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Stress and Long-term Potentiation.

By

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A dissertation submitted for the degree of Doctor of Philosophy of the University of Dublin, Trinity College, Dublin 2, Ireland.

This research was conducted in the department of Pharmacology and Therapeutics in the Faculty of Health Sciences.

October 2000

To my parents, Alan and Susan Shakesby, without whose hard work and unfailing support I would not be where I am today. We made it.

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Declaration

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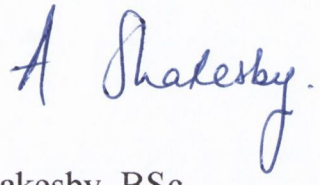

Alison Shakesby. BSc.

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Abbreviations

5-HT	5-hyroxytryptamine (serotonin)
ACh	acetylcholine
ACTH	adrenocorticotropin hormone
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
Ca^{2+}	calcium ion
CAMKII	calcium/calmodulin kinase II
cAMP	cyclic adenosine mono-phosphate
CNS	central nervous system
CRH	corticotrophin-releasing hormone
CSF	cerebro-spinal fluid
DG	dentate gyrus
DRN	dorsal raphe nuclei
EEG	electroecephalogram
EPSP	excitatory post-synaptic potential

FLX	fluoxetine
GABA	gamma-amino butyric acid
GR	glucocorticoid receptors
HFS	high frequency stimulation
HPA	hypo-thalamic pituitary adrenal
I/O	input/output
i.p.	intra-peritoneal
K^+	potassium ion
LFS	low frequency stimulation
LTD	long term depression
LTP	long term potentiation
MAOI	mono-amine oxidase inhibitor
MCPG	methyl-4-carboxyphenylglycine
Mg^{2+}	magnesium ions
mGluR	metabotropic glutamate receptors
MR	mineralocorticoid receptors

MRN	median raphe nucei
NA	noradrenaline
PKC	protein kinase C
PS	population spike
PTX	picrotoxin
VOCC	voltage operated calcium channel

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Summary.

Stress is a physiological reaction which the body copes with by a variety of mechanisms in order to restore homeostasis. Amongst these is an increase in plasma glucocorticoids, catecholamines and increases in brain neurotransmitters. Stress has been shown to have detrimental effects upon synaptic plasticity, blocking a form known as long-term potentiation (LTP) which is believed to be, in part, responsible for memory formation.

The effect of stress on synaptic plasticity was assessed in anaesthetised rats. Electrodes were implanted into the CA1 region of the hippocampus and field excitatory postsynaptic potentials (EPSP) amplitudes were measured. The ability of rats to express LTP was assessed using a stimulation protocol of single high frequency stimulation (HFS) for induction. Rats were stressed by placement for 30 minutes upon a raised platform. The effect upon the ability to induce LTP was then investigated.

Initially it was shown that exposure to acute episodes of stress could block the induction of LTP to HFS. This was then followed by investigation into some of the factors that may be involved in this phenomenon. Investigation into the potential involvement of the major neurotransmitters within the hippocampus was carried out. Alteration of excitatory glutamatergic receptor function failed to significantly alter the block of LTP in stressed animals. This included blockade of NMDA receptors and also by enhancing AMPA mediated transmission. Decreasing local hippocampal inhibition in stressed animals by the use of a GABA-ergic antagonist followed by HFS produced a delayed attenuation in EPSP amplitude that lasted for more than 60 minutes.

Next, the role of other neurotransmitters present within the hippocampus were investigated. These used agents that increase or reduce extracellular 5-HT levels

and enabled the induction of LTP in response to HFS in stressed animals. However, elevation of 5-HT in non-stressed animals produced a block in the ability of HFS to induce LTP. The involvement of two 5-HT receptor subtypes, 5-HT_{1A} and 5-HT_3, was investigated with the use of antagonists and found to have little or no effect upon the stress-induced block of LTP. Increasing hippocampal levels of noradrenaline (NA) were also observed not to reverse the block of LTP in stressed animals.

From the results obtained it can be concluded that there is a significant involvement of the serotonergic system in the stress-induced block of LTP. Stress has been shown to raise hippocampal levels of 5-HT and increases in 5-HT levels in non-stressed animals has been shown to block LTP induction, which was also observed here with fluoxetine and fenfluramine, both of which raise extracellular levels of 5-HT. These observations implicate elevated levels of 5-HT as a cause of the block of LTP by stress. In stressed animals it was also shown that reduction of 5-HT levels can restore the ability to induce LTP as could further elevation of levels. This indicates a dynamic relationship between 5-HT levels and synaptic plasticity. How this relationship is mediated is not known. 5-HT_{1A} and 5-HT_3, receptors do not appear to be involved. Neither does NA or the excitatory AMPA or NMDA glutamate receptors. Inhibitory GABA-ergic interneurones do appear to have a role in the mediation of stress effects on synaptic plasticity but the results here showed a shift in plasticity towards a depression rather than recovery of potentiation.

There are implications for the treatment of disorders that arise from stress exposure whereby treatments that are presently used clinically as antidepressants can have other uses in maintaining the ability of the hippocampus to exhibit normal plasticity.

I. Introduction

1.0 Introduction

1.1 The hippocampus.

Anatomically the hippocampus follows the line of the lateral ventricles. The large growth of the neo-cortex through evolution is thought to have pushed the hippocampus down in the gross structure of the brain. The hippocampus for many years now has been the primary site implicated in the processes of certain types of memory and learning. Given its central location and connections with other brain structures, it is ideally situated as a primary processing site for such a function.

The primary input is from the entorhinal cortex, an old evolutionary structure that was primarily associated with smell. Sensory inputs come via the perforant pathway and from there are relayed to the granule cells of the dentate gyrus. These granule cells give rise to axons that project and synapse upon pyramidal cells in the CA3 region. Schaffer colateral fibres then lead from CA3 to the pyramidal cells present in CA1 area whose projections synapse at various parts of the CNS. All of these excitatory pathways are believed to be glutamatergic.

Apart from the primary excitatory pathways that form the main part of the hippocampus, there are other neurones and transmitters that play vital modulatory roles in hippocampal functioning. There is a large number of gamma-amino butyric acid (GABA) interneurones throughout the entire structure. These are responsible for controlling the overall activity of other neurones particularly the within dentate gyrus (DG) and CA1 regions. Also present are 5-hydroxytryptamine (5-HT) releasing neurones that originate in the raphe nuclei. These are responsible for modulating the activity of both principle cells and interneurones present within the hippocampus. Acetylcholine (ACh) containing neurones as well as noradrenergic neurones also terminate within the hippocampus providing another potential source for modulation.

1.2 Plasticity at glutamatergic synapses.

1.2.1 Long term potentiation.

Long term potentiation (LTP) is a widely investigated phenomenon seen in several regions of the central nervous system (CNS). It is believed to provide a model of the neuronal mechanism underpinning memory formation, memory storage and certain types of learning (Bliss and Collingridge, 1993). LTP can be recognised electropysiologically by long lasting increases in population spikes or of glutamatergic excitatory post-synaptic potentials (EPSP) that can last between hours and days experimentally, indicating a long-term, activity dependent increase in synaptic efficacy and strength. LTP can be induced by either hetero- or homo-synaptic inputs and has been studied in many brain regions including the hippocampus, cerebellum, striatum and neocortex. More recent work has discovered that other forms of synaptic plasticity could be induced such as depotentiation and long-term depression (LTD). In addition, N-methyl-D- aspartate (NMDA) receptor independent forms of LTP have been found expanding the classical understanding of LTP (reviews can be found by Bennett, 2000, Malenka, 1994, Malenka and Nicoll, 1999, McEachern and Shaw, 1996). Also, the stimulation parameters for the induction of LTP could be varied for example theta burst stimulation or LTP can be induced pharmacologically.

Glutamate is found in the L- stereo-isomeric form in almost all excitatory synapses and is considered to be the primary excitatory transmitter. Its ionotropic effects are mediated via activation of 3 main receptor subtypes that were originally characterised by their respective agonists. The subtypes are NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors which are ligand-gated ion channels. An entire family of metabotropic glutamate receptors reliant on second messengers as a transduction mechanism has been cloned and characterised using selective agonists and antagonists.

The classical view of LTP is that it can be induced by high frequency stimulation (HFS) of an afferent pathway and was first described fully by Bliss and Lomo (1973). In the hippocampus, induction of LTP in the CA1 and dentate gyrus areas are NMDA receptor-dependent (Collingridge *et al*, 1983), although recent work has found that there are forms of NMDA receptor-independent LTP. Generally activation of NMDA receptors can provide sufficient entry of calcium ions (Ca^{2+}) into the post-synaptic cell to initiate calcium induced calcium release from intracellular stores. Under standard conditions, NMDA receptors are prevented from allowing calcium entry into the cell by a magnesium (Mg^{2+}) block. Under conditions of low stimulation, glutamate binds to both NMDA and AMPA receptors but the resulting EPSP is mediated primarily by AMPA receptors. By stimulating afferent fibres at high frequency, the result is EPSP summation (Gustafsson ***et al.***, 1987) and a large depolarisation sufficient to remove the Mg^{2+} block from the voltage dependent NMDA receptors.

Elevation in Ca^{2+} levels are thought to be the most critical initial step activating second messenger cascades. It was reported by Lynch *et al,* 1983, that LTP could be blocked by the Ca^{2+} chelator EGTA. The second messenger cascades that are thought to be involved in LTP induction and formation are complex. The Ca^{2+} that enters the cell due to the activation of NMDA receptors activates, among others, calcium-calmodulin kinase II (CaMKII). This particular kinase is required for the induction of LTP in the hippocampus and also for spatial learning (Bach *et al,* 1995).

The most dominant recent theory on LTP expression is that it is mediated by activation of post-synaptic silent synapses (Feldman *et al*,1999; Isaacs *et al*, 1999). It is thought that there is a reservoir of AMPARs within the post-synaptic neurone. Activation of the CAMKII and then through phosphorylation of as yet unidentified protein residues, allows the rapid insertion of additional AMPARs into the post-synaptic membrane from the intracellular store, thereby increasing the post-

synaptic response of the cell. Other changes that may occur with postsynaptic AMPA receptors are an increase in receptor binding and affinity as well as altered kinetics of the channels. However, some silent neurones have been found to be presynaptically silent (Gasparini *et al*, 2000), depending upon the release of glutamate from the synapse as a major factor in plasticity rather than postsynaptic mechanisms.

The links for LTP as a cellular mechanism for learning and memory are still an intensively researched area. For example, certain NMDA receptor antagonists that block LTP can also interfere with certain types of learning (Walker and Gold, 1994) although this does not prove that LTP is required for learning. Pharmacological manipulations of LTP and learning are reviewed by Izquierdo, 1994. Interestingly, certain endogenous rhythms such as theta rhythm can provide the optimum conditions for LTP induction (Larson and Lynch, 1988). Doyere *et al*, 1993 showed that LTP could be induced in the hippocampus by high frequency stimulation trains and also by theta rhythms that occurred during a learning task. Buszaki, 1989, produced a theory by which experimental LTP could account for memory traces *in vivo* by considering the neuronal firing patterns that occur in natural behaviour and applying them to conditions that are used experimentally. Additional and more recent evidence for a link between LTP and learning has been reported. Rioult-Pedotti *et al*, 2000 reported that synaptic strength was enhanced in animals that underwent learning. Also it has been reported that LTP induced in the dentate gyrus following learning disrupted the retention of the task and this was dependent upon NMDA receptors (Brun *et al*, 2001). Saturation of LTP has also been shown to inhibit learning although where further potentiation could occur learning was unimpaired (Moser *et al*, 1998).

1.2.2 LTD

Long term depression (LTD) is a persistent activity dependent decrease in synaptic strength. It can be induced by the application of low frequency stimulation within the range of 1-5 Hz (Dudek and Bear, 1992). Unlike LTP, which is seen in both young and mature hippocampus, LTD is more easily induced by LFS in the developing hippocampus (Wagner & Alger, 1995; Dudak & Bear, 1993). However, Otani & Connor (1998), reported that LTD could be induced in adult rat hippocampus in vitro in the presence of picrotoxin, the GABA receptor antagonist. Heynen *et al*, 1996 reported that homosynaptic LTD was inducible in the adult rat hippocampus *in-vivo*. Also, LTD formation has also been reported in adult rats when exposed to acute stress prior to LFS (Xu *et al.*, 1997). Recently different forms of LTD have been illustrated by Kemp *et al*, 2000 to differing stimulation protocols and at various stages of development. In the adult hippocampus differing stimulation protocols were shown to produce either NMDAR-dependent of independent forms, whereas another stimulation protocol would only induce LTD in the young hippocampus but not adult

LTD induction has been shown to be dependent upon the activation of both NMDAR and post-synaptic entry of Ca^{2+} (Mulkey and Malenka, 1992, Dudek and Bear, 1992) and mGluRs. Calcium is believed to be the important key step in the induction of both LTP and LTD. Increases in postsynaptic levels of calcium is believed to trigger activation of phosphatases within the post synaptic cell leading to both short and long term reductions in transmission. There was an apparent paradox that an increase in postsynaptic Ca^{2+} should be required for both LTP and LTD induction. This was resolved by the hypothesis that varying amounts of Ca^{2+} could differentially activate secondary systems involving activation or deactivation of enzymes, transcription factors or receptors (Teylor *et al*, 1994). The "silent synapse" theory may also be relevant to LTD but in this case AMPA receptors

being removed from the post-synaptic membrane thereby reducing cellular responses to stimulation.

1.3 Endogenous neuromodulators of hippocampal function.

1.3.1 GABA.

Gamma-amino-butyric acid (GABA) is the most abundant inhibitory neurotransmitter within the mammalian central nervous system. GABA is synthesised in nerve terminals by the enzyme glutamic acid decarboxylase (GAD), of which there are two isoforms. Both isoforms are co-expressed in GABA-ergic neurones (Erlander *et al.* 1991). The two isoforms are suggested to be required for different functions. GAD67 is thought to maintain basal levels of GABA within the neurone, while GAD65, the smaller of the two isoforms, is thought to be available for rapid activation in times of high demand (Martin *et al.* 1991).

