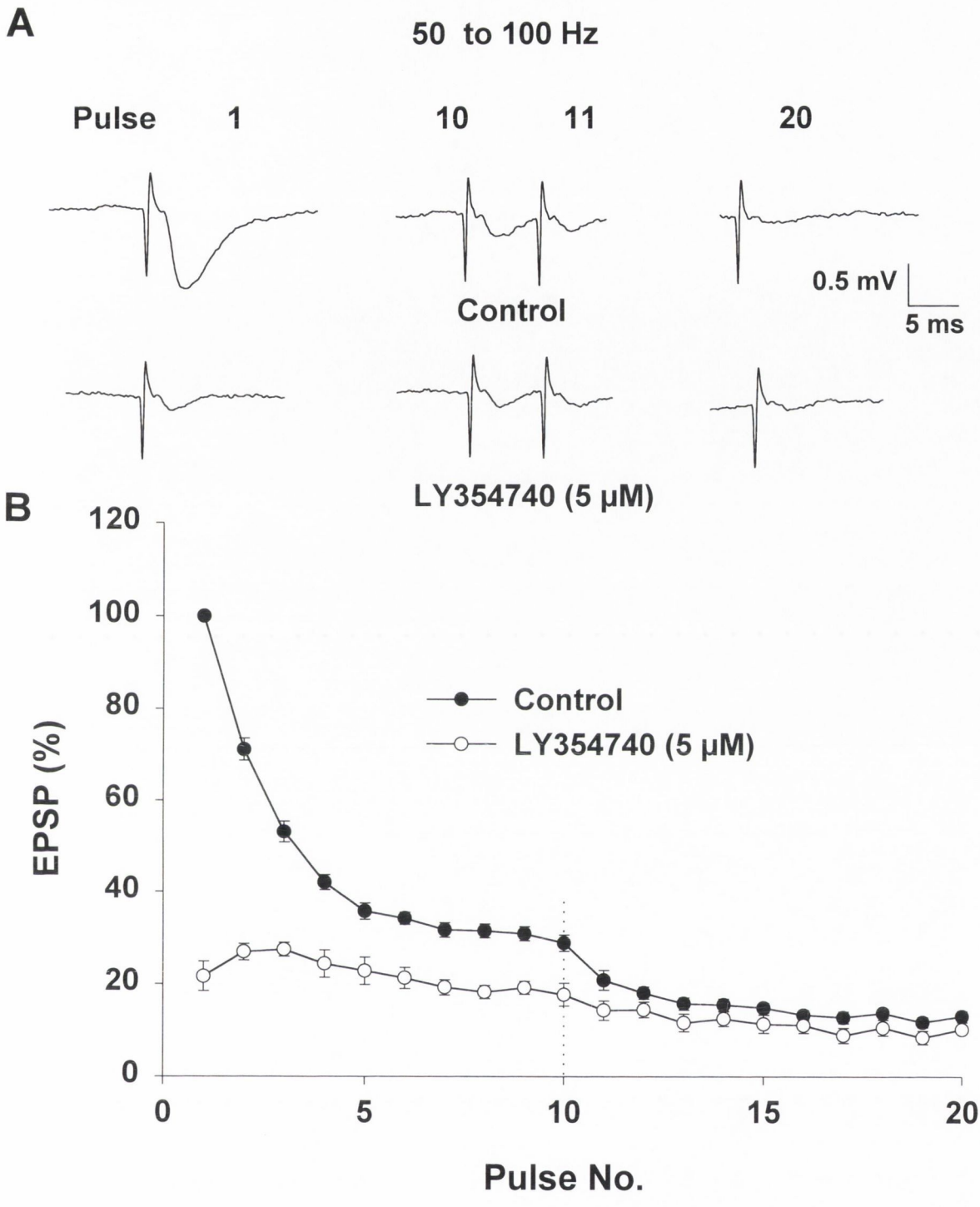


Figure 5.5 Modulation of synaptic depression by LY354740 is frequency dependent. (A) Typical example of EPSPs recorded during a train delivered at 50 Hz (1st and 10th pulses) and after the firing rate was increased to 100 Hz (11th and 20th) in control (upper) and in the presence of LY354740 (5 µM), lower. (B) Summary data (n=14) showing EPSP amplitudes normalised to the first EPSP in control conditions, before and after the change in rate from 50 Hz to 100 Hz. Control (filled circles) and LY354740 (5 µM) (open circles). The steady-state EPSP amplitudes in control and LY354740 are the same.

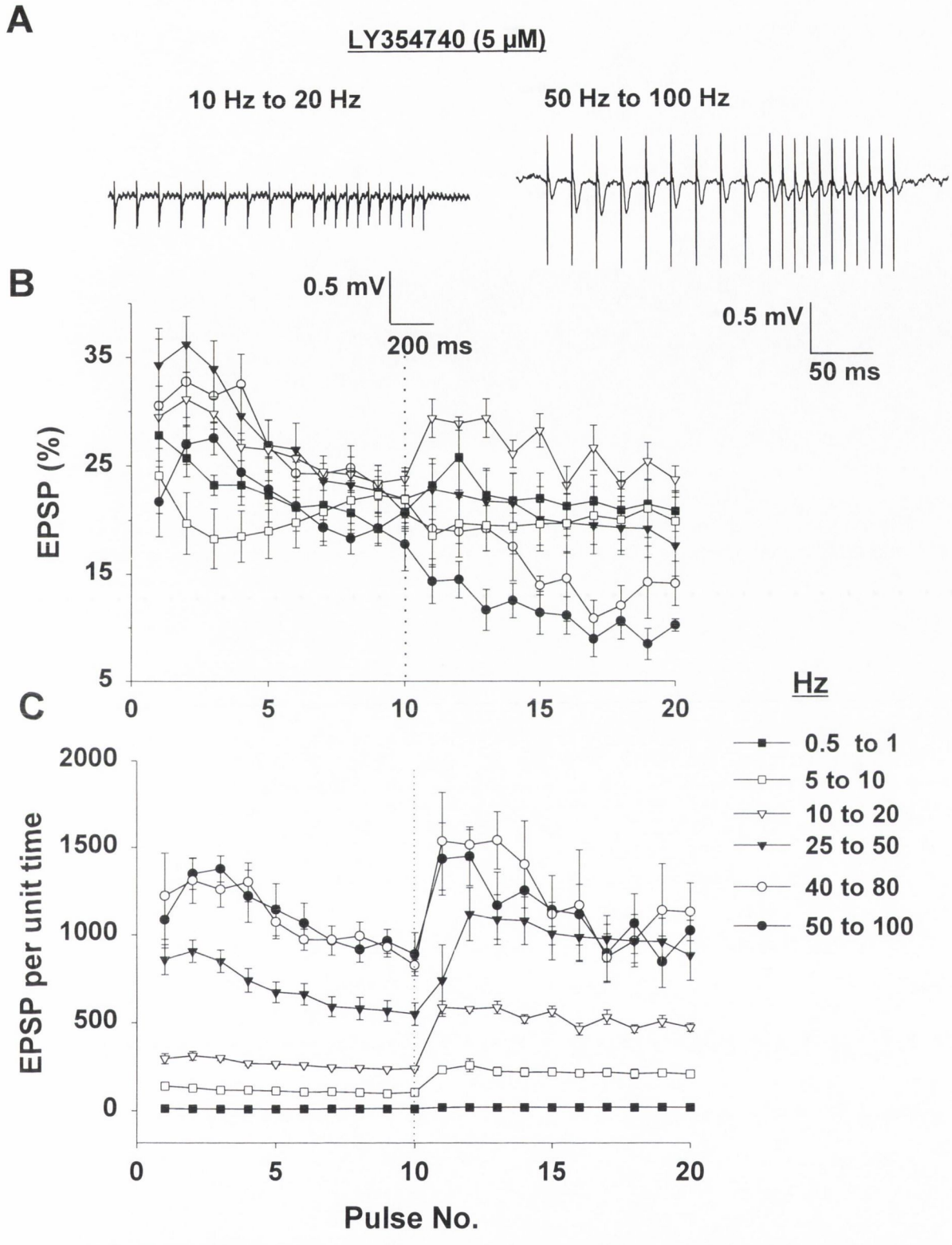


Figure 5.6 The effect on synaptic transmission of an instantaneous increase in the presynaptic firing rate of 100 % is modulated by LY354740. (A) Typical example of trains of EPSPs recorded before and after a 100 % increase in the firing rate in the presence of LY354740 (5 µM). Left, from 10 Hz to 20 Hz and right, from 50 Hz to 100 Hz. (B) EPSP amplitudes normalised to the first EPSP in each train and plotted against stimulus number in the train. (C) Normalised EPSP per unit time (EPSP amplitude (%) x frequency, expressed as arbitrary units) for the data shown in B. Dotted line represents the change in firing rate. Symbols indicate frequencies. Mean and SEM for 7-14 slices.