GABA is released from nerve terminals in response to an incoming depolarisation as with other neurotransmitters. The post-synaptic response to GABA, however, is a hyper-polarisation of the cell reducing the overall excitability to other incoming stimuli. As such GABA is usually thought to have a modulatory effect within the CNS to control the overall level of neuronal excitability. GABA can play an important role in feedback mechanisms. Although GABA is largely considered as an inhibitory neurotransmitter, some groups have reported that GABA can induce depolarisations in some areas of the CNS (Staley *et al.* 1995). It has also been reported that GABA can raise intracellular Ca^{2+} levels in the cortex (Takebayashi *et al.* 1996).

GABA acts via three different receptors. These belong to two different superfamilies of receptor, the ligand-gated and metabotropic._The ligand-gated channel ($GABA_A$) is the main GABA receptor for GABA-ergic transmission within the CNS (Bormann, 1988). It is a five sub-unit receptor able to gate for chloride ions (Cl^-). It is the flux of Cl^- through the open channel that is responsible for the subsequent hyper-polarisation of the cell (Bormann, 1988). The $GABA_A$ receptor

can be subject to modulation by various ligands such as benzodiazepines, barbiturates, neurosteroids, and ethanol. Convulsant agents such as picrotoxin can physically block the channel. Facilitation of GABA-ergic transmission produces anxiolytic effects. Benzodiazepines are used clinically for this purpose. Antagonists such as bicuculline and picrotoxin are anxiogenic, presumably due to a reduction in overall CNS inhibition.

$GABA_B$ receptors are found both pre- and post-synaptically and are responsible for slow GABA-mediated IPSPs. They are thought to act as auto-receptors pre-synaptically to regulate release of GABA from GABA-ergic terminals. A third receptor, $GABA_C$ shares some of the properties of $GABA_A$ receptors such as Cl^- conductance, and is found throughout the hippocampus, cortex, retina and cerebellum.

1.3.2 GABA, LTP and LTD

Given its role as the major inhibitory transmitter, GABA has been of much interest with regard to LTP. It has been noted in other brain areas that activation of $GABA_A$ receptors leads to inhibition of LTP formation. For example exogenously applied GABA to rat superior cervical ganglia was shown to inhibit the induction of LTP (Gonzalez Burgos *et al.* 1997). This inhibition was reversed completely by the application of PTX. Widespread activation of neurones by strong electrical stimulation such as HFS would lead to the release of GABA along with glutamate. GABA would be responsible for the hyperpolarisation of the post-synaptic cell thereby limiting the excitatory effect of HFS. Consistant with this, Wigstrom & Gustaffson (1985) found that inhibition of $GABA_A$ receptors in the rat hippocampus facilitated the induction of LTP. With continued stimulation, released GABA starts to activate pre-synaptic $GABA_B$ receptors initiating negative feedback such that the release of GABA from those terminals and hence overall system inhibition is reduced.

GABA is also implicated in LTD induction but evidence for its role in LTD induction remains contradictory. Wagner and Alger, 1995, found that the blockade of $GABA_B$ autoreceptors lead to an increase in inhibition in CA1 and that this was sufficient to produce a significant attenuation of LTD in young animals. $GABA_A$ blockade using bicuculline failed to produce any greater LTD than without the blockade leading to the conclusion that autoreceptor mediated inhibition of GABA release is sufficient to produce maximal LTD. Conversely, Steele and Mauk, 1999, reported that enhanced GABA-ergic activity favoured the induction of LTD rather than LTP and also widened the range of stimulation frequencies that induced LTD. Facilitation of LTD in the presence of greater inhibition was also demonstrated by Yang, 1994.

1.3.3 5-HT.

5-hydroxytryptamine (5-HT) or serotonin is a neurotransmitter that is present widely throughout the CNS. It is synthesised from L-tryptophan by two sequential enzyme steps that add a hydroxyl group to position 5 of the indole ring and then a decarboxylation to form 5-HT. It is found stored in synaptic vesicles where it can be released via the calcium-dependent process of exocytosis. It acts both as a neurotransmitter and as a neuromodulator. (Zimmerman, 1993)

5-HT containing neurones are well represented throughout the CNS. A major source of 5-HT afferents is the raphe nuclei which is subdivided mainly into dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN). Afferents arising from the DRN innervate the frontal cortex and the amygdala. Afferents from the DRN also innervate the periacqueductal gray and periventricular areas. MRN arising afferents provide a major pathway to the hippocampus which also receives an input from the DRN (Azmitia, 1978).

1.3.4 5-HT and the hippocampus.

The hippocampus is well innervated with serotonergic afferents (Conrad *et al.*, 1974). 5-HT pathway stimulation leads to attenuation of pyramidal cell firing within the hippocampus by the synaptically released 5-HT (Blier *et al.*, 1985). There is a well defined firing pattern exhibited by the hippocampal serotonergic neurones with long duration and a slow, regular firing rate. This particular pattern has been put down to a pacemaker effect of a calcium-dependent potassium current. The level of activity of 5-HT neurones also changes during the sleep-wake cycle being inactive during the REM phase of sleep while having discharge frequencies of 3 and 6Hz during quiet and arousal states respectively (Trulson *et al*, 1979). There are several distinct receptor subtypes present both pre- and post- synaptically for 5-HT (summarised in table 1.1). Many of these can be found pre-synaptically

acting as auto-receptors such as the 5-HT_{1A}, $_{1B}$ and $_{1D}$ sub-types. Due to the large number of receptor subtypes present, 5-HT can mediate either excitatory or inhibitory effects.

Some subtypes seem to mediate their effect through alterations in potassium channel function. The 5-HT_{1A} receptor subtype is found pre-synaptically on the soma and dendrites of DRN cells and is thought to regulate the overall discharge activity of 5-HT neurones. Within the CA1 area of the hippocampus 5-HT_{1A} receptors are located on the postsynaptic membranes of both pyramidal cells and inhibitory interneurones. Once activated this receptor produces an inhibitory effect by G-protein mediated opening of potassium channels, thus hyperpolarising the membrane (Aghajanian *et al.*, 1987, Innis *et al.*, 1988). Thus activation of these receptors in the raphe nuclei leads to a reduction in the amount of 5-HT released from hippocampal terminals.

Other receptors, such as the 5-HT_{1B} autoreceptor, act through decreasing the calcium entry via voltage-dependent calcium channels. 5-HT_{1B} receptors are located pre-synaptically within the hippocampus and inhibit release of 5-HT. Both 5-HT_{1A} and 5-HT_{1B} receptor sub-types are negatively coupled to adenylate cyclase and generally lead to inhibition of the neurones upon which they are present, as do the other members of the 5-HT_1 group.

Excitatory effects of 5-HT appear to be mediated via the 5-HT_4 subtype and have been found to be positively coupled to adenylate cyclase (Shenker *et al.*, 1987). 5-HT_3 receptors are located mainly on inhibitory interneurones and increase inhibition by direct ionotropic depolarisation of the cells. Connections between the two groups of neurones, 5-HT and GABA-ergic, have been found anatomically (Freund *et al.*, 1990). See table 1.1.

Activation of 5-HT_{1A} receptors has been found to have both facilitatory and detrimental effects upon learning depending upon task conditions (Balleine *et al*,

1996). Stimulation of the post-synaptic 5-HT_{1A} receptors in the hippocampus has been found to impair the acquisition of information in spatial learning tasks (Herreman *et al*, 1995), while activation of these receptors has also been found to improve performance by decreasing latency in radial arm maze trials (Buhot M-C *et al*, 1995).

1.3.5 5-HT and synaptic plasticity

The role of 5-HT in LTP is believed to be as that of an inhibitory modulator and that by causing inhibition 5-HT blocks LTP induction. It has been suggested that the cause of the attenuation of LTP induction by 5-HT is caused by hyperpolarising the postsynaptic neurones (Villani *et al*, 1993). Also, it has been reported that in rat visual cortex LTP inhibition is caused by activation of phospholipase C system via the 5-HT_{2C} receptor subtype (Edagawa *et al*, 2000). LTP induction is not just blocked when HFS is used but also when theta rhythm stimulation paradigms are used. Staubli and Otaky, 1994, reported that 5-HT decreased facilitation seen in response to theta burst *in-vitro* and that 5-HT also decreased theta-stimulated AMPA receptor currents and also LTP induction by primed burst stimulation (Corradetti *et al*, 1992).

Table 1.1.

5-HT receptor subtype	Transduction Mechanism	Presence in the hippocampus
5-HT_{1A} 5-HT_{1B} 5-HT_{1C} 5-HT_{1D} 5-HT_{1E} 5-HT_{1F}	7 transmembrane spanning, G-protein coupled receptor. Negatively coupled to adenylate cyclase	(+) (+) (+)
5-HT_{4} 5-HT_{6} 5-HT_{7}	7 transmembrane spanning, G-protein coupled receptor. Positively coupled to adenylate cyclase	(+) (+)
5-HT_{2A} 5-HT_{2B} 5-HT_{2C}	7 transmembrane spanning, G-protein coupled to phospholipase C.	(+) (+) (+)
5-HT_{3}	Ligand-gated ion channel	(+)

Table 1.1

5-HT receptor sub-types and their transduction mechanisms. Each receptor sub-type has its own distinct distribution throughout the brain which determines the functional significance of each. This table shows the sub-types that are present throughout the hippocampal formation and indicates the transduction mechanism by which activation produces an effect.

1.3.6 Noradrenergic modulation of synaptic plasticity.

Noradrenaline (NA) is a catecholamine with actions peripherally and centrally. It has also been implicated to act as a modulator of synaptic plasticity. The locus coerulus (LC) provides the hippocampus with major adrenergic inputs (Loy *et al*, 1980). Adrenergic receptors are found throughout the hippocampus on excitatory and inhibitory interneurones (Madison and Nicoll, 1982 and Bergles *et al*, 1996). Receptor activation leads to alterations in channel function and mobilisation of intracellular second messengers leading to modulation of the excitability and activity of their target neurones (Madison and Nicoll, 1982, Segal, 1982).

Evidence for the involvement of NA in LTP has been found in the DG area of the hippocampus. NA application both *in vivo* and *in-vitro* has been found to produce potentiation of perforant path evoked field potentials. Interestingly, this effect was mediated by different receptors, β-receptors *in vitro* and α-receptors *in vivo* (Chaulk, *et al*, 1998). Direct LC activation has been shown to produce a β-receptor dependent increase in population spike in the anaesthetised rat (Neuman & Harley, 1983; Dahl & Sarvey, 1989).

There has been conflicting evidence over the role of NA in LTP induction in the CA1 area of the hippocampus. As early as 1982 a study by Dunwiddie and colleagues indicated that there was no effect of NA on the plasticity of the Schaffer collateral and CA1 synapses. This was supported by other work later by Katsuki *et al*, 1997, who reported that NA was ineffective in modulating the induction of LTP in response to theta burst stimulation. However, more recent studies have found that a modulatory role for NA may exist. Activation of β-receptors, which have been reported to alter the excitability of neurones (Madison and Nicoll, 1982) and also to alter the activity of NMDA receptors (Raman *et al*, 1996), was found to enhance the ability of weak tetanic stimulation to induce LTP in the CA1 area (Sah and Bekkers, 1996). Also it has been reported that in the adult rat hippocampus NA

promotes the induction of LTP *in vitro* using single 100Hz tetanus and appears to be mediated through the activation of α1 receptors rather than the previously reported β-receptors, which were ineffective in this study (Izumi and Zorumski, 1999).

The conflicting evidence is quite likely due to the differences in the protocols that are used and particularly in the stimulation parameters of the studies mentioned here. The hippocampal network of neurones appears to be exquisitely sensitive to changes in these particular parameters. Such differences between studies makes it difficult to produce a consistent hypothesis for the involvement of potential modulators such as NA.

1.4 The stress response.

1.4.1 General introduction

Stress has become an ever increasing factor in the daily lives of the masses where there is less leisure time and more responsibilities than ever before. With the pressure for maximal performance from people, usually in the work place, together with the continued demands outside of work, the problems associated with stress and the number of people requiring treatment and help will only increase. Stress has been closely linked with both anxiety and depression. The effects of stress on the body and mind are still not well understood and will continue to be an area of research for many years.

The way in which the term stress is commonly used is rather vague. For the purpose of research and experimentation, stress has been defined in various ways, but the common factor is always that stress involves a move away from the standard state of homeostasis by the action of or exposure to a stressor. How the body deals with this in its attempt to re-establish homeostasis is therefore known as the stress response.

The stress response is a complex series of physiological responses to the exposure of stress. It is mediated and controlled by the hypothalamic-pituitary axis located in the brain. The stress response results in much higher circulating levels of the hormones adrenaline, noradrenaline, and glucocorticoids than under normal physiological conditions. The end result is commonly known as the "fight or flight" response where the body is mobilised and in a heightened excitatory state.

1.4.2 The hypothalamic-pituitary adrenal axis.

The primary control centres for the regulation of the physiological response to stress are the hypothalamic-pituitary adrenal axis (HPA) and the sympathetic nervous system. They are responsible for control of the release of the glucocorticoids mentioned previously and also catecholamines. In both normal conditions and a stress situation, corticotrophin-releasing hormone (CRH) is released from the paraventricular nuclei of the hypothalamus. The released CRH proceeds to act upon the pituitary gland stimulating the release of adrenocorticotropin hormone (ACTH) into the circulation, which has its action on the adrenal gland. Stimulation of the adrenal gland, specifically the cortex, causes the release of corticosteroids into the peripheral circulation. Control of the HPA axis and its responses to specific stressor types are reviewed by Jessop (1999).

Both the hypothalamus and the pituitary gland are exquisitely sensitive to peripheral circulating levels of glucocorticoids. A rise in the peripheral levels of glucocorticoids causes a reduction in the amount of ACTH produced by the pituitary gland, thus suppressing adrenal output (Axelrod & Reisine, 1984). Homeostatic control of glucocorticoid release and adrenal output is thus kept within narrow limits although there is a diurnal cycle that is observed within those limits. Given the nature of the negative feedback system, it should be somewhat surprising that levels of glucocorticoids should rise so rapidly in response to stressful situations. However, it has been reported that mechanisms exist so that stress can initiate ACTH release independently of circulating levels of glucocorticoids (De Souza & Van Loon, 1982; Marti *et al* , 1999).