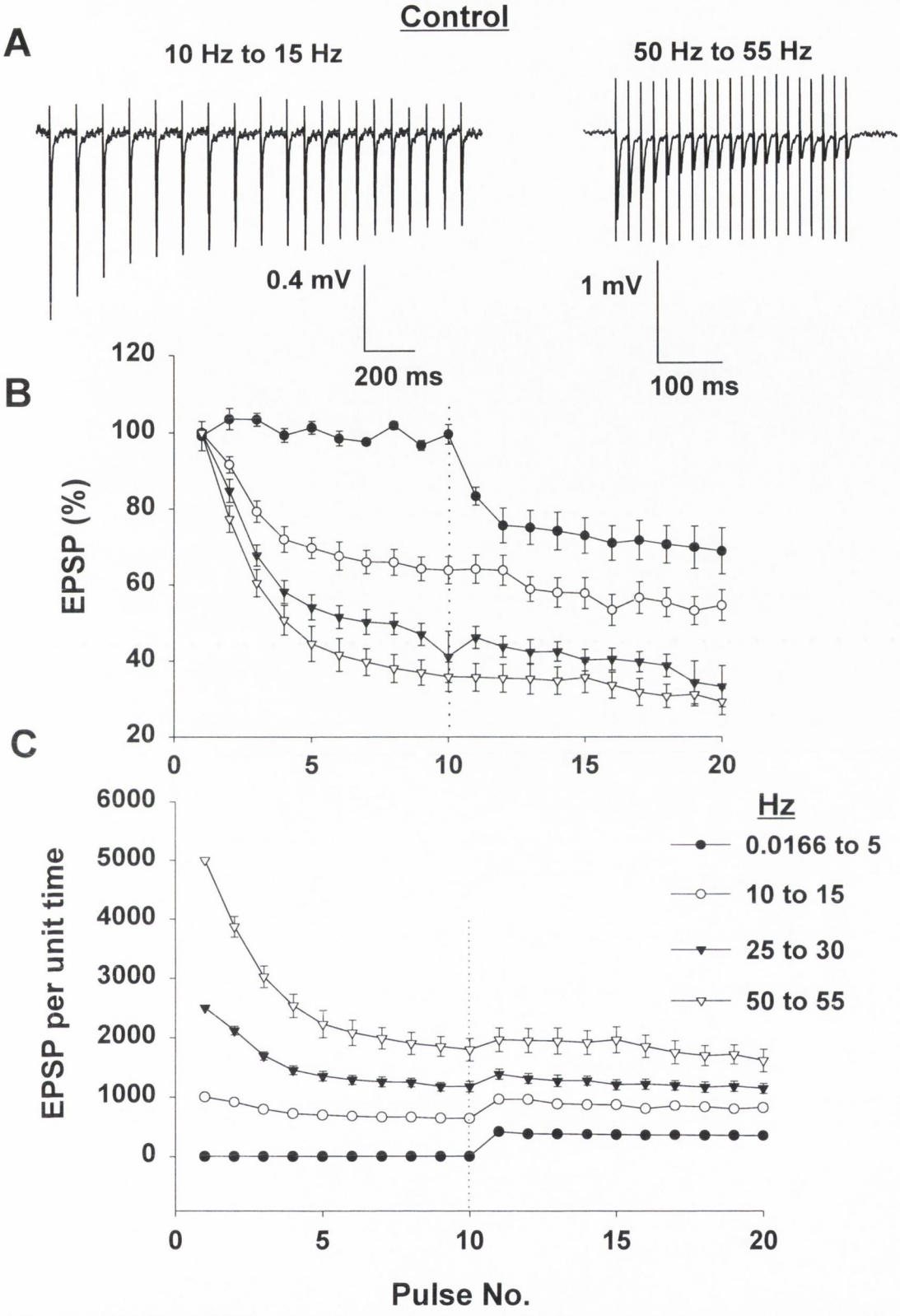


Figure 5.7 The effect on synaptic transmission of an instantaneous increase in the presynaptic firing rate of 5 Hz. (A) Typical example of trains of EPSPs recorded before and after a 5 Hz increase in the firing rate. Left, from 10 Hz to 15 Hz and right, from 50 Hz to 55 Hz. (B) EPSP amplitudes normalised to the first EPSP in each train and plotted against stimulus number in the train. (C) Normalised EPSP per unit time (EPSP amplitude (%) x frequency, expressed in arbitrary units) for the data shown in B. Dotted line represents the change in firing rate. Symbols indicate frequencies. Mean and SEM for 6-7 slices.