More recently, it has been found that control of glucocorticoid levels occurs in two phases. A short-term phase is controlled at the pituitary level, while a more long-term control is mediated via the hippocampus and the hypothalamus (Ritchie &

Nemeroff, 1991). These control measures come into effect in response to elevated levels of corticosterone such as those that are seen in response to stressful stimuli.

Under normal, standard conditions, the output from the HPA axis is not continuous (for review see Carroll & Mendels, 1976). The CNS exerts an inhibitory influence for much of the time over the axis thus reducing its output. In rats, corticosterone levels rise to a maximum of approximately 16μg/dl in the late afternoon, while during the night they rest between 1-5μg/dl (Dallman *et al.*, 1987). The main rise in levels is around the time immediately before and during the time that the animals wake. The same phenomenon can be observed in human cortisol levels. When an animal is maximally stressed, corticosterone levels have been found to reach levels of up to approximately 100μg/dl.

1.4.3 Glucocorticoids.

Corticosterone is a glucocorticoid that has the basic steroid structure and is synthesised from the standard steroid precursor of cholesterol. It is the primary stress hormone that is produced by the rat (Bohus & de Kloet, 1981), compared to cortisol in humans (Baxter *et. al.*, 1979). Corticosterone and other circulating glucocorticoids are highly lipid soluble, accessing the cerebro-spinal fluid (CSF), completely crossing cell membranes where they bind to receptors present in the cytosol. The resulting complex then translocates to the nucleus producing conformational changes leading to alterations in the transcription rate of the appropriate genes and protein synthesis (McEwen & Weiss, 1970). It is these products that are responsible for the resulting physiological effects. It is due to this mechanism of action that the responses to steroids often take hours or days rather than seconds or minutes. There are, however, some effects that can be attributed to glucocorticoids that are non-genomic in origin. The evidence for non-genomic actions of oestrogens, androgens and corticosteroids are reviewed by Moore and Evans, 1999. Acute glucocorticoid administration in the hippocampus has been shown to increase levels of excitatory amino acids *in vivo* (Venero & Borrell, 1999).

The physiological response to stress is not as simple as would be initially thought– it can be subject to a variety of other influencing factors. For instance, it has been shown to be subject to modification according to early experiences. In rats, Meaney *et al.*, 1988, showed that early handling of rats modified the response of the adult animals to acute stress episodes. This shows that glucocorticoids can have developmental effects upon the CNS and that early experiences can alter the way that stressful events are later dealt with in adulthood (Rosenfeld *et al.*, 1993).

Receptors for glucocorticoids can be found distributed throughout the body and scattered throughout the CNS. There is a particularly high concentration of

receptors to be found within the hippocampal structures (McEwen *et al.*, 1986). Two classes of corticosteroid receptor are known: type 1 and type 2. Type 1 receptors are also known as mineralocorticoid receptors (MR). At basal levels of glucocorticoids, they are thought to be the receptor that is principally occupied and primarily involved in the regulation of basal activity of the HPA axis. Type 2 receptors are also known as glucocorticoid receptor (GR) and are thought to require higher concentrations of corticosterone to be occupied due to a lower affinity, as such they are the receptor type deemed to be principally involved in the stress response (Oitzl & de Kloet, 1992). Experimental work using selective antagonists of both the GRs and MRs, revealed that the hippocampus can have a significant role in the control of the diurnal cycle of plasma corticosterone levels. MRs were found to be responsible for the feedback inhibition on the HPA axis at the peak of the diurnal cycle, while GRs were responsible for disinhibition of the axis (Van Haarst *et al*, 1997).

Glucocorticoid GR and MR receptor density has been found to alter under various conditions, which would lead to an altered response of the various systems to stressful stimuli. This has particular relevance when associated with development and ageing. Following adrenalectomy in rats, an increase in receptor number was observed. In aged rats, by contrast, there is a reduced number of both MR and GR receptors to be found (Morano *et al*, 1994). There is potential for a positive feedback loop, in which increased corticosterone levels cause an associated decrease in receptors.

The effects of chronic exposure to stress on cell numbers have also been looked at by several laboratories. Hippocampal cell loss is associated with ageing, and raised plasma corticosterone levels have been observed in both aged rats and primates (Sapolsky *et al.* 1986). Bodnoff *et al.*, 1995, found that chronic exposure of animals to both stress and corticosterone, produced no effect on cell counts taken from the hippocampus in mid aged and young animals. Kerr *et al.*, 1991, also found that in young rats, chronic stress failed to cause any degradation in hippocampal cell

number. Older animals, however, did show a decline in cell number. There may be implications for such conditions as Alzheimer's Disease. Interestingly, early handling of animals has been shown to reduce the loss of pyramidal cells due to ageing (Meaney *et al.*, 1988).

1.5 Stress, synaptic plasticity and learning.

1.5.1 General introduction.

The relationship between the glucocorticoids and learning is one that is still under intense investigation. It has been found that there could be an inverted-U-like relationship between plasma corticosterone levels and primed burst potentiation (Diamond *et al*, 1992) and both LTP (Bennet *et al*, 1991) and learning. There is also some evidence that there are differing roles for the two receptor types for corticosterone. It has been noted that both MR and GR antagonists have effects in rats, concluding that GRs were involved in memory consolidation while MRs in situation evaluation and the selection of the response (Oitzl & de Kloet, 1992). The duration of GR blockade that is required for facilitation of learning in rats is reported to be variable. Oitzl *et al*, 1998, reported that acute blockade was sufficient for facilitation and also that continuous blockade was required

It has been quite widely documented that excessively high levels of acute stress have a detrimental effect on learning (Diamond & Rose, 1994), synaptic plasticity, and also LTP formation (Bennet *et al.*, 1991, Foy *et al.*, 1987, Kerr *et al.*, 1991). Previous work by a variety of sources have shown that stress was capable of altering conditions within the hippocampus such that LTP induction was blocked to the standard stimulation paradigm (Foy *et al.*, 1987, Bodnoff *et al.*, 1995, Smriga *et al.*, 1996). GR activation has been shown to facilitate the induction of LTD in the hippocampus by Coussens *et al*, 1997 as well. However, stress exposure of 90 minutes tail shock was sufficient to facilitate classical conditioning (Shors *et al*, 1992).

Exposure to a stressful environment has been shown to cause learning deficits in hippocampal dependent learning tasks such as spatial learning using the Morris water maze. (Diamond *et al*, 1996; Diamond *et al*, 1999). As well as acute stress

exposure producing learning deficits, chronic stress has been shown to cause similar impairment (Nishimura *et al*, 1999). These effects of stress have been shown to last over periods of time from one hour to 3 weeks following stress exposure still producing impairment in performance of rats in spatial learning tasks (Richter-Levin, 1998). In contrast, it was reported by Luine *et al*, 1996, that the use of restraint stress produced a reversible impairment of performance in the water maze so that several days following stress exposure performance of rats returned to that seen in non stressed animals. Connections between stress and impairment of learning and LTP have been shown with impaired performances in spatial learning and also impaired LTP in the DG area of the hippocampus, indicating the involvement of hippocampal mediated processes (Wang, *et al*, 2000).

As mentioned previously, early handling can have effects on the way a stressful response is dealt with in adulthood. Wilson *et al.*, 1986, found that LTP in the hippocampus of young animals could be potentiated by early handling. The reduction in the amount of glucocorticoids produced in response to a stress situation enabled facilitation of LTP.

1.5.2 GABA, 5-HT and stress.

The relationship between GABA and stress has been assessed in a variety of different ways. Not entirely unexpectedly, the relationship appears, at the moment, to be subtle and complex. Receptors for GABA have been identified in both the pituitary gland and the hypothalamus. As GABA tends to have a primarily inhibitory action, it would seem reasonable to assume that GABA would therefore play a role in the inhibition of the HPA axis (De Souza & Appel, 1991). Exposure to ether stress has been shown to activate the hypothalamic GABA system which is then thought to inhibit further responses of the HPA axis to stress (Maney *et al*, 1983). Hypothalamic concentrations of GABA were reported to rise by 69% (Acosta & Rubio, 1994) in response to ether stress but to decrease in response to cold stress and this effect was mediated via $GABA_A$ receptors. However, GABA concentrations and turnover has been shown by some groups not to alter under stressed conditions (Maney *et al*, 1983; Yoneda, *et al*, 1983). Responses of GABA levels appears to be dependent upon the type of stress used and the area of the brain assessed. Additionally, GAD-65 is thought to be responsible for the processing of signals from the environment. Mice deficient in this enzyme have been shown to exhibit spontaneous seizures when placed under mild stress (Kash *et al.*, 1997).

Within the CNS itself there are changes in neurotransmitter levels that are produced in response to stress exposure. Noradrenaline and 5-HT levels have been monitored in animals while they were exposed to a stressful situation and both were noted to rise considerably during these episodes. This has been reported to occur in several brain regions including the hippocampus (Abercrombie *et al*, 1988)

There have also been clearly established links between stress, 5-HT, particularly the $5\text{-}HT_{1A}$ receptor subtype, and corticosterone. The degree to which receptors in differing brain regions are affected appears to be dependent upon the type of stress involved and whether the exposure is acute or chronic. The $5\text{-}HT_{1A}$ receptor seems

to be particularly vulnerable to changes in HPA influence, with increases in receptor density being reported in animals that have undergone adrenalectomies within the hippocampal region. Facilitation of 5-HT_{1A} receptor function through use of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, has been shown to reduce the effects of stress and be involved in the adaptive responses of an animal to stressful stimuli (Graeff *et al*, 1996, MacBlane and Handley, 1994).

Reduced 5-HT levels within the CNS have been traditionally implicated in the aetiology of depression. Disorders of the 5-HT system have more recently been associated as an influence on a range of psychiatric and behavioural disorders such as alcohol dependence, affective disorders and dementias (reviewed by Lesch, 1998). Treatments for depression have focussed mainly on altering 5-HT levels with both older and newer generation drugs. These have worked either by increasing levels via inhibition of the enzyme mono amine oxidase (MAO), that is responsible for the metabolism of 5-HT, or by blocking uptake into the terminals such as the tri-cyclic group and the more selective serotonin selective re-uptake inhibitors (SSRI). All of these treatments have been found to require a period of around 14 days for a clinical effect to be seen. This has been attributed to the acute effects of decrease in the firing rate of serotonergic neurones due to increased activation of somatodendritic 5-HT_{1A} receptors.

There are a variety of treatments for depression that currently prescribed clinically. Amongst them are tianeptine and fluoxetine. Tianeptine is part of the tri-cyclic anti-depressant family. It is novel in several ways. First, though it is related to the tri-cyclic group of drugs, it differs structurally as a result of chemical modifications. It has actions that include facilitation of 5-HT re-uptake in the rat brain (Fattaccini *et al.*, 1990; Kamoun *et al.*, 1989; Broqua *et al.*, 1992; Kato & Weitsch, 1988). In terms of its antidepressant action, this makes tianeptine unusual. Most commonly prescribed modern anti-depressants work by inhibiting the re-uptake of 5-HT in the CNS.

Tianeptine is presently of great interest within research circles for its effects upon memory formation and as an anti-stress agent. Tianeptine has been reported by Delbende *et al.*, 1994, to lower the amount of corticosterone produced by exposure to tube restraint stress when administered chronically in rats. They concluded that the primary site of action of tianeptine was the HPA axis and under stress conditions could be responsible for reducing the activation of the axis. It has also been reported to facilitate both reference and working memory (Kamoun *et al.*, 1989).

Fluoxetine is more well known as Prozac. It differs from tianeptine in its primary mode of action, leading to increase in the overall levels of 5-HT by inhibiting re-uptake from the synaptic cleft (Wong *et al.*, 1995). It is considered highly selective for the 5-HT system producing no changes in the levels of other neurotransmitters such as noradrenaline (Fuller & Wong, 1977) and dopamine (Wong & Bymaster, 1976). It is desirable as a clinical drug as it is orally effective and has a long duration of action; it has been shown to be able to reduce the uptake of 5-HT for up to 24 hours after administration (Wong *et al.*, 1975).

Interestingly, some other drugs such as fenfluramine that affect 5-HT levels, by contrast, are not reported to show any signs of antidepressant activity (Lichtemberg *et al*, 1992), even though, like fluoxetine, it is a 5-HT re-uptake inhibitor and also acts as a 5-HT releasing agent. It has been found to be difficult to distinguish between agents that cause increases in extracellular 5-HT acting as re-uptake inhibitors and those that act by releasing 5-HT from nerve terminals. At low doses fenfluramine is primarily thought to raise levels by acting through uptake inhibition as the drug is a substrate for the transport system (Berger *et al*, 1992). At higher doses, higher concentrations are found in 5-HT terminals and the mechanism is then considered to be due to release of 5-HT from serotonergic terminals (Berger *et al*, 1992, Mennini *et al*, 1981). Another agent that is reported to raise 5-HT levels but have only been shown to express efficacy in animal models is 5-methoxy-6-methyl-2-aminoindan (MMAI) (Scorza *et al*, .1999). D-Fenfluramine has been

found to be anxiogenic in human subjects (Graeff ***et al,*** 1996). The property of fenfluramine to release 5-HT from serotonergic terminals seems to affect terminals arising from the DRN (Viana *et al*, 1996).

1.6 Aims

The first study here investigated the effect of acute stress exposure upon the ability of hippocampal CA1 neurones to express LTP in rats. It has been shown previously that exposure to stress can block the induction of LTP (Foy *et al.*, 1987; Bodnoff *et al.*, 1995). Field excitatory postsynaptic potentials (EPSPs) were recorded from the CA1 area of the hippocampus. The ability of high frequency stimulation (HFS) to induce LTP was assessed in anaesthetised rats that either were not exposed to a stress-inducing situation or those that had been exposed to a stress protocol of 30 minutes upon a raised platform.

Subsequently, the studies investigated the effects of pharmacological intervention in order to ascertain some of the factors involved in the effects of stress on synaptic plasticity.

- Investigation of glutamatergic transmission by excitatory NMDA receptor blockade or AMPA receptor facilitation in anaesthetised animals following exposure to stress and the effect that this intervention had on LTP inducibility in response to HFS.
- The role of GABA-ergic inhibition by using antagonists of the $GABA_A$ receptor on the stress–induced block of LTP both before and after stress exposure was also investigated.
- Changes in extracellular hippocampal levels of 5-HT and noradrenaline (NA) have been reported to occur following exposure to stress (Abercrombie *et al*, 1988). Therefore the effect of agents that increased (fluoxetine and fenfluramine) and decreased (tianeptine) levels of extracellular 5-HT in both stressed and non-stressed animals.
- Increases in NA levels following stress exposure were investigated for their effects on the inducibility of LTP in response to HFS.

II. Methods

2.0 Methods.

2.1 Animals.

Adult male Wistar rats (in-bred strain, Bio Resources Unit, Trinity College Dublin) of weight 280-350g were used in all experiments. This weight range corresponds to an age of 3-4 months. Animals were group-housed 6 or less to a cage under a 12-hour light/dark cycle, and allowed unlimited access to both food and water. Atmospheric temperature was maintained between 19-23^{0}C. Animals were transported from their home cage to the laboratory at a similar time each day (0900-0930) and handled in a similar manner to limit possible changes in animal response to being transferred. All animals were weighed prior to transport to determine the dose of anaesthetic required for each animal.

2.1.1 Anaesthesia.

Animals were subjected to anaesthesia prior to all surgical procedures. Anaesthesia was induced with urethane (2.1g/kg) Urethane induced anaesthesia was found to induce a reliable and stable form of anaesthesia which was maintained over several hours without the need for supplementation. At the end of each experiment the animal was killed with either a lethal dose of sodium pentobarbital (Euthatal 800mg/kg) or exsanguination and decapitation.

2.2 Surgery.

Once each animal had been sufficiently anaesthetised, the hair covering the top of the scalp was removed using a standard pair of scissors. Lignocaine (0.5 ml, 1% adrenaline) was injected sub-cutaneously over the area of skull where electrodes were to be implanted (between the ears and down as far as the nose) in order to minimise bleeding upon scalpel incision. A scalpel incision was made from between the ears and to between the eyes. The skin and covering membranes were removed exposing the top of the skull, which was then dried. The co-ordinates for both recording and stimulating electrodes, reference and earth screw were marked out using a waterproof marker.

A screw acting as a reference electrode was placed 8.0 mm anterior of the bregma and 0.5 mm left of the centre line. An earth screw which acted as a ground electrode was placed 7.0 mm posterior to the bregma and 5.0 mm right of the centre line. The recording electrode was placed 3.4 mm posterior to the bregma and 2.5 mm left of the centre line. Finally, the stimulating electrode was placed - 4.2 mm posterior to the bregma and 3.8 mm left of the centre line.

A dental drill RS-541 242 and dental drill bits sizes 2 (1mm) and 3 (1.5mm) for the electrodes and screws respectively were used to penetrate the skull. The holes were drilled carefully to ensure that no penetration of the dura mater occurred.

2.3 Electrodes.

The monopolar recording electrode and the bipolar stimulating electrode were prepared for each experiment and only used once. Teflon coated tungsten wire (750μM external diameter and 625μM internal tungsten diameter, Advent Research Materials Ltd.) was used with 2 lengths of approximately 1 inch soldered to dual pin socket connector. Prior to being soldered, the outer Teflon insulation on the outside of the wire was first removed to enable a good electrical contact. The 2 lengths of wire were then twisted around one another to form a single length and then sealed together with cyanoacrylate. The soldered connections were then covered by dental acrylic cement. Each electrode was trimmed at a slight angle to a length of 1-1.5cm and straightened as much as possible prior to use.

Reference and ground electrodes consisted of stainless steel screws (Bilaney, Germany) with soldered single pins attached.

Once the holes had been drilled, the screws were screwed into the respective places. The dura mater was pierced with a syringe needle to allow insertion of the electrodes. Once in place the electrodes were fixed in place a small amount of cyanoacrylate and then dental cement.

2.4 Stress protocol.

In order to produce a state of anxiety prior to surgery and implantation, animals were placed on a raised platform of dimensions 30 x 27 cm and 130 cm above the ground. Exposure to a novel environment, such as a raised platform, in mice and rats has been shown to be sufficient to raise corticosterone levels (Hennessy & Levine, 1978). This protocol was chosen as it had been reliably used for previous work in the department and shown to produce changes in synaptic plasticity. The platform was positioned so that movement by the animal caused the platform to move slightly. All rats were exposed to this environment for 30 minutes followed by immediate anaesthesia with urethane. All animals exposed showed physical symptoms of stress including immobility, defaecation and urination. These physical symptoms were monitored for each animal to assess uniformity of response (see table 2.1).

To control for diurnal changes that may occur in basal levels of hormones and neurotransmitters all animals were exposed to the raised platform at the same time of the day, shortly after 9.00 a.m. and before 9.45 a.m. Animals that were not subjected to the platform were anaesthetised at around the same time

Table 2.1

	Number of faecal deposits	Incidence of urination	Exhibition of piloerection	Time spent immobile (min)
Rat 1	9	1	+	24
Rat 2	10	3	+	18
Rat 3	10	3	+	25
Rat 4	7	2	+	26
Rat 5	6	2	+	26
Average	8.7	2	+	23

Table 2.1

Individual animal responses to stress exposure upon a raised platform show a high degree of consistency. Each of the examples shown was chosen randomly from the data collected and the average responses for animals stressed in one calendar month (n=17). All values were taken for the time that the animal was on the platform only. A positive (+) response for piloerection was taken if observed for greater than 15 minutes of duration. The time that an animal was immobile was always counted to the nearest whole minute.

2.5 Electrophysiological procedures

2.5.1 Excitatory Post Synaptic Potential (EPSP) recording.

For recording of EPSPs in the brain, the rat head was immobilised so that the top of the skull was completely horizontal in stereotaxic equipment prior to electrode insertion. EPSPs were recorded ipsilaterally from the CA1 region of the dorsal hippocampus. Electrode (both stimulating and recording) depth was 2.0-2.5 mm, optimum depth for each experiment being determined electrophysiologically according to criteria determined by Leung (1980). The electrodes were lowered slowly into the brain and the evoked responses monitored constantly to stimulation with a pulse of 0.1ms duration, 2ms delay, at 6.2V at a frequency of 0.1 Hz. Both the monopolar recording and bipolar stimulating electrode were lowered initially to 1.5 mm before being lowered in 10μm degrees. The recording electrode was positioned first. Correct positioning within CA1 was also verified by the presence of paired pulse facilitation. Electrode positions were verified after death using a Vibroslice or manual dissection.

EPSPs were recorded using a MacLab system by Scope versions 3.28 and 3.5. The stimulation protocols used were controlled by MacLab and Scope programmes. The settings for the standard recording of the EPSP over the length of an experiment were as follows: a stimulation frequency of 0.033 Hz with an inter-stimulation delay of 5ms. Output current is determined by an input-output curve and the taken as 50-60% of maximum for test stimulation. In order to prevent electrical interference from both the immediate environment and 50 cycle noise, the recording area was surrounded by a metal Faraday cage and each piece of equipment was grounded.

It was observed that in a comparison of relative field EPSPs that were located in both stressed and non-stressed animals, that there was no appreciable difference in the quality and size of EPSPs located indicating that stress produced no appreciable alterations in baseline synaptic transmission.

2.5.2 Input-output curves.

An input-output curve was determined prior to the start of every experiment and used to establish the test stimulation current. Unless otherwise stated the test and conditioning stimuli were set to evoke responses that were 50-60% of the maximum EPSP amplitude. It was found that there was no effect of the stress protocol on a typical input/output curve compared to those taken from the average non-stressed animals. See Fig. 2.1

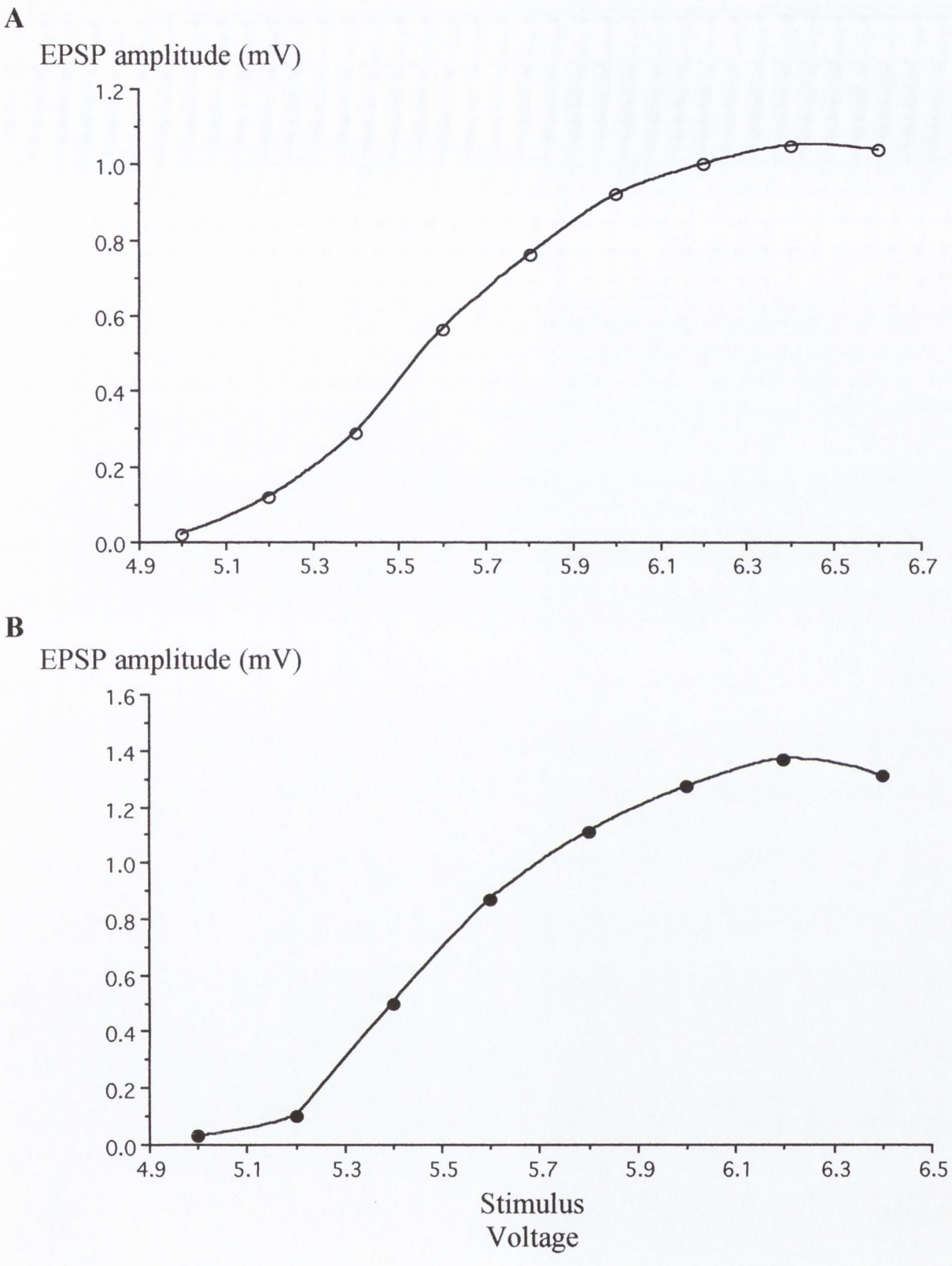

Fig. 2.1 Exposure of animals to acute stress prior to obtaining an input/ouput curve does not significantly alter the typical responses obtained. A and B show typical responses to increasing stimulus intensity in a stressed animal (A, open circles) and a non-stressed animal (B, filled circles). Each point is the average of two responses at that output level.

2.6 Stimulation and Experimental Protocols.

2.6.1 High frequency stimulation protocol.

The following high frequency stimulation protocol was used as standard in experiments at an intensity that was used as the conditioning stimulus (50% of the intensity that produced maximum EPSP response):

200 pulses of 0.1ms duration with a 5ms interval between pulses with a two second delay between each one of 10 trains of 20 pulses.

Observed potentiation in the EPSP amplitude was termed as LTP when greater in value than 15% of baseline and maintained beyond 60 minutes following the tetanus. The average value of 30 minutes prior to tetanus was used as the baseline value. In trials where stress was employed, the baseline value was considered to be the 30 minutes immediately prior to tetanus.

2.6.2 Low frequency stimulation protocol.

Two low frequency stimulation protocols were employed, each at conditioning stimulus intensity. The 10 Hz protocol used a 0.1 second inter-stimulus interval, while the 3Hz protocol used an interval of 0.33 seconds both for 900 pulses lasting 90 seconds and 5 minutes respectively.

2.6.3 Stress protocol

In experiments where animals were stressed according to the procedure previously outlined, a baseline was recorded for a standard 30 minutes. A single burst of HFS was then applied and the degree of LTP assessed. The stress procedure was deemed to have been successful when EPSP changes were less than ±15% from baseline values pre-HFS.

Recordings were then continued for at least 60 minutes before any pharmacological intervention. The specific timings of drug administration varied according to the characteristics of the particular drug in question and will be mentioned later.

2.7 Temperature of animals

Body temperature of animals was monitored throughout the recording period of each experiment. This was assessed by measuring directly rectal temperature with an anal thermometer (Ama-Digit, range –40-120°C). Body temperature was maintained between 36-38°C with the aid of an electrically heated blanket placed underneath the animal and a small cloth blanket covering over the animals back. There was no observable difference in the field EPSP amplitudes within that body temperature range over the time course of an experiment, remaining stable throughout except for drug or stimulation intervention, although a comparison with non-anaesthetised implanted animals was not carried out.

2.8 Analysis of data.

2.8.1 EPSP measurement.

Quantification of the recorded EPSP was taken as the difference from the baseline immediately prior to stimulation and the peak amplitude in mV. In no experiment was there any sign of EPSP amplitude contamination by a population spike.

Standard baseline values were measured as the EPSP values taken 30 minutes prior to any experimental protocol. All EPSP values were expressed as a percentage of the mean EPSP baseline value to eliminate inter-experimental EPSP variation. All EPSP values were averaged in ten-minute epochs from the start of the baseline recordings.