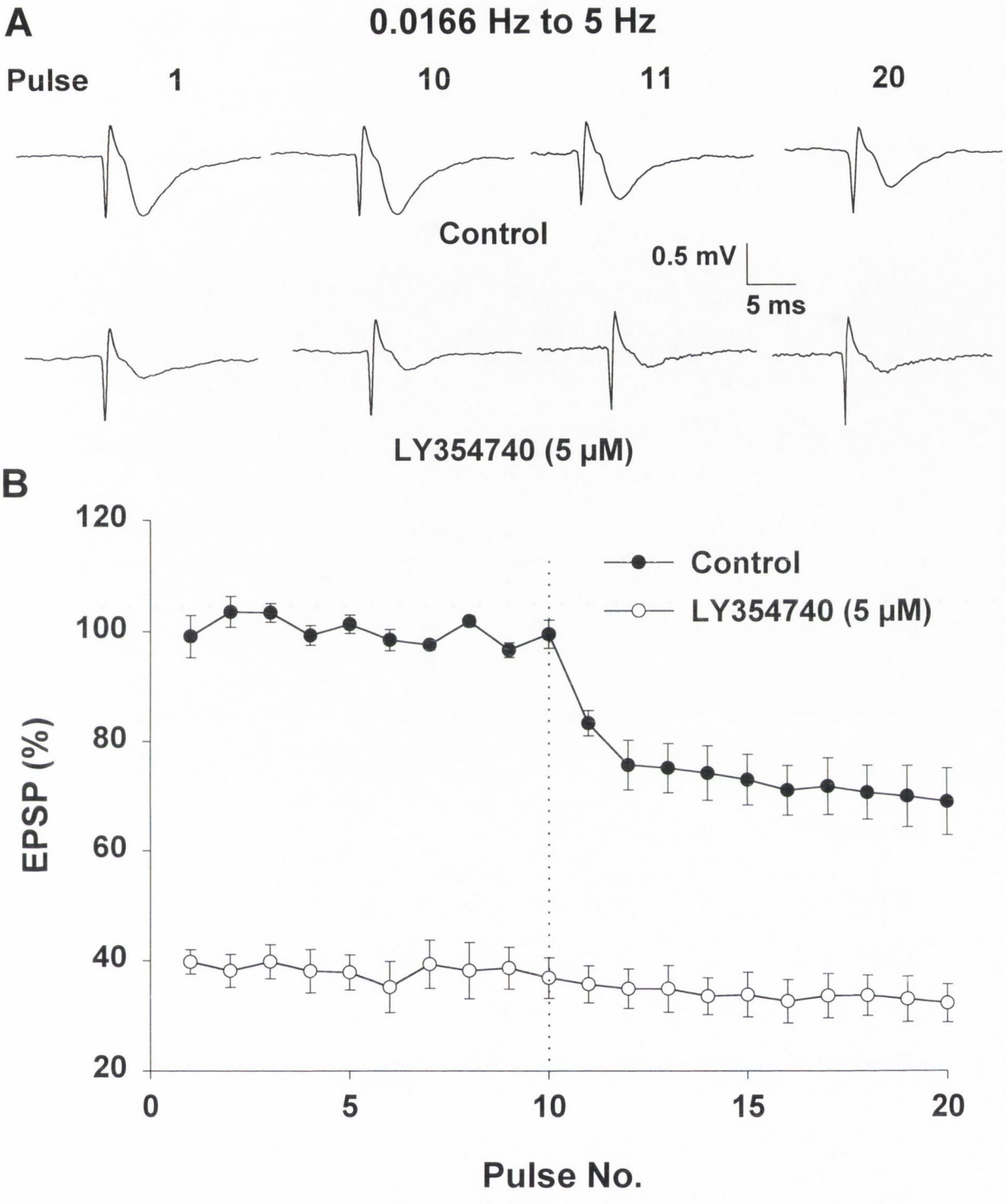


Figure 5.8 Modulation of synaptic depression by LY354740 is frequency dependent. (A) Typical example of EPSPs recorded during a train delivered at 0.0166 Hz (1st and 10th pulses) and after the firing rate was increased to 5 Hz (11th and 20th) in control (upper) and in the presence of LY354740 (5 µM), lower. (B) Summary data (n = 6-7) showing EPSP amplitudes normalised to the first EPSP in control conditions, before and after the change in rate from 0.5 Hz to 1 Hz. Control (filled circles) and LY354740 (5 µM) (open circles).

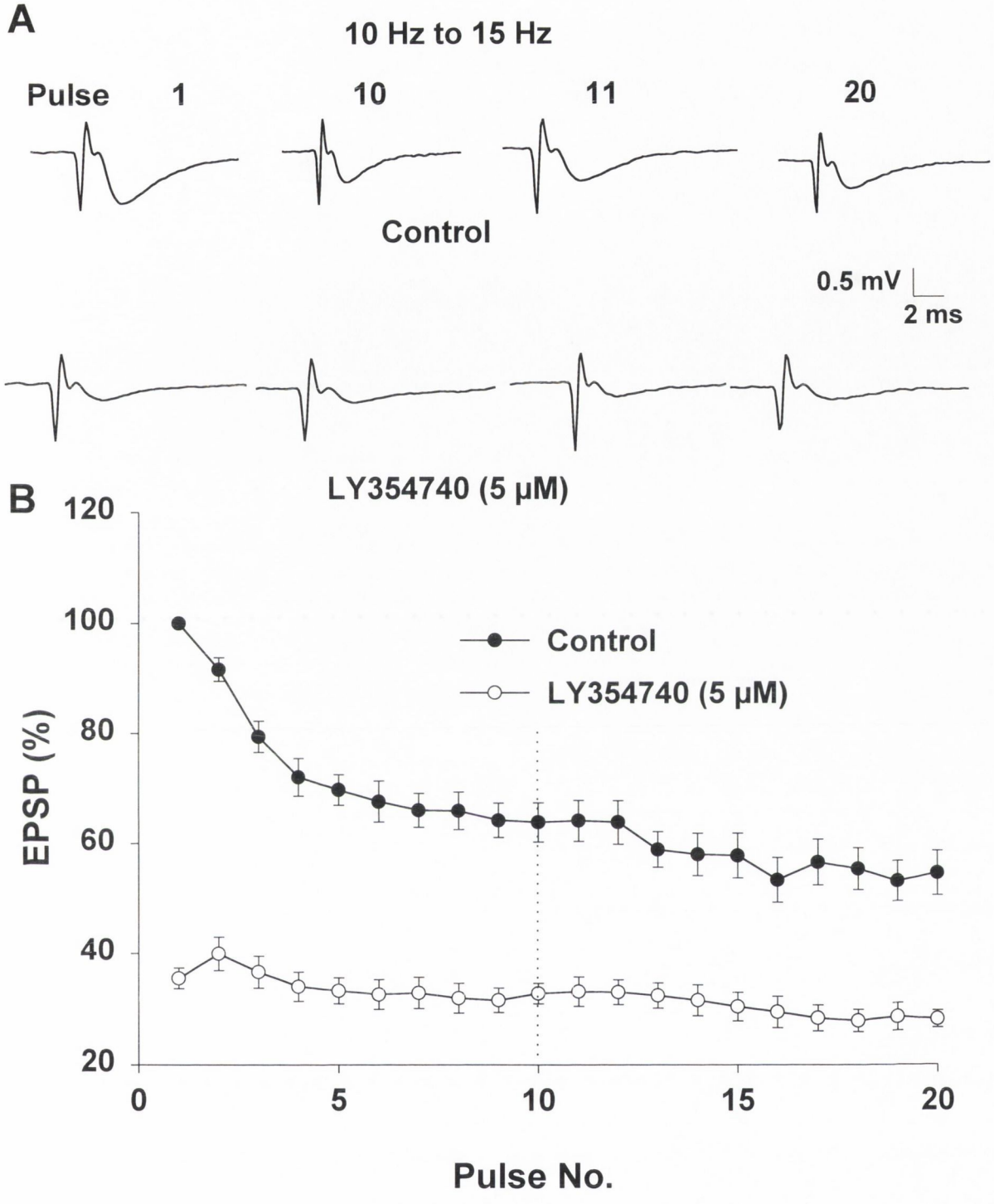


Figure 5.9 Modulation of synaptic depression by LY354740 is frequency dependent. (A)Typical example of EPSPs recorded during a train delivered at 10 Hz (1st and 10th pulses) and after the firing rate was increased to 15 Hz (11th and 20th) in control (upper) and in the presence of LY354740 (5 µM), lower. (B) Summary data (n = 7) showing EPSP amplitudes normalised to the first EPSP in control conditions, before and after the change in rate from 10 Hz to 15 Hz. Control (filled circles) and LY354740 (5µM) (open circles).