2.8.2 Statistical analysis.

Data were pooled between animals and averaged over consecutive 10-minute periods. Standard error of the mean was calculated from these values. For intra-experimental statistical analysis, a paired t-test was used for assessment of significance between specific time points from the experiments. The baseline value was taken from the first 10 minutes and used as a control for any treatment such as drug injection or high/low frequency stimulation. If there was no further treatment to be applied then analysis was taken at 60 and 120 minutes post treatment. For analysis of any further time points following additional treatments, the 10 minute time point prior to treatment was taken as the baseline for comparison. Analysis was then carried out as before on values taken from the initial 10 minutes following treatment and also at 60, and 120 minutes. Inter-experimental analysis was done using unpaired t-test across experiments at the equivalent time points that were mentioned for the intra-experimental analysis. In both cases, statistical significance was taken with confidence limits greater than 95%.

2.9 Drug preparations.

2.9.1 Bicuculline

Bicuculline (Sigma) was prepared by dissolving in a small volume of 0.1M HCl and then made up with saline. The final concentration of HCl was approximately 3%. The solution was administered i.p. at 10ml/kg (1mg/kg). For the vehicle control a solution of saline and 0.1M HCl was used. Bicuculline concentrations of 0.25 and 0.5 mg/kg were also assessed (Ammasari-Teule *et al.*, 1991).

2.9.2 Picrotoxin

Picrotoxin (Sigma) was prepared in a solution of ethanol and saline (50:50). It was found that in lower ethanol concentrations the picrotoxin remained undissolved. Vehicle controls consisted of a solution of 50% ethanol and saline. Picrotoxin was also administered i.p. at a dose of 2.5 mg/kg and 1mg/kg (2.5mg/ml).

2.9.4 Tianeptine

(±)Tianeptine (kindly provided by Servier) was dissolved in distilled water at a concentration of 10mg/ml and administered i.p. at a concentration of 5mg/kg. When lower concentrations of drug for administration were used, the stock solution was subsequently lower but in the same ratio i.e. when 0.5mg/kg was given, the stock solution was 1mg/ml. It was stored at room temperature.

The individual stereo-isomers (+) and (-) forms of tianeptine, S-16190-1 and S-16191-1, were prepared and stored in an identical manner to the racemic compound. These were prepared at a concentration of 1mg/ml and administered at 0.5mg/kg i.p.

2.9.5 Fluoxetine

Fluoxetine hydrochloride (Sigma) ((±)-N-methyl-γ-[4-(trifluoromethyl)phenoxy]-benzenepropanamine)hydrochloride) was prepared in distilled water at a concentration of 5mg/kg and administered at 10mg/kg i.p. Solutions were freshly prepared each day before use and the compound was stored at room temperature and away from light.

2.9.6 CPP.

CPP ((±)-3-(2-carboxypiperazin-4-yl) propanephosphonic acid) (Sigma) was dissolved in distilled water and prepared freshly each day for administration. The solution was vortex mixed for 30 seconds to ensure thorough mixing.

2.9.7 Desipramine

Desipramine (Sigma) was dissolved in distilled water at a concentration of 5mg/ml and administered at a dose of 10 mg/kg.

2.9.8 CX546

CX546 (1-(1,4-benzidioxan-6-yl carbonyl) piperidine) (Sigma) was prepared in a solution of 30% 2-hydroxyproplyl-β-cyclodextrin (Sigma) at a concentration of 20mg/ml and administered i.p at either 20 or 30mg/kg. Each day CX546 was prepared freshly and the drug stored refrigerated in the dark. Cyclodextrin was prepared as a solution in distilled water.

2.9.9 Fenfluramine

(±)Fenfluramine HCl was stored at room temperature and dissolved in distilled water prepared daily. It was administered i.p at a concentration of 10mg/kg.

2.9.10 Nisoxetine

Nisoxetine (Sigma) was dissolved in water to a concentration of 10mg/ml and administered at a dose of 10mg/kg, i.p. Once dissolved, nisoxetine was stored at room temperature.

III. Results.

3.0 Results.

3.1 Stress.

3.1.1 HFS in non-stressed control animals.

This particular set of experiments was carried out in order to ensure that under standard animal handling and transportation conditions, the induction of LTP by HFS was consistent in urethane anaesthetised rats. The experiment was occasionally repeated at various times throughout the thesis, although not all are included, to ensure that the induction of LTP remained consistent. The animals were not exposed to any stress-inducing protocol or drug intervention but were prepared as outlined in the Methods section.

Baseline EPSP recordings were established (103.7±2.3% in the 10 minutes prior to HFS, n=8) before exposure to a single high frequency tetanus. There was an rapid increase in EPSP amplitude from all animals (155.5±15.0% immediately following tetanus $P<0.01$, paired t-test). This increase in the EPSP remained relatively stable for the period of recording (142.8±8.0% and 135.5±7.0% at 60 and 120 minutes post tetanus respectively, both $P<0.01$ paired t-test compared to baseline). See Fig. 3.1.

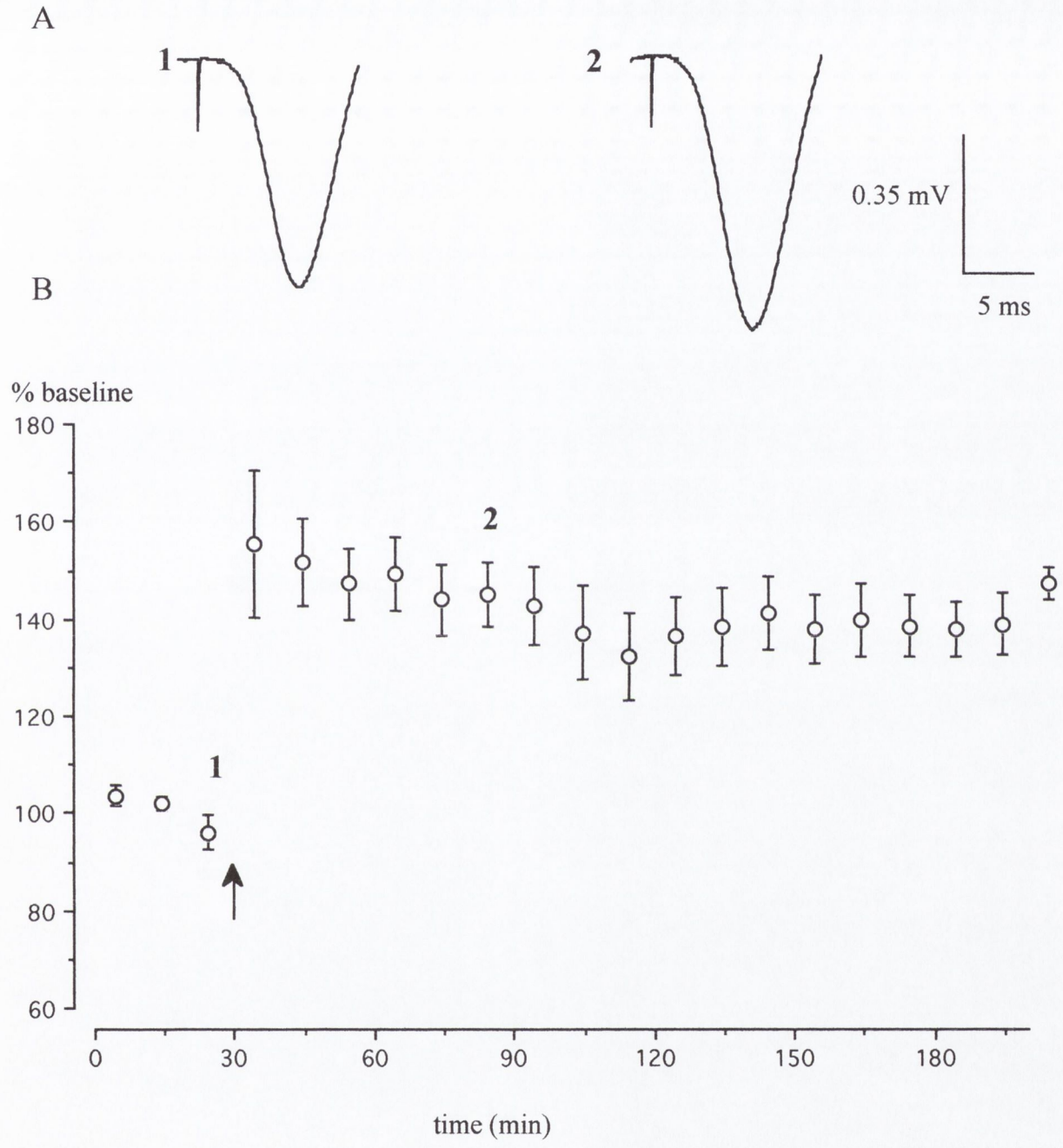

Fig. 3.1. The effect of a single tetanus of high frequency stimulation (HFS) on synaptic transmission. A) Typical traces of EPSP prior to HFS and 60 minutes after HFS. Each trace is an average of 20 sweeps representing 10 minutes recording. B) Graphical representation of the effect of HFS (arrow) on EPSPs. Results are mean±sem, n=8.

3.1.2 The effects of stress on HFS-induced LTP.

This set of experiments was designed to assess the effectiveness of stress on the ability of high frequency stimulation to induce LTP in the hippocampus. A baseline EPSP amplitude of 100.9±1.9% (n=6) was established for 30 minutes before an initial tetanus of HFS. This produced a non-significant ($P>0.05$) increase in the EPSP amplitude to 114.2±5.0. Two hours after the tetanus the EPSP amplitude was 88.8±2.4%. A second tetanus elevated values from pre-tetanus levels 102.3±5.3%. After a further hour of recording, the EPSP amplitude was 88.4±5.4% (n=6). See Fig. 3.2.

This stimulation protocol was successful in showing two things. Firstly, LTP induction was successfully blocked by the raised platform stress protocol. Secondly, the block of LTP induction lasted at least 5 hours after the stress was initially applied. As a result, the protocol was then adopted for further experiments where prior to the second burst of HFS a pharmacological intervention could be applied.

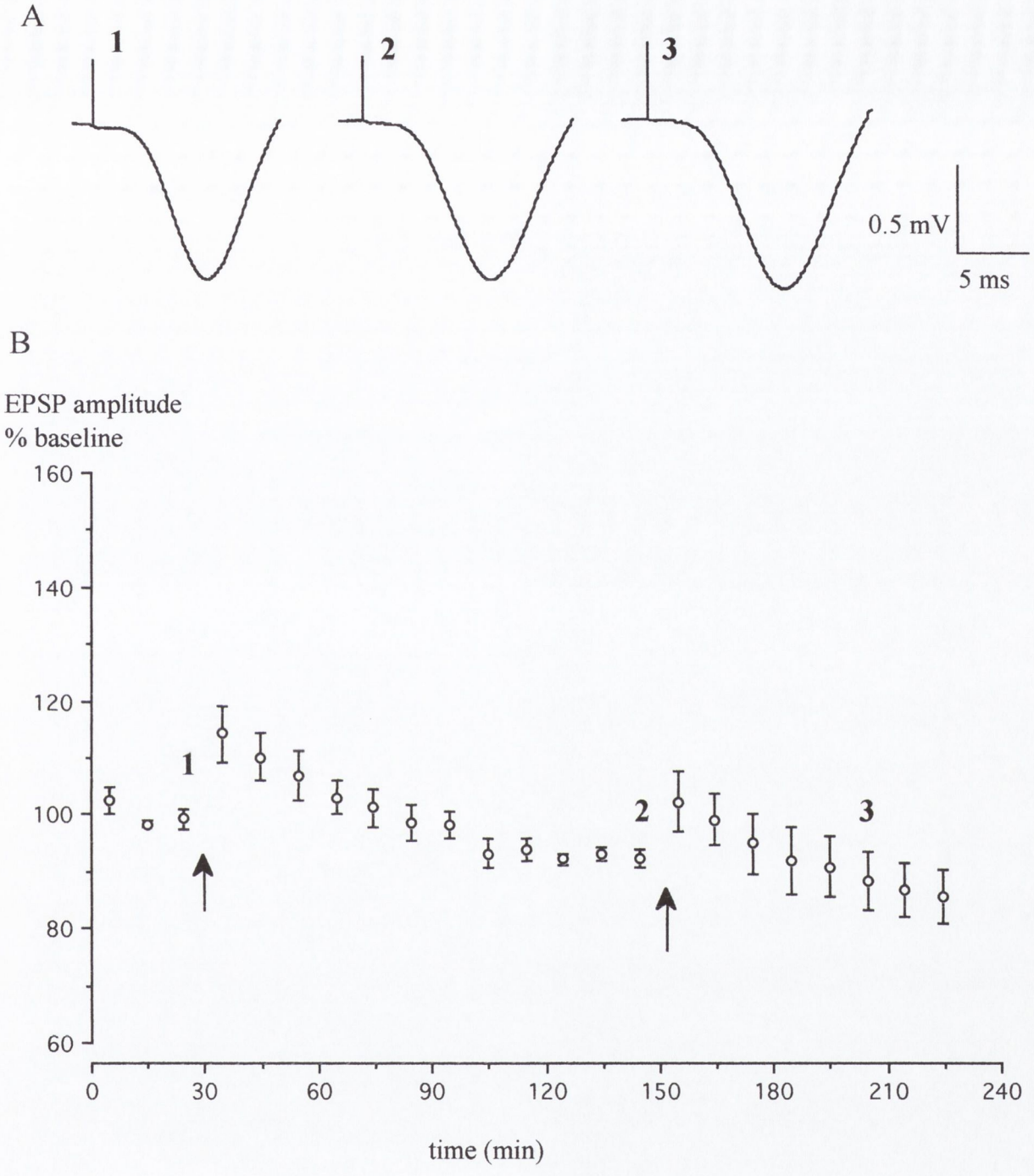

Fig. 3.2. Exposure to acute stress blocks the induction of LTP in response to HFS in the anaesthetised rat. A) typical traces of field EPSPs at baseline (1), prior to second HFS (2) and 60 minutes following second tetanus (3). B) Shows the EPSP response to HFS (filled arrows) as a percentage of baseline, n=6. Results are mean±sem.

3.2 Intervention of GABA-ergic antagonists.

3.2.1 The effect of picrotoxin on stress-induced block of LTP induction.

Following the protocol outlined in Section 3.1.2, a baseline of 99.0±1.7% (n=5) was obtained. The stress was sufficient to block the induction of LTP (105.1± 10.5% 60 minutes following HFS). Picrotoxin (PTX) was administered i.p at 2.5mg/kg 100 minutes after the initial tetanus. PTX showed no effects on baseline transmission at this dose (101.0±5.6% immediately prior to second tetanus) which was applied 20 minutes later. The EPSP amplitude transiently increased to 114.0±25.3% immediately after HFS. There was a large degree of variation in response to the second tetanus across individual experiments with one particular animal showing an STP in excess of 300%. Approximately 20 minutes after the second tetanus, attenuation of the EPSP amplitude occurred (63.6±12.0% at 20 minutes) and was maintained at this level for the remainder of the recording period (69.5±11.6% and 62.5±19.0% at 60 and 120 minutes post tetanus respectively). ($P<0.05$, paired t-test). See Fig.3.3

Vehicle experiments for PTX involved injection of an equivalent volume of vehicle (100% EtOH). Baseline values were established for 30 minutes. Immediately prior to the first HFS, the EPSP baseline was 97.8±2.1% (n=5). The tetanus produced no enhancement in the EPSP of these stressed animals (103.6±2.7% 10 minutes post-tetanus). The vehicle injection produced no effect (106.9±5.9% immediately prior to tetanus) and there was no change in EPSP amplitude in response to the second tetanus (107.0±6.9% in the 10 minute period immediately following tetanus). Sixty minutes post-tetanus the EPSP was stable (96.4±5.4%), and remained stable for the duration of the recording period (93.8±4.1% 120 minutes post-tetanus).

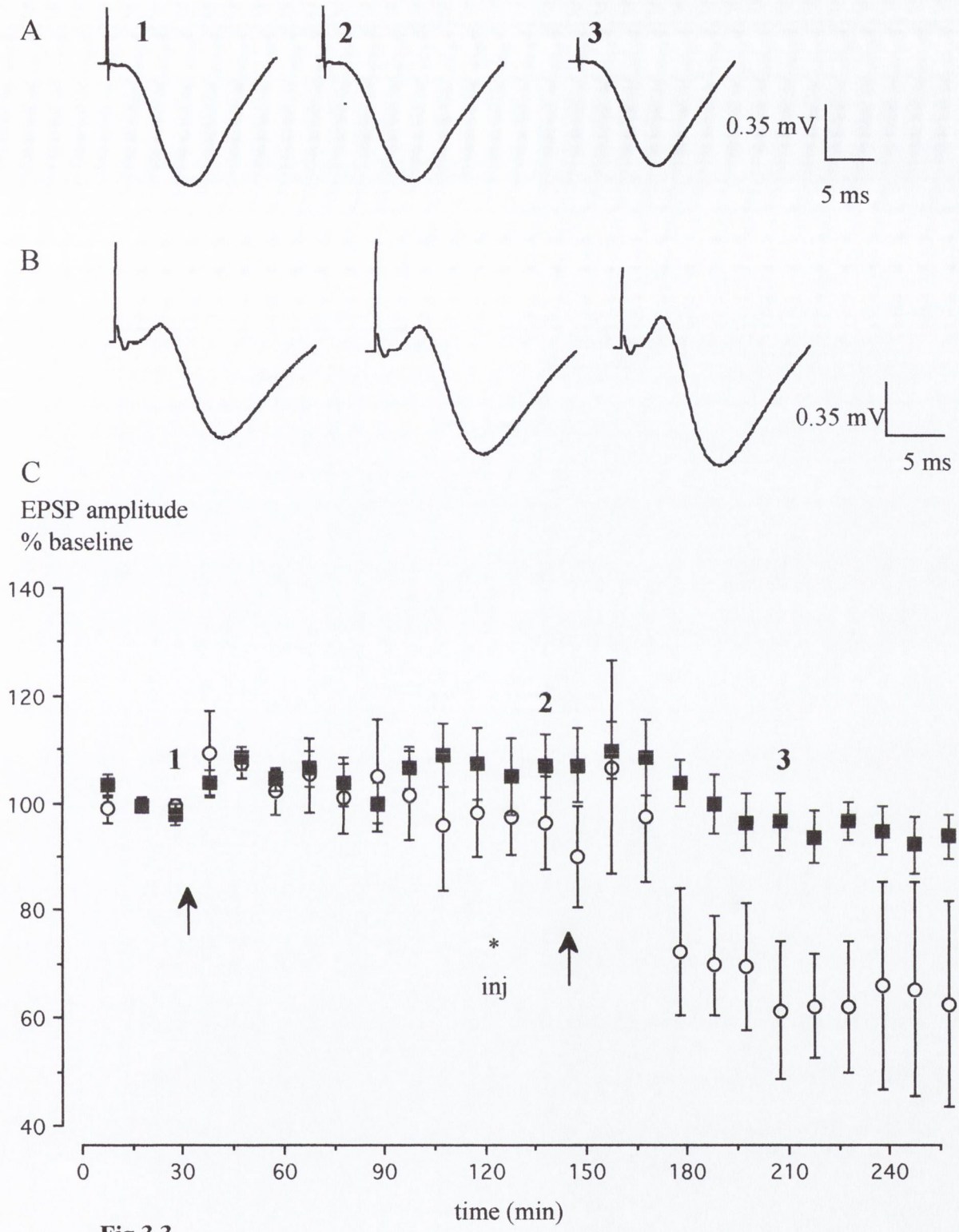

Fig 3.3

The effect of picrotoxin on the EPSP response to HFS in stressed animals. A and B) Typical traces of field EPSPs for baseline EPSP 10 minutes prior to first tetanus (1), the 10 minute period before PTX (A) or vehicle injection (B) (2), and 60 minutes following the second HFS (3). C) Graphical representation of the effect of either PTX (2.5mg/kg, i.p, open circles) or vehicle (filled squares) on the response to HFS (arrows). Results are mean±sem, n=5 for both % of baseline.

3.2.2 The effect of PTX on non-stressed control rats.

PTX was administered as before 20 minutes prior to HFS. PTX produced no significant effect on the baseline EPSP value (101.8±2.2% in the 10 minute period following the first tetanus and 99.6±1.9% immediately prior to the second tetanus, n=5). Immediately following the tetanus, it was shown that there was no change of the EPSP amplitude (108.8±4.5%). At 60 minutes post-tetanus (105.9±3.5%) and 120 minutes (93.4±14.2%) there was no significant change. See Fig. 3.4

As in section 3.2.1 vehicle control experiments were carried out in non-stressed animals. Baseline was 99.3±4.1% (n=5) in the 10 minutes immediately prior to vehicle injection. Vehicle was administered i.p. 20 minutes prior to the second tetanus consistent with PTX experiments. HFS produced an enlargement in the EPSP amplitude (139.5±9.0% immediately following tetanus and 123.3±12.0% 60 minutes post tetanus, $P<0.01$, paired t-test compared to pre-tetanus baseline for both). See Table 3.1

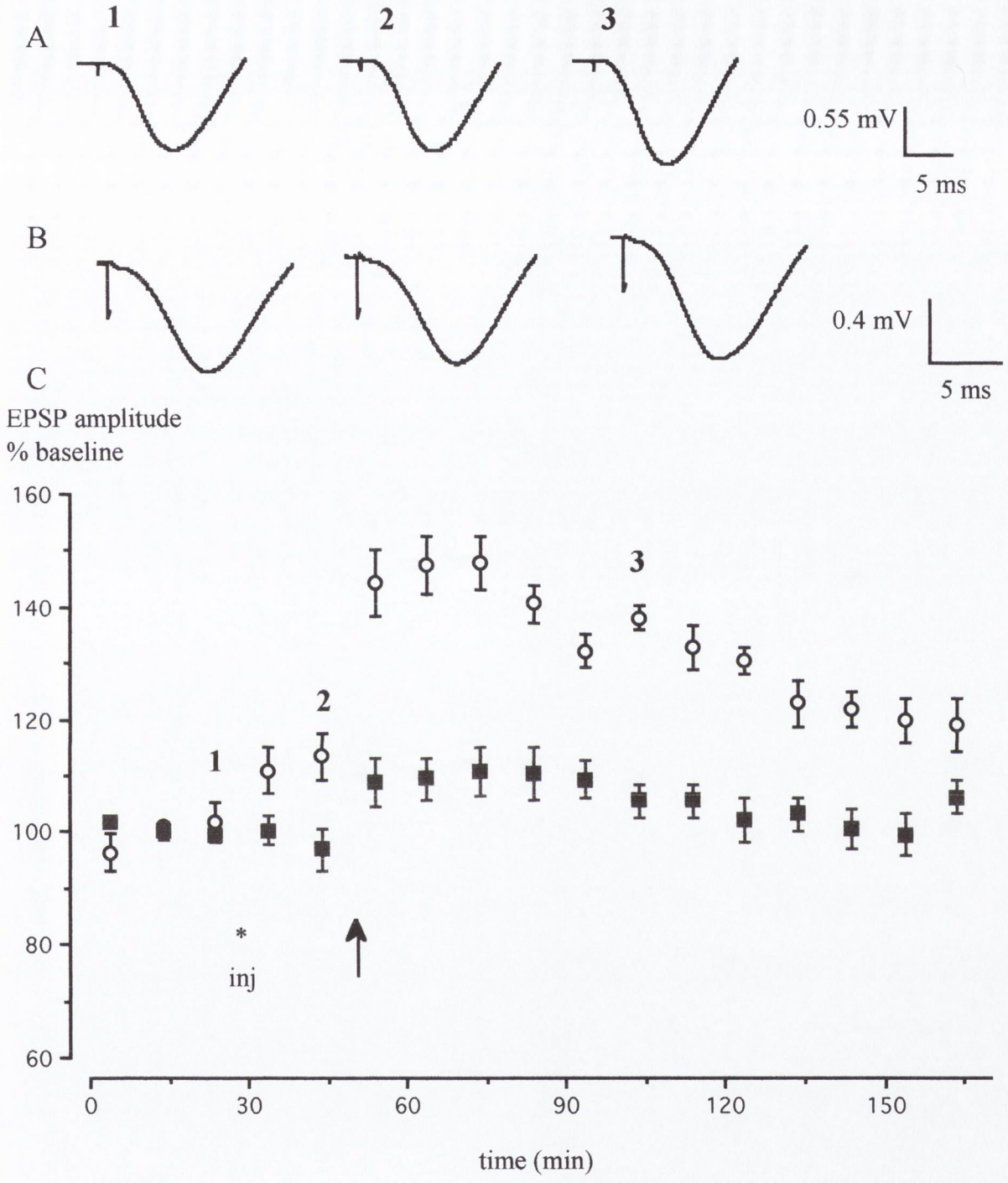

Fig 3.4

The effect of picrotoxin on LTP induction in non-stressed anaesthetised animals. A and B) Typical example of traces of baseline EPSP 10 minutes prior to injection (1), after vehicle (A) or PTX (B) 10 minutes prior to HFS (2), and 60 minutes after HFS (3). C) Graphical representation of the effects of both picrotoxin (filled black squares) and vehicle (100% EtOH, open circles) injection (*). HFS is shown by arrow. Results are mean±sem, n=5 for PTX and n=4 for vehicle as a % of baseline. $P<0.05$ immediately following HFS at 60 and 120 minutes post tetanus, unpaired t-test.

3.2.3 PTX effects in the awake animal prior to stress.

The experiments in 3.2.1 and 3.2.2 assessed the effects of PTX when administered after stress and anaesthesia. The following set of experiments investigated whether PTX administration before exposure to acute stress and the subsequent anaesthesia, could interfere in the block of LTP to stress. See Table 3.2.

PTX (2.5mg/kg i.p) was administered prior to rats being stressed. The animals were observed while upon the platform for any behavioural effects such as twitching and convulsions. Fully developed convulsions were only observed in one animal. In all others, twitches and an increase in distress, such as defaecation and urination, above that caused by the raised platform, was observed. The time-course of these observations was uniform in the animals at 19-20 minutes following the injection. The physical effects of the PTX were seen for the remainder of the time on the platform. Animals were subjected to platform exposure for the standard 30 minutes.

Following standard procedure, a stable baseline was achieved at 101.6%±3.5 (n=5). An applied tetanus produced no appreciable effect (102.2±3.8%). There was a slight decrease in the EPSP amplitude over the recording time (83.5±16.4%, 60 minutes post-tetanus). This was not statistically significant from the baseline value.

3.2.4 Effects of PTX in the awake non-stressed animal.

The effect of the same dose of PTX in awake animals was examined. PTX was injected and then animals were placed back into their home cage for 30 minutes instead of upon the raised platform. Upon observation, the animals showed signs of stress, as seen in animals that were on the platform, after 15 minutes post-injection. These included some of the classical signs of stress such as defaecation, urination and piloerection. The signs of stress continued up to the administration of anaesthesia. Animals often also showed mild signs of convulsions in the form of uncontrollable twitches.

HFS caused an immediate increase in the EPSP amplitude (114.3±10.9% immediately after tetanus from 99.7±2.6% 10 minutes prior to tetanus, n=5), which lasted only a few minutes before returning to baseline levels. Immediately prior to the second tetanus the EPSP amplitude was 85.8±9.3%. As seen with the first tetanus, there was an increase in amplitude to pre-tetanus baseline values. This effect was transient and not significant (75.3±13.7% 120 minutes post-tetanus). See Fig. 3.5.