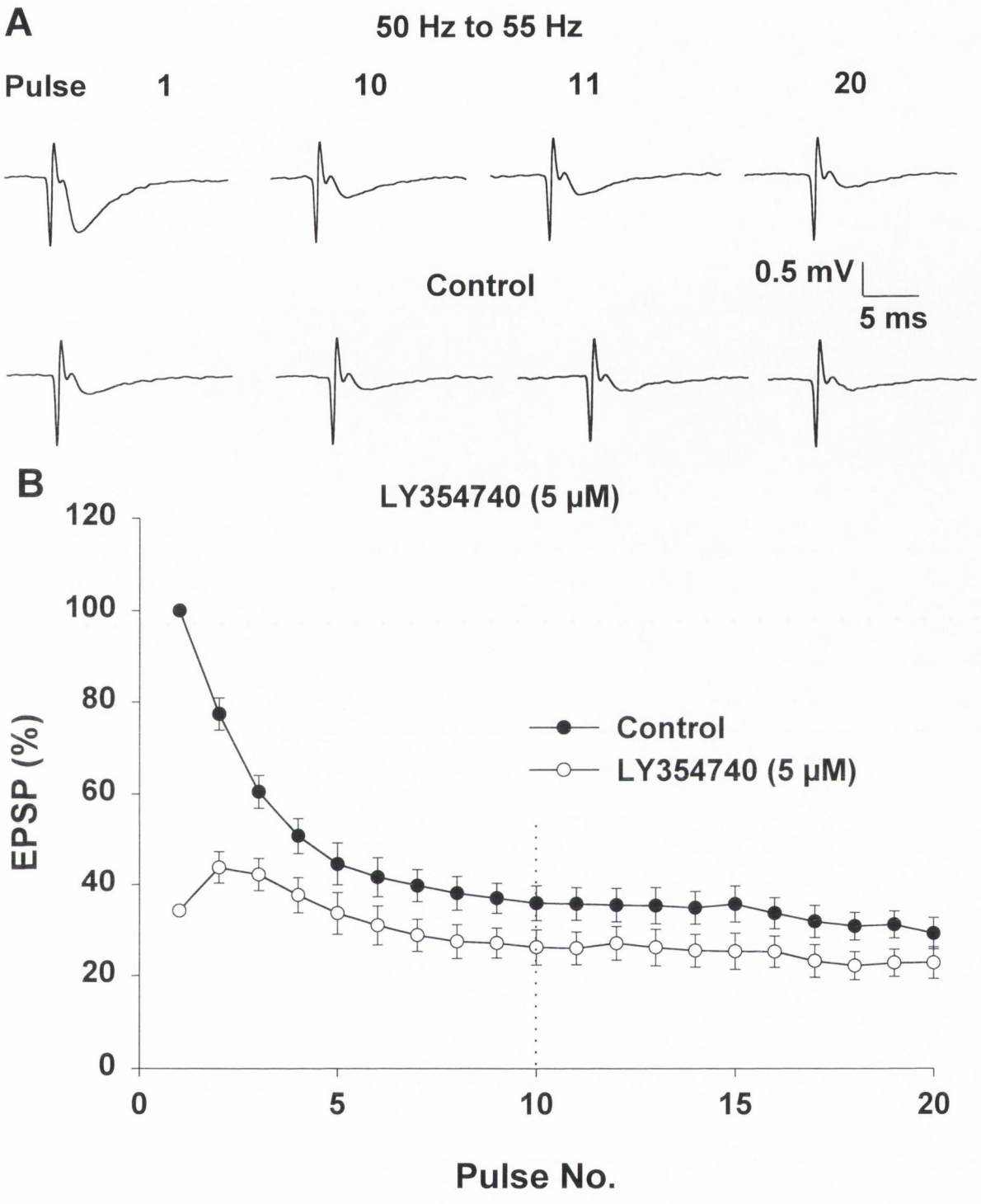


Figure 5.10 Modulation of synaptic depression by LY354740 is frequency dependent. (A) Typical example of EPSPs recorded during a train delivered at 50 Hz (1st and 10th pulses) and after the firing rate was increased to 55 Hz (11th and 20th) in control (upper) and in the presence of LY354740 (5 µM), lower. (B) Summary data (n = 7) showing EPSP amplitudes normalised to the first EPSP in control conditions, before and after the change in rate from 50 Hz to 100 Hz. Control (filled circles) and LY354740 (5 µM) (open circles).

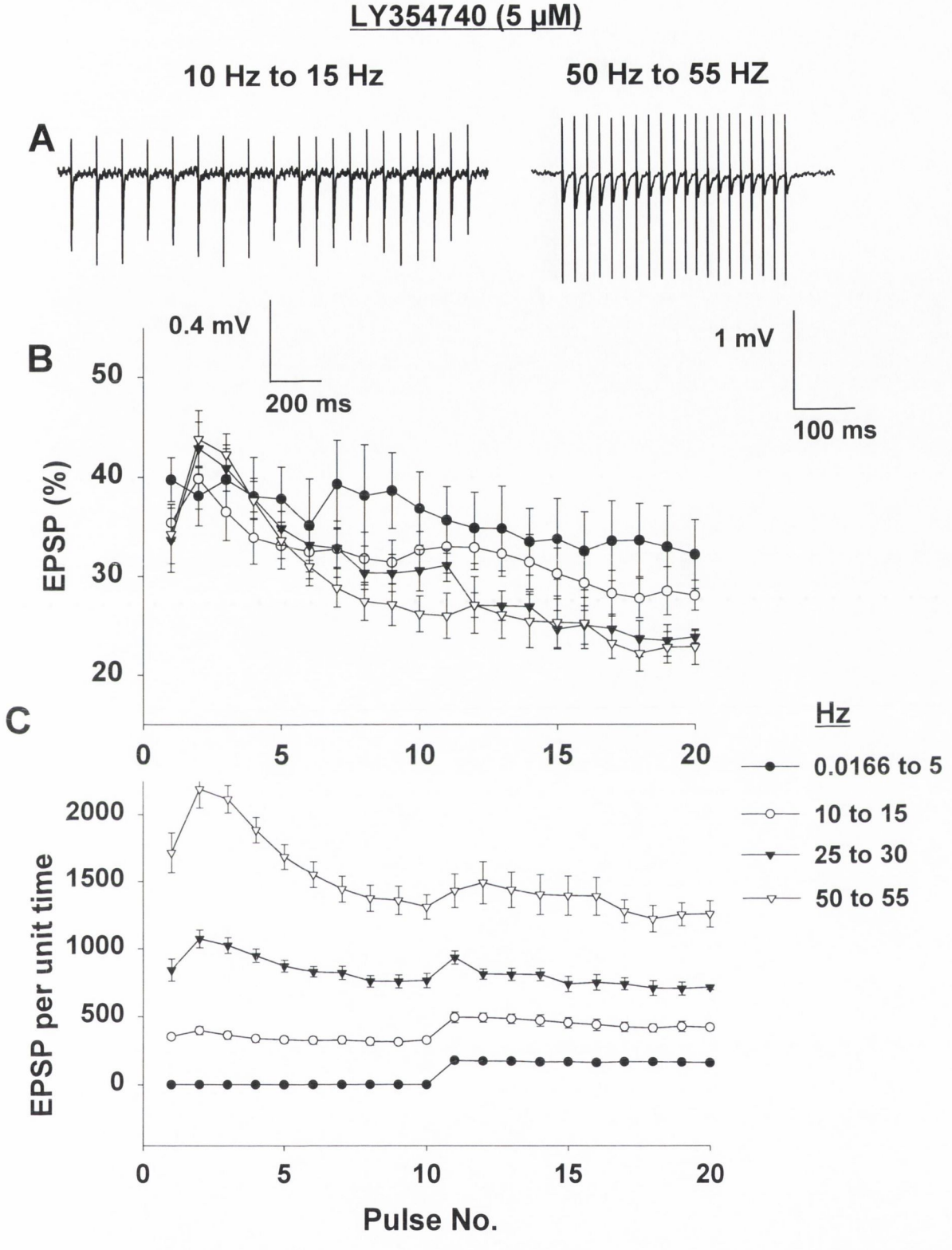


Figure 5.11 The effect on synaptic transmission of an instantaneous increase in the presynaptic firing rate of 5 Hz is modulated by LY354740. (A) Typical example of trains of EPSPs recorded before and after a 5 Hz increase in the firing rate. Left, from 10 to 15 Hz and right, from 50 to 55 Hz. (B) EPSP amplitudes normalised to the first EPSP in each train and plotted against stimulus number in the train. (C) Normalised EPSP per unit time (EPSP amplitude (%) x frequency, expressed in arbitrary units) for the data shown in B. Dotted line represents the change in firing rate. Symbols indicate frequencies. Mean and SEM for 6 -7 slices.

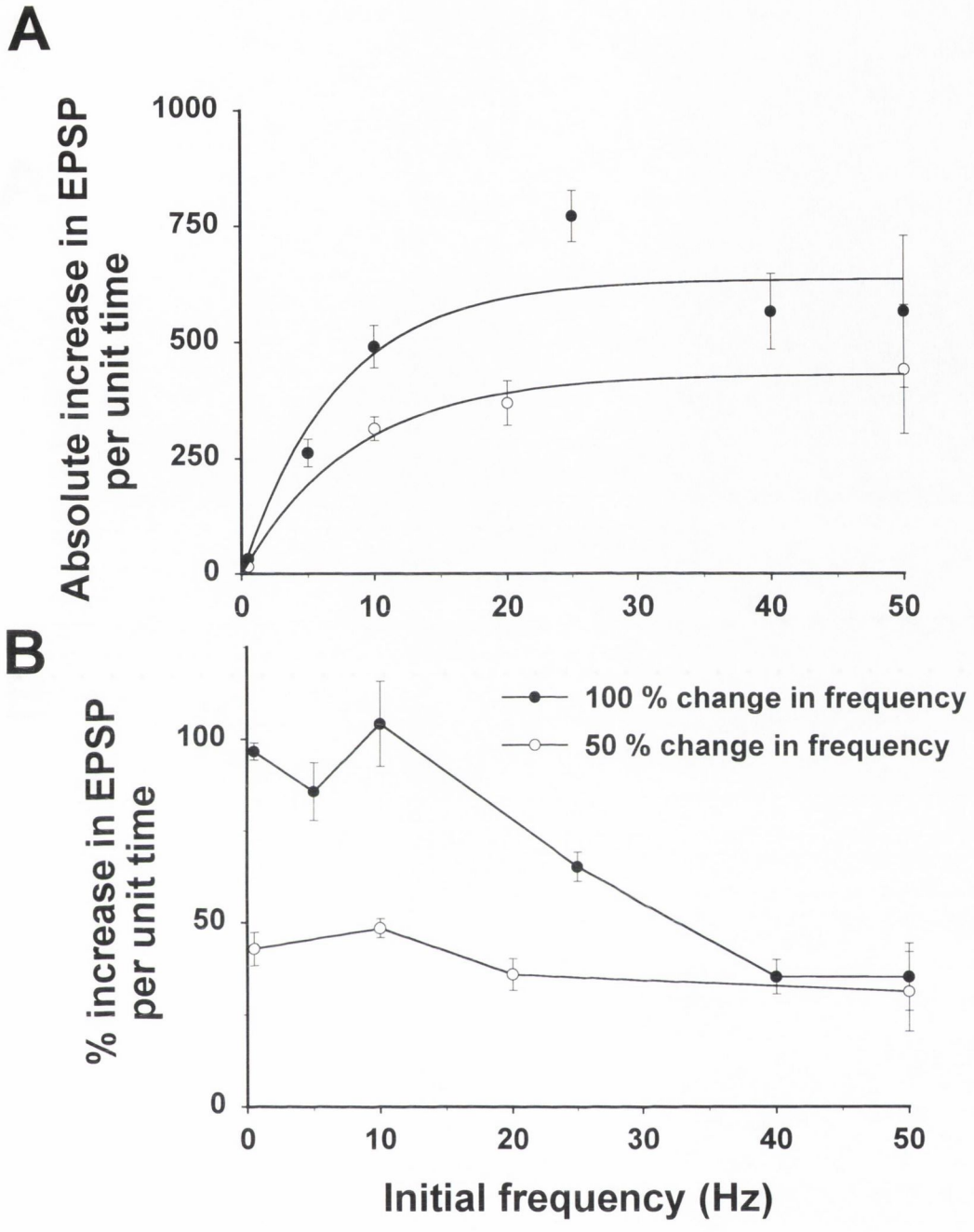


Figure 5.12 The transient postsynaptic response to fixed rate changes over 10 Hz in the medial perforant path is constant. Absolute (A) and percentage (B) increases in EPSP per unit time (EPSP amplitude (%) x frequency, expressed in arbitrary units) associated with an instantaneous increase in firing rate of either 50 % (open circles) or 100 % (filled circles) plotted as a function of the initial stimulation frequency (n = 4 -7).

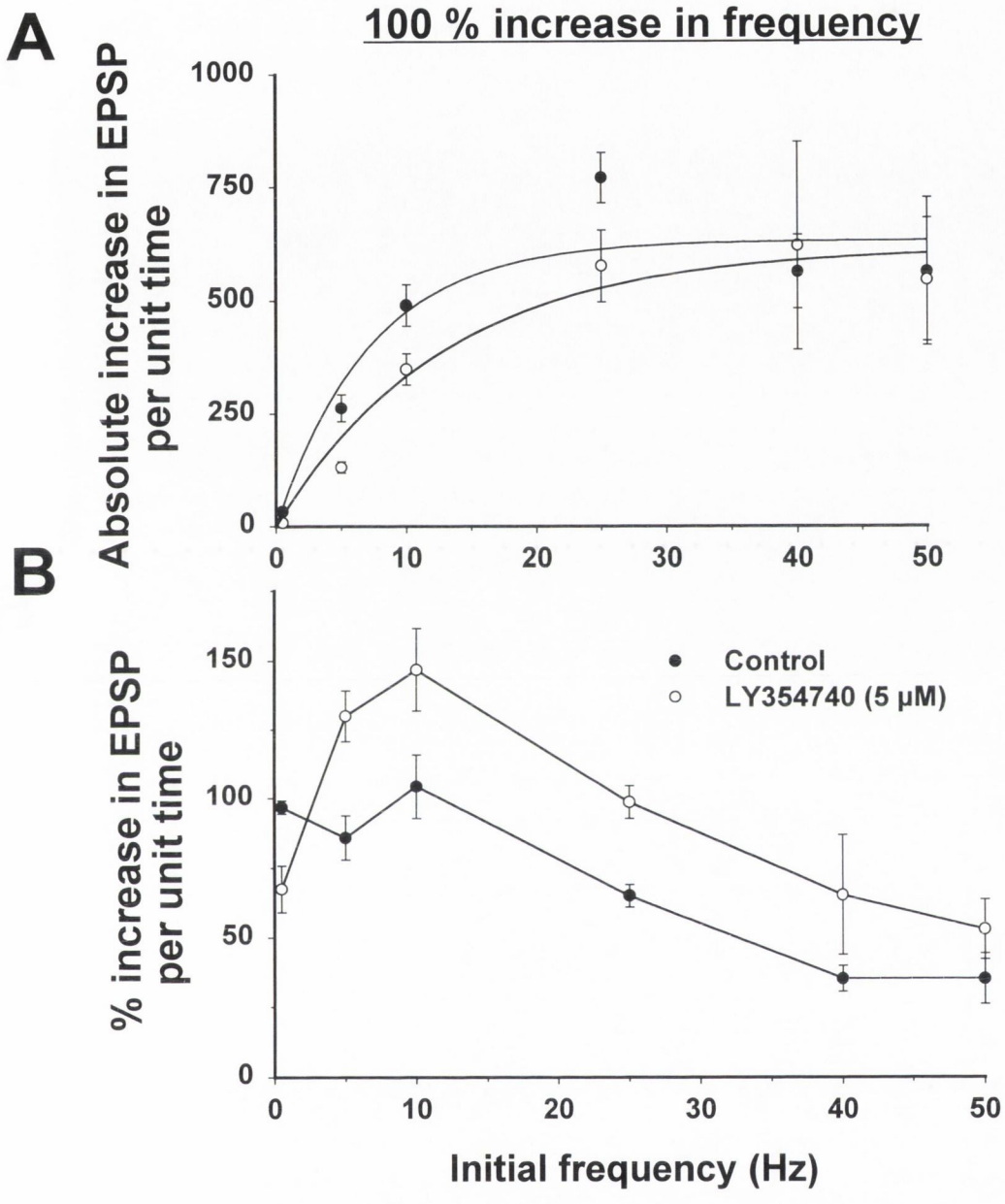


Figure 5.13 Group II mGluR activation reduces the ability of the medial perforant path-granule cell synapses to respond to fixed percentage increases in firing rate with equal sensitivity. (A) The absolute increase in the EPSP per unit time (EPSP amplitude 9%) x frequency, expressed in arbitrary values) before and after a 100 % increase in firing rate is reduced by LY354740 (open circles) only at low frequencies. (B) In contrast the percentage increase in the EPSP per unit time associated with a 100 % increase in firing rate is increased by LY354740 (5 µM) (n = 7-14).