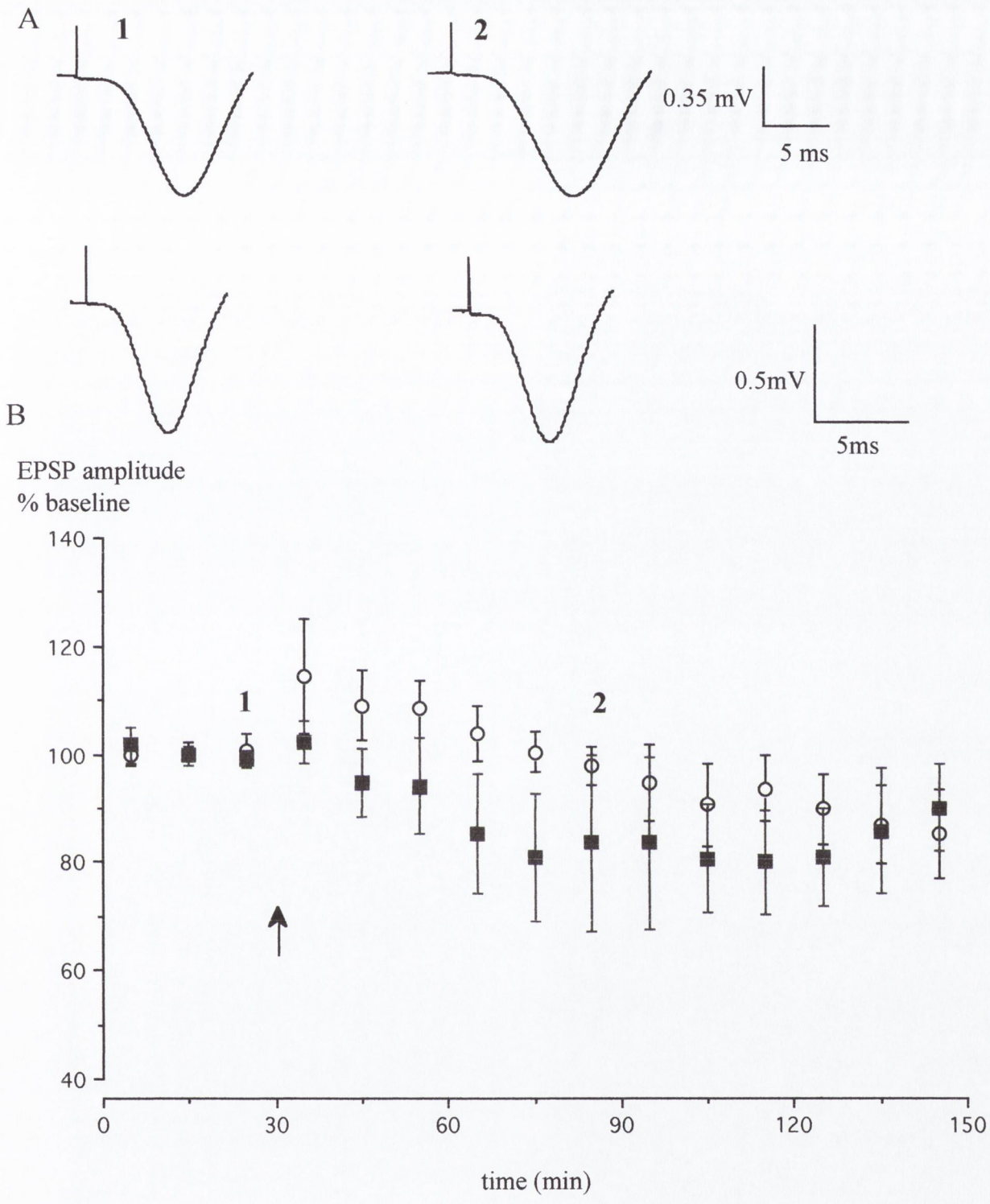

Fig. 3.5 The effect of picotoxin injection (PTX) before anaesthesia. A) Typical traces of field EPSPs in animals exposed to raised platform (A) or left in home cage (B) prior to HFS (1) and 60 minutes following tetanus (2). B) Shows the effect of PTX injection (2.5mg/kg, i.p) prior to anaesthesia on the response to HFS in platform exposed animals (filled squares), n=4 and home cage (open circles), n=5

Table 3.1

	Anaesthetised		Awake	
	PTX alone	**PTX + elevated platform**	**PTX + elevated platform**	**PTX alone**
Baseline	101.8±2.2	99.0±2.1	103.4±5.0	99.7±5.0
Post HFS 1	----	109.1±0.7	----	----
Post PTX injection	99.6±1.9	92.8±8.7	----	----
Post-HFS 2	108.8±4.5	114.0±27.3	102.2±3.7	114.3±10.6
+ 60 mins	105.95±3.6	65.8±12.3 **	83.6±16.1	94.8±7.1
+ 120 mins	93.4±14.2	71.0±5.9	90.1±8.0	93.8±11.4

Table 3.1.

PTX administered to animals after stress (anaesthetised animals) initiated a delayed attenuation in EPSP amplitude that was not seen in non-stressed or awake animals. Results are shown at the equivalent time points across experiments. Results are expressed as mean±sem. ** $P<0.01$ unpaired t-test compared to non-stressed anaesthetised group, n=5 for all groups.

Table 3.2

	Stressed	PTX stressed	Vehicle stressed
Baseline	101.8±2.2	99.0±2.1	97.8±2.1
Post HFS 1	99.6±1.9	109.1±0.7	103.6±2.7
Post injection	----	92.8±8.7	106.9±5.9
Post HFS 2	108.8±4.5	114.0±27.3	107.0±6.9
+60 mins	106.0±3.6	65.8±12.3 *	96.4±5.4
+120 mins	93.4±14.2	71.0±6.0 *	93.8±4.1

Table 3.2.

Attenuation of EPSP amplitude following PTX in stressed animals was not due to vehicle. Table shows 3 groups of animals. Control group were stressed but received no other intervention. PTX stressed group was stressed animals that received PTX injection following anaesthesia. Vehicle stressed were animals that had been stressed and injected with vehicle alone instead of PTX at the same time point. Vehicle studies showed no significant differences to control stressed animals that were not treated with PTX. Results are expressed as mean±sem. n=5 for all groups.

* $P<0.05$ unpaired t-test compared to vehicle stressed animal group.

3.3 The effect of NMDA receptor antagonist CPP in stressed animals.

CPP is reported to be a competitive NMDA receptor antagonist. As NMDA receptors are so widely implicated by published literature in the field of LTP induction, the potential role of NMDA receptors in the stress-induced block of LTP was investigated using CPP.

Once a stable baseline had been established, a burst of HFS was given to ensure that stress had been effective in blocking LTP. A small STP lasting approximately 15 minutes occurred in 3 of the 5 animals (100.6±2.2% 10 minutes prior to tetanus, 118.3±3.4% at 10 minutes post tetanus and 105.8±2.9% 20 minutes post tetanus, n=5). CPP (15mg/kg i.p.) was administered 60 minutes prior to a second tetanus to allow sufficient absorption time, after which there were no observable effects upon the field EPSP amplitude in any animal (103.2±2.9% 10 minutes prior to tetanus). The second tetanus following CPP injection caused no effect on the field EPSP amplitude in 2 of the 5 animals and a continuing attenuation of amplitude in the remaining 3 animals (70.9±15.8% and 59.0±18.0% at 60 and 120 minutes post tetanus respectively, $P<0.05$ at 120 minutes, paired t-test compared to post injection baseline, $P>0.05$ unpaired t-test to vehicle injected animals). See Fig. 3.6.

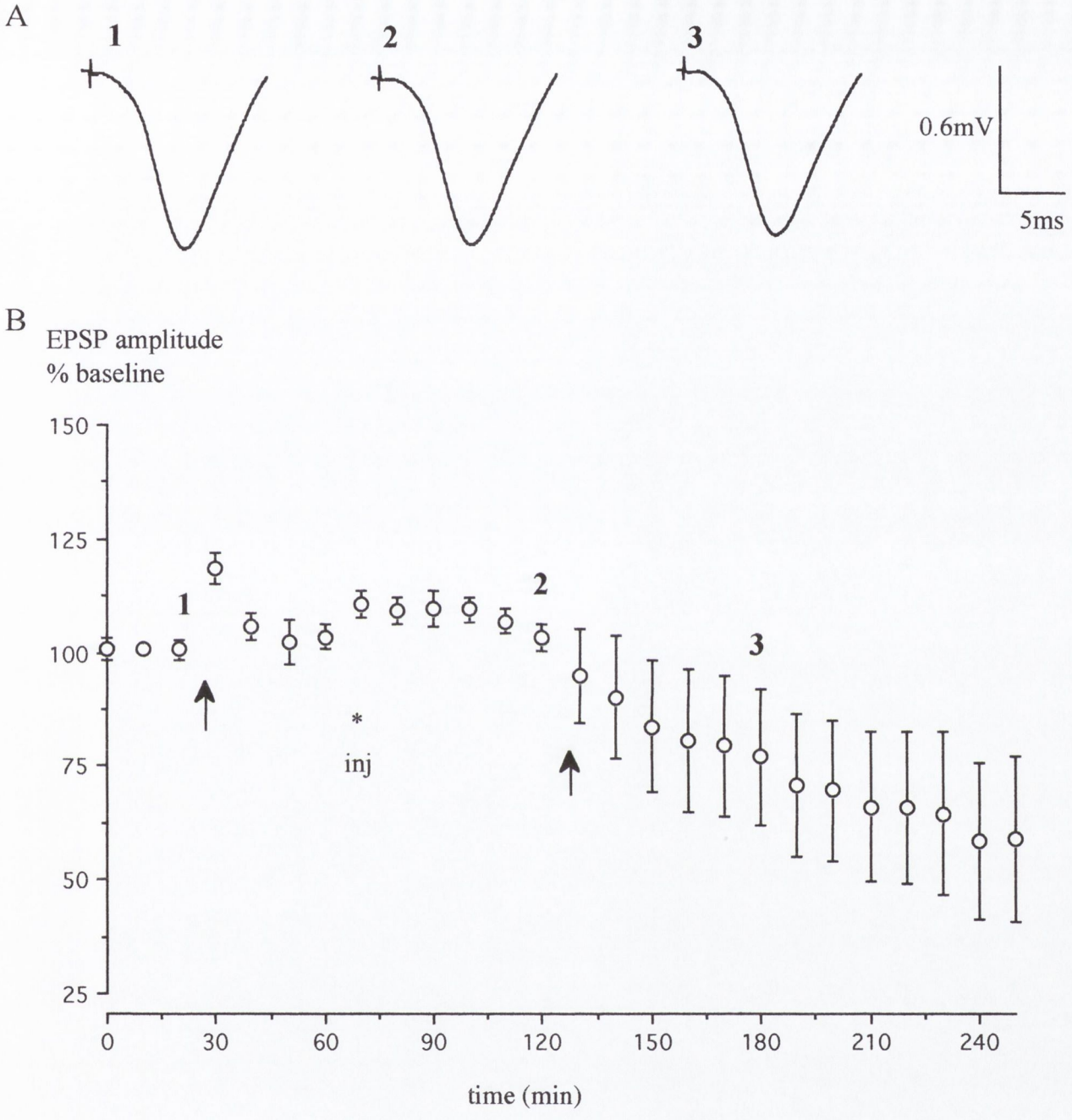

Fig. 3.6 The effect of the NMDA receptor antagonist, CPP, on the stress-induced block of LTP. A) Typical traces of field EPSPs 10 minutes prior to the first tetanus (1), 50 minutes after CPP (10mg/kg i.p) injection (2) and 60 minutes following second tetanus (3). B) CPP injection (*) 60 minutes prior to second tetanus (arrows) produces attenuation of the field EPSPs. Results are mean±sem, n=5.

3.4 The effect of the ampakine, CX546, in stressed animals.

CX546 is reported to act as an ampakine through modulation of the kinetics of postsynaptic AMPA receptors in a positive way so that the resulting post synaptic response is greater than in control conditions (Arai *et al,* 1996). Here CX546 was used to ascertain whether the facilitation of the postsynaptic AMPA receptors transmission could be, at least in part, responsible for a recovery of LTP in stressed animals.

A stable baseline was established in all animals for at least 30 minutes before any intervention (104.9±1.7%, n=5). 20 minutes prior to the first tetanus an injection of vehicle was given to each animal. This produced no visible changes in EPSP amplitude in that time (105.0±3.1% immediately prior to tetanus). Exposure to the first tetanus showed a small increase in EPSP amplitude in all animals that did not exceed the specified 15% for exclusion (111.2±4.2% immediately following tetanus and 113.9±3.3% prior to drug injection). Administration of CX546 (30mg/kg i.p.) 20 minutes before a second tetanus showed a slight increase in amplitude from pre-injection values (118.8±6.9% immediately before tetanus). The second tetanus produced an immediate increase in EPSP amplitude (136.3±8.7%) but this was not long lasting in nature as over 2 hours EPSP amplitude drifted towards pre-tetanus values (129.6±4.9% and 118.9±7.4% at 60 and 120 minutes post tetanus respectively). See Fig. 3.7.