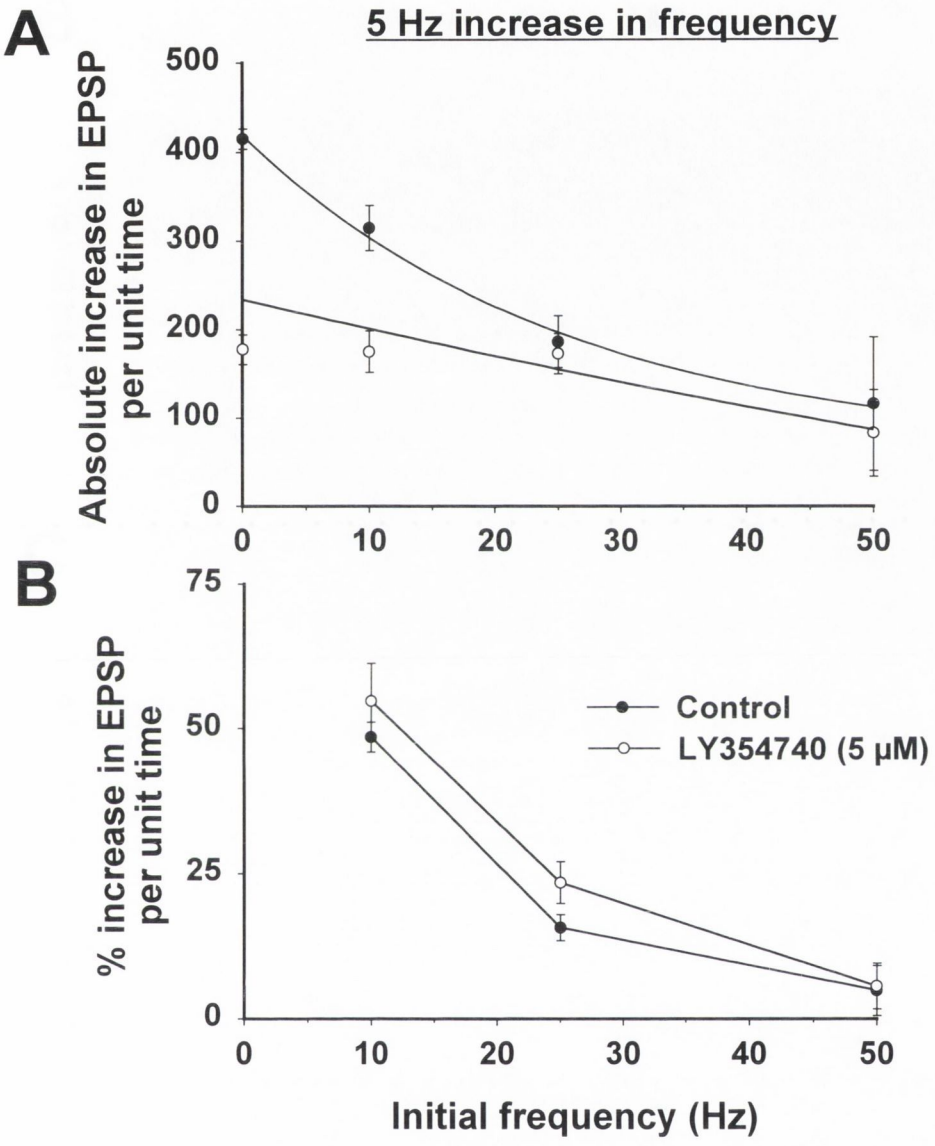


Figure 5.14 Group II mGluR activation reduces the ability of the medial perforant path-granule cell synapses to respond differentially to fixed amplitude increases in firing rate. (A) The absolute increase in the EPSP per unit time (EPSP amplitude (%) x frequency, expressed in arbitrary units)) plotted against the initial stimulation frequency, before and after a 5 Hz increase in firing rate is reduced by LY354740 (open circles) only at low frequencies. (B) In contrast the percentage increase in the EPSP per unit time associated with a 100 % increase in firing rate is increased by LY354740 (5 µM) (n = 6 - 7).

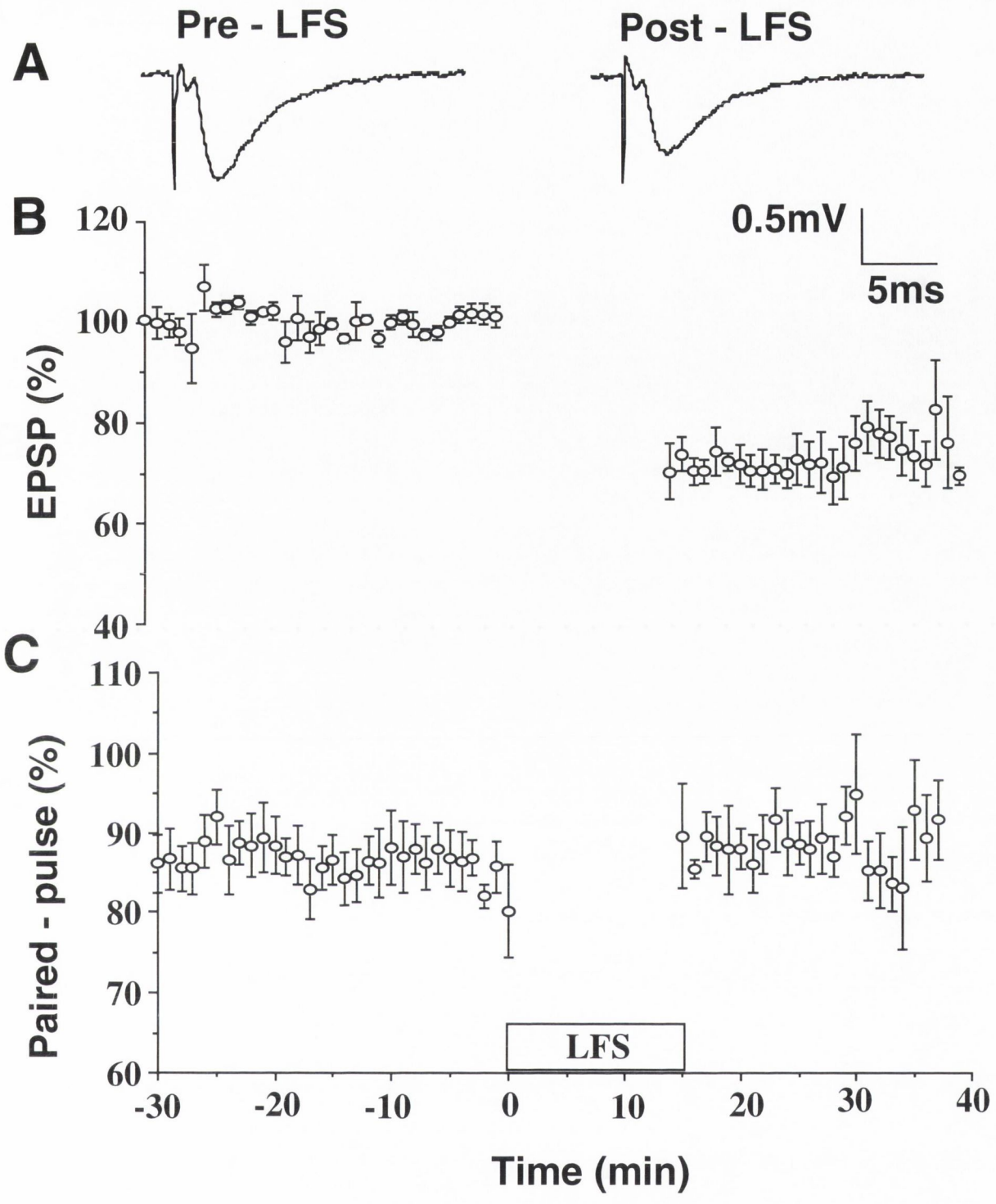


Figure 5.15. LFS induces LTD in the medial perforant path. (A) Typical traces showing paired-stimuli (25 Hz) before and after LFS-induced (900 pulses at 1 Hz) LTD. (B) Normalised EPSP amplitude before and after LTD induction (n = 5) (C) Paired-pulse depression is unaffected by LTD induction.

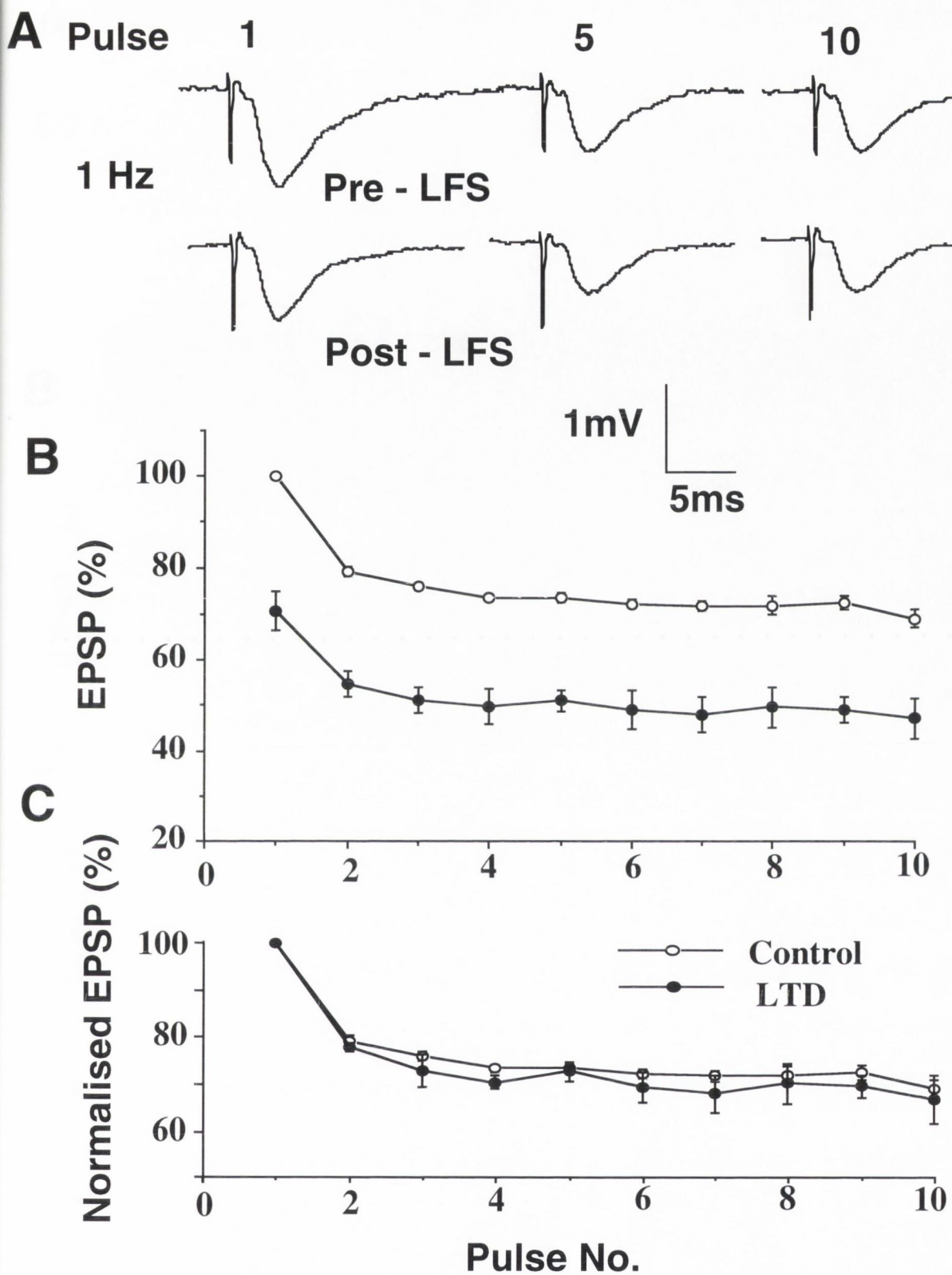


Figure 5.16 Short - term depression induced by 1 Hz stimulation is unaffected by LTD induction. (A) Typical example of the 1st, 5th and 10th EPSPs in a train delivered at 1Hz before (upper) and after (lower) LTD induction by LFS. (B) Trains of EPSPs elicited at 1 Hz normalised to the first EPSP, in control (open circles) and after LTD induction (filled circles) plotted as a function of pulse no. (C) Data in B normalised to the initial control pulse, shows the rate of onset and extent of synaptic depression is unaffected by LTD (n = 5).

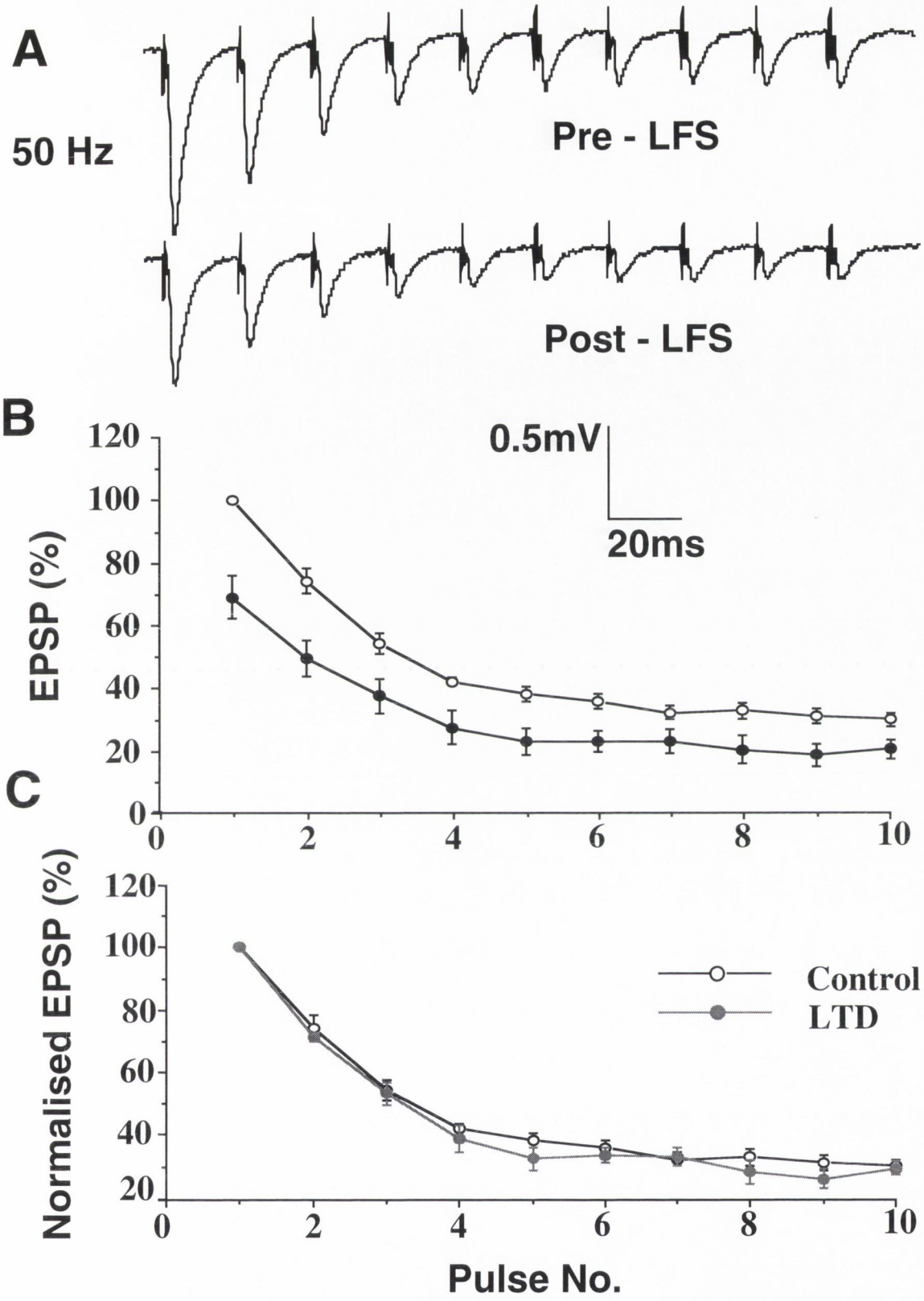


Figure 5.17 Short-term depression induced by 50 Hz stimulation is unaffected by LTD induction. (A) Typical example of the 1st, 5th and 10th EPSPs in a train delivered at 50 Hz before (upper) and after (lower) LTD induction by LFS. (B) Trains of EPSPs elicited at 1 Hz normalised to the first EPSP, in control (open circles) and after LTD induction (filled circles) plotted as a function of pulse number. (C) Data in B normalised to the initial control pulse shows the rate of onset and extent of synaptic depression is unaffected by LTD (n = 5).

5.5 Discussion

5.5.1 Rate vs Temporal coding

The findings of this study into activity-dependent synaptic transmission in the medial perforant path of the dentate gyrus are in broad agreement with similar studies in the cortex. (Markram and Tsodyks, 1996; Abbott et al., 1996; Varela et al., 1997; Tsodyks and Markram, 1997). Specifically the ability of postsynaptic neurons to decipher the information encoded within their presynaptic inputs, on the basis of a rate code, is limited to frequencies below 20-25 Hz. Above this the time-averaged postsynaptic response is inversely proportional to the presynaptic input rate and is therefore independent of the rate at which the action potentials arrive at the presynaptic terminal. Within this range the limited capacity for rate coding is compensated for by an increased sensitivity to derrivatives in the firing rate. In fact it is *only the change* in rate that can be signalled. Furthermore within this range the medial perforant path-granule cell synapses can respond to relative rather than absolute temporal changes in firing rate. Functionally these synapses are subject to the same dynamic gain control mechanism that exists in the cortex (Abbott et al., 1997; Tsodyks and Markram., 1997; Varela et al., 1997). Furthermore the activation of presynaptic group II mGluR can extend the rate coding capacity of these synapses possibly at the expense of temporal coding, and this may be a role that these autoreceptors perform *in situ* (Tsodyks and Markram, 1997; Varela et al., 1997).