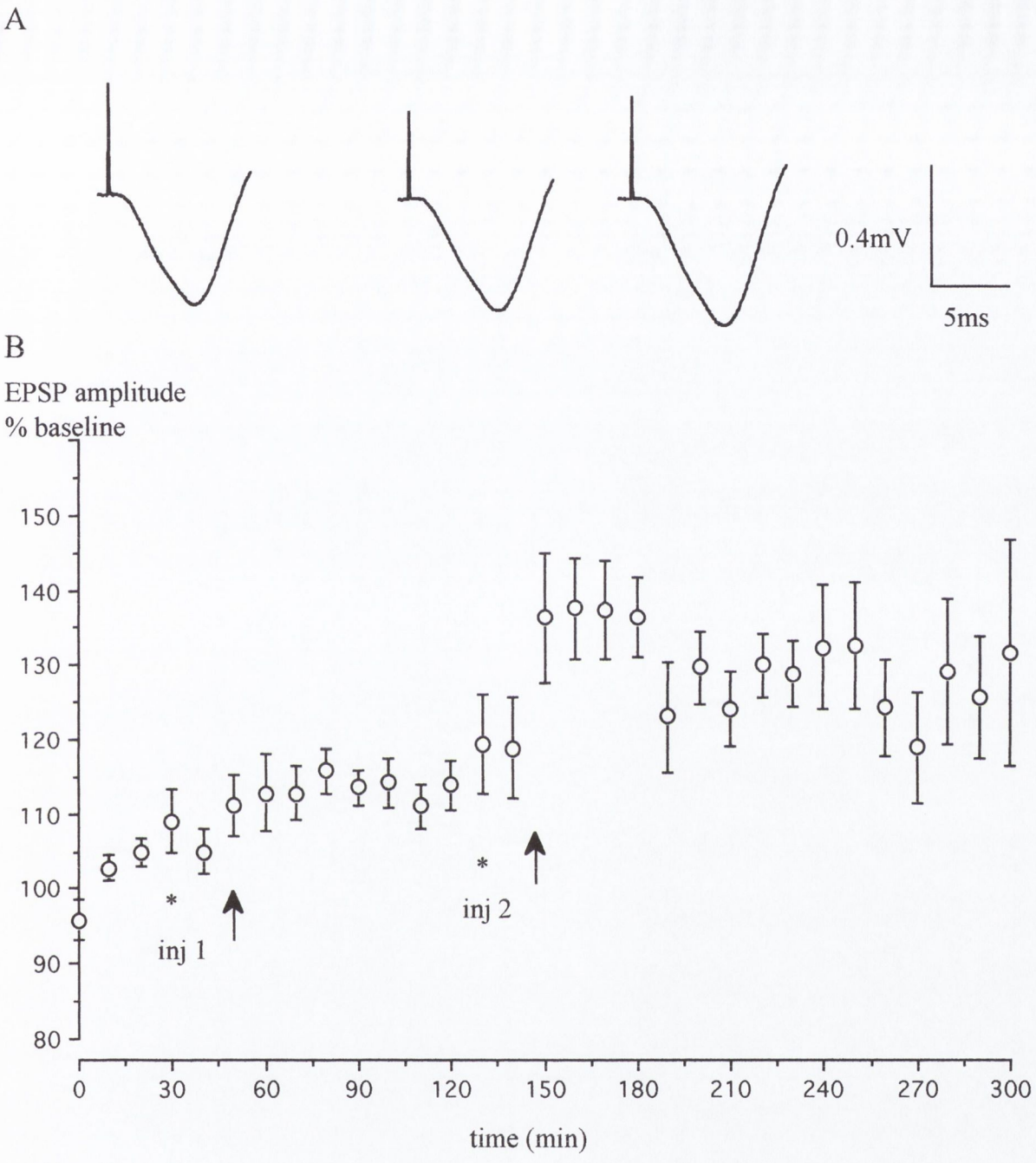

Fig. 3.7 The ampakine CX546 partially reversed the effects of stress on synaptic plasticity. A) Shows typical traces of field EPSPs in a single experiment using CX546. From left to right shows an average baseline trace 10 minutes prior to tetanus, 10 minutes following CX546 injection and 60 minutes following second tetanus. B) Shows the effects of CX546 injection (* inj 2) and vehicle (* inj 1) in stressed animals, n=5. HFS tetani are represented by filled black arrows. Results are expressed as mean±sem.

3.5 The effect of tianeptine on synaptic plasticity.

Tianeptine is part of the tri-cyclic anti-depressant family. It has actions that include facilitation of 5-HT re-uptake in the rat brain (Fattaccini *et al.*, 1990, Kamoun *et al.*, 1989 & Broqua *et al.*, 1992, Kato & Weitsch, 1988). In terms of its antidepressant action, this makes tianeptine unusual. Most commonly prescribed modern anti-depressants work by inhibiting the re-uptake of 5-HT in the CNS. With reports that increase extracellular concentrations of 5-HT can lead to impairment of LTP (Abercrombie *et al*, 1988 and Kalen *et al*, 1989), the effect of reducing potentially stress-elevated levels of 5-HT was assessed.

3.5.1 Effects of tianeptine in non-stressed rats.

The dose of tianeptine that has been routinely administered in previous animal studies was 10mg/kg i.p both acutely (Delbende *et al*,1991) and chronically (Kuroda and McEwen, 1998). Here tianeptine at a dose of 5mg/kg i.p was found to produce a facilitatory effect on EPSP baseline transmission in non-stressed animals. There was a delay of several minutes from the time of tianeptine injection to the observed EPSP potentiation (117.9±8.6% 30 minutes following injection from 99.8±2.2% in the 10 minutes preceeding injection, n=4, $P>0.05$). For the following 60 minutes EPSP amplitude was stable (125.7±12.4% and 129.6±7.0%, 60 and 90 minutes post injection respectively, $P<0.05$ at 90 minutes, paired t-test to 10 minutes before injection). There was then a gradual further increase in EPSP amplitude over the next hour before stabilising again to end of the recording period (140.3±13.4% 180 minutes post injection, $P>0.05$). See Fig.3.8.

To assess the effect of tianeptine on the stress-induced block of LTP, a dose that did not produce baseline effects was used. The dose of tianeptine was reduced to 1mg/kg i.p. At this dose in non-stressed animals, tianeptine did not affect baseline transmission within 40 minutes (103.9±2.8% 40 minutes post tianeptine injection, n=5). Tianeptine, 1mg/kg, in non-stressed animals produced no significant effects on the ability of a high frequency tetanus to induce potentiation compared to control non-stressed animals (140.6±4.5%, $P<0.01$ compared to baseline, n=5 and 155.5±15.0%, n=8 respectively, 10 minutes after HFS). The resulting LTP was stable for the remainder of the recording period (143.0±16.25% and 136.9±9.4% at 60 and 90 minutes post HFS, $P<0.05$, paired t-test). See Fig. 3.9.

Vehicle experiments showed that there was no effect of water in LTP induction in non-stressed animals. Stable baseline EPSPs were established (104.4±2.9% immediately prior to vehicle injection, n=5). Vehicle administration caused no facilitation or attenuation of the EPSP amplitude (95.4±3.6%, immediately prior to tetanus). HFS produced a stable potentiation as in control animals (135.0±7.3% immediately following HFS, $P<0.01$ paired t-test compared to post-injection baseline) which was maintained (130.6±6.3%, and 126.1±6.3% $P<0.05$ at 60 and 120 minutes post HFS respectively, paired t-test compared to post-injection baseline). There is no statistical significance between vehicle and tianeptine in non-stressed animals at 60 minutes post HFS (unpaired t-test, $P>0.05$).

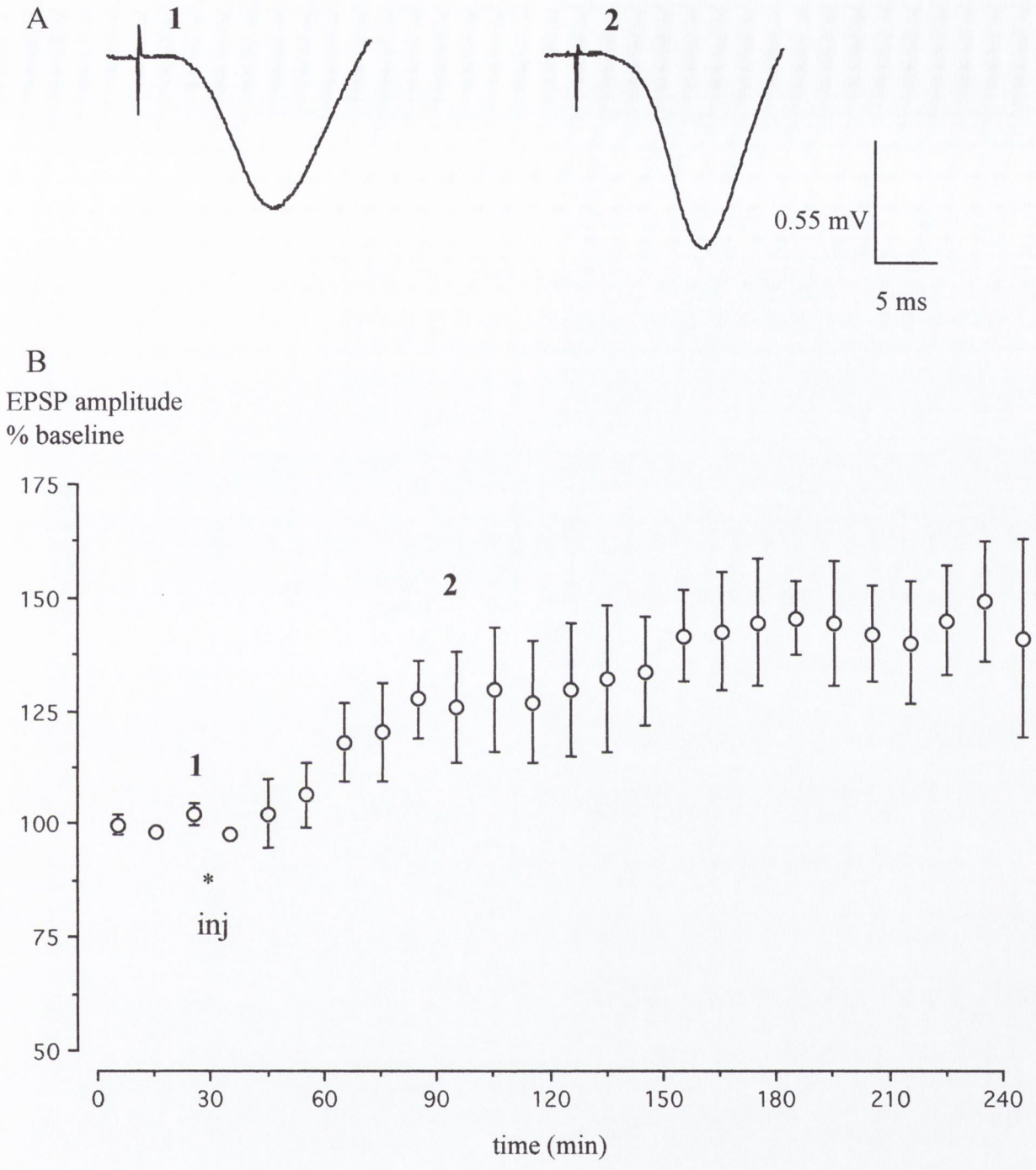

Fig 3.8 The effect of tianeptine on baseline synaptic transmission in non-stressed animals. A) Typical trace of field EPSP before tianeptine (5mg/kg, i.p.) (1) and trace of EPSP 60 minutes following tianeptine injection (2). B) Graphical representation of the effect of tianeptine (injection shown by *) on field EPSPs over time. Results are mean±sem, n=4.

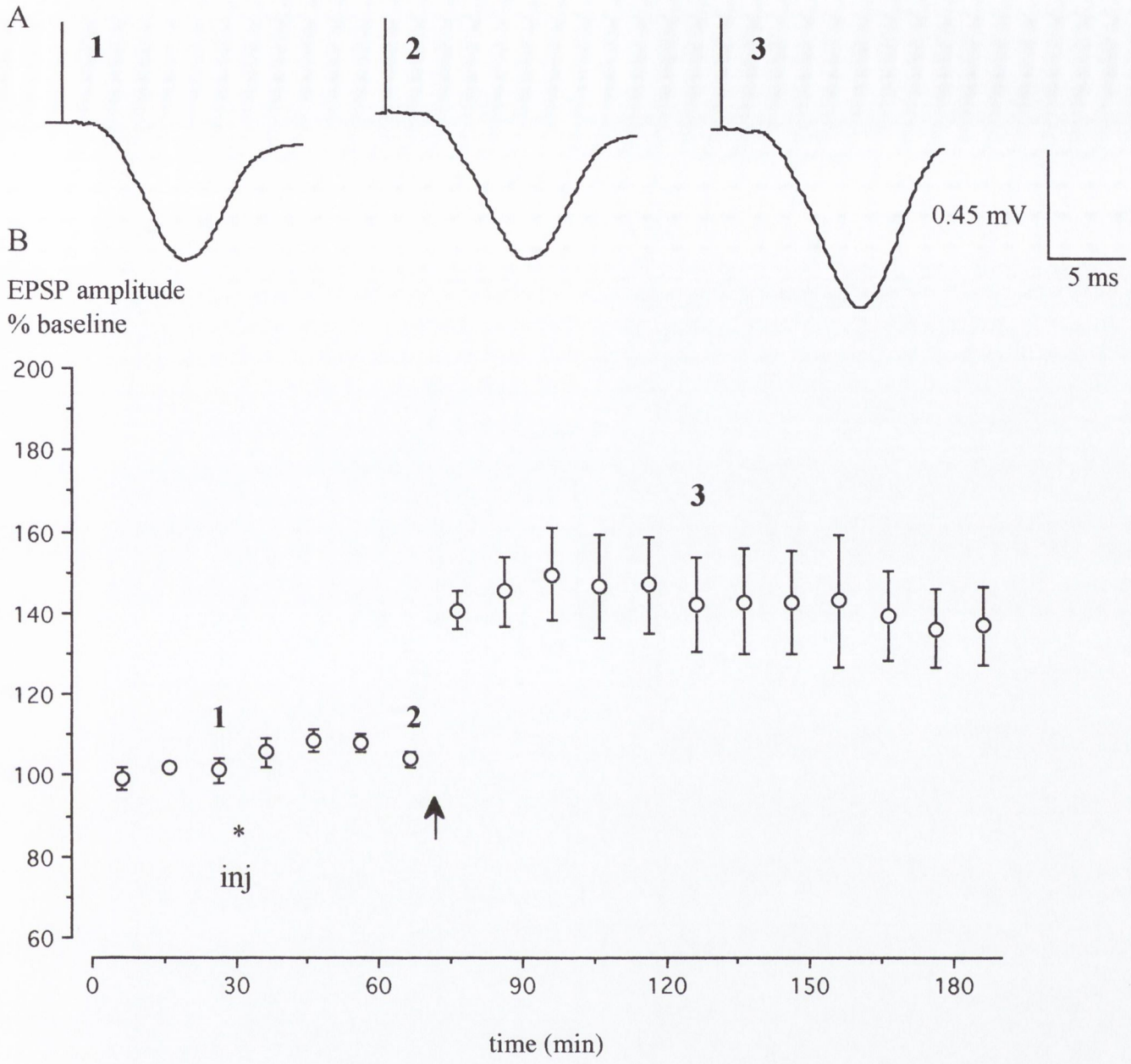

Fig. 3.9 The effect of tianeptine on synaptic transmission in non-stressed rats. A) trace of average baseline field EPSP (1), 30 minutes after tianeptine (1mg/kg, i.p.) (2). 60 minutes following HFS tetanus (3), each trace is the average of 20 sweeps representing a 10 minute epoch, n=5. B) Graphical representation of the effect of tianeptine on EPSP amplitude in unstressed animals over time. Tianeptine injection is (*) 