The rate and temporal coding properties descibed here are a result of depression being the predominant form of short-term plasticity expressed at these synapses under normal conditions. If the medial perforant path did not display synaptic depression then there would be no limiting

frequency to rate coding. Furthermore the response to fixed percentage rate changes would increase linearly as a function of the initial input rate. In contrast the response to fixed increment changes would be constant across all initial frequencies (Abbott et al., 1997). In practice this would mean that an increase in the presynaptic input rate from 5 Hz to 10 Hz or from 50 Hz to 55 Hz would have the same effect, in contrast a doubling of the firing rate from 20 Hz to 40 Hz would be registered as a smaller change than a change from 50 Hz to 100 Hz. Synaptic depression enhances the computational processing power at these synapses (Zador and Dobrunz, 1997; O'Donovan and Rinzel, 1997; Liaw and Berger, 1996). The ability to discriminate between what may be a significant change in the activity of a slowly firing afferent from random fluctuations ("noise") in a more rapidly firing afferent would be beneficial for information processing (Abbott et al., 1997; Tsodyks and Markram, 1997). In the same way, the ability to respond to fractional changes in input rates would allow neural circuits to respond equally, to inputs that deviate over many orders of magnitude (Zador and Dobrunz, 1997). When the time-averaged postsynaptic response before and after a fixed percentage rate change, was plotted as a function of the initial stimulation rate, the response plateaued at about ~25 Hz. This effectively means that coding of neural information on the basis of the mean input rate, within a given period of time (temporal integration), is restricted to firing rates less than ~25 Hz. Two other groups have found limiting frequencies within a similar range. Abbott's group found a limiting frequency of 10 Hz in cat visual cortex. In the rat neocortex Tsodyks and Markram reported imiting frequencies of between 10 and 25 Hz, although the high calcium extracellular media both groups used would favour enhanced synaptic depression and as a consequence a lower limiting frequency (Abbott et al., 1997, Tsodyks and Markram, 1997).

The response to fixed percentage rate changes was roughly proportional to the magnitude of the change in rate for frequencies over ~20 Hz. That is, the time-averaged postsynaptic response to a 100 % change in firing rate was twice that of a 50 % change. The analysis of the relative (%) change in the EPSP per unit time reinforces the hypothesis that there is equal sensitivity at higher frequencies, but goes further to suggest that in relative terms the sensitivity to fixed rate changes is greatest at low rates of synaptic transmission (0-10 Hz). Previous studies have contained their analysis to the absolute increases in the synaptic drive. Abbott's group found there is an equalisation of the sensitivity to fixed percentage rate changes in input rate over ~10 Hz. This bestows synapses with the ability to respond to relative rather than absolute changes in rate, and as such allows an equal response to a rate change of 10 - 20 Hz and 50 - 100 Hz and so on. Otherwise the rapidly firing afferents would drown out slowly firing afferents.

Again these findings are in agreement with previous studies which have found that synaptic depression increases the sensitivity with which synapses respond to fixed increment (5 Hz) changes in input rate when the initial input rate is low (0 - 10 Hz), in contrast to this, at higher rates the sensitivity to change is reduced. As in other brain regions this makes common sense as a 5 Hz change in a slowly firing afferent is more likely to represent a significant change in the information that is being encoded than a 5 Hz change in a rapidly firing afferent (Abbott et al., 1997).

5.5.2 Modulation of neural coding by activation of presynaptic group II mGluR.

The group II mGluR induced frequency-dependent reduction in synaptic depression extends the limiting frequency for rate coding. That is, the input rate above which the time-averaged postsynaptic response is independent of the input rate. Therefore when these receptors are activated, as is the case in the presence of LY354740, the range of frequencies over which the postsynaptic neurons can glean information from the firing rate of the presynaptic inputs is extended.

As activation of presynaptic group II mGluR reduces the rate and extent of synaptic depression, one would expect it to have a considerable effect on the temporal coding properties of these synapses. If group II mGluR activation reduced synaptic depression equally well at low and higher firing rates, then one would expect to see the curve relating the absolute increase in the EPSP per unit time to frequency for fixed percentage / increment rate changes to take a parallel shift downwards. This only occurs at low frequencies (< 10 Hz). At frequencies higher than this, there is less equality about the increase in the EPSP per unit time than in control. In fact the curves in LY354740 and control converge towards 50 Hz. This is exactly what one would expect in a situation where synaptic depression is reduced in a frequency-dependent manner. In effect it means that the ability of the postsynaptic cell to respond to relative changes in rate equally at mid- and high frequency input rates is somewhat diminished.

Similarly there is a tendency towards a less differentiated response to small fluctuations (e.g. 5 Hz) in input rate on both slowly and rapidly firing

afferents, consistent with a shift towards a synapse displaying less synaptic depression.

Obviously the absolute change in the time-averaged postsynaptic response associated with changes in input rate are going to be smaller in the presence of LY354740 compared to control. This is especially true at low frequencies where the EPSP amplitude is only 20-30 % of the control value. It is worth noting that the relative (%) changes in the EPSP per unit time in LY354740 were elevated on control over almost all frequencies, particularly at the lower range of frequencies.

5.5.3 LTD induction does not affect short-term depression in the medial perforant path.

In the neocortex pairing of presynaptic stimulation with postsynaptic depolarisation induces a form of long-term potentiation which appears to result from an increase in the probability of neurotrasnsmitter release from the presynaptic terminals. (Markram and Tsodyks, 1996). Markrams' group have suggested that this reflects a re-distribution of synaptic efficacy (as opposed to a linear increase in synaptic gain), which is only revealed by examining the response to trains rather than single or paired stimuli as is common practice. Although the initial response to a train of stimuli is potentiated, subsequent stimuli depress at a faster rate to the same steady-state depressed level as before the induction of LTP.

In the hippocampus efforts to replicate these studies have failed, this is most likely because the LTP typically recorded in the hippocampus is postsynaptic in origin. (Selig et al., 1999) As such no interaction between long-term and short-term plasticity would be expected to occur. The

results presented here are in agreement with previous research in the CA1 area of the hippocampus, where the responses to long depressing trains of stimuli were uniformly altered by the induction of LTP (Panaceau et al., 1998; Selig et al., 1999). That is no redistribution of synaptic efficacy occured. Nevertheless these results compound the argument for studying synaptic transmission using more physiologicaly accurate stimulation parameters (Dobrunz and Stevens, 1999, Varela et al., 1997). Studies using single pulses delivered at very low frequencies do not reflect the *in vivo* firing patterns within the CNS, and therefore they ignore the context-dependence that shapes synaptic transmission from one moment to another (Shelig et al., 1999).

In the hippocampus the gain at which presynaptic inputs are "heard" by the postsynaptic cell is set by linear scaling mechanisms such as LTP and LTD. In contrast short-term plasticity enables the postsynaptic neuron to response to transient fluctuations in the presynaptic input it receives. The input specificity of both short and long-term plasticity ensures some degree of selectivity is maintained. Possibly large or long lasting fluctuations in the presynaptic input are turned into LTP changes after a certain threshold is reached.

In conclusion these results in the medial perforant path of the dentate gyrus are in agreement with previous work in the neocortex which suggests that short-term depression acts as an automatic gain control mechanism, favouring slowly firing afferents at the expense of more rapidly firing afferents (Abbott et al., 1997). At low firing rates, when depression is not particularily strong, temporal integration (rate coding) is likely to represented in the neural code, at higher firing rates when depression becomes more prominent, temporal coherence (temporal

coding) may become more important, possibly acting as a complement to the limited rate coding mechanism (Tsodkys & Markram, 1997a/b). Furthermore activation of group II mGluR, but not LTD induction, reduces short-term depression and as a consequence shifts the balance of neural coding towards rate coding and away from temporal coding and as such might point towards a physiological role for these receptors in the living brain.

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VII Publications

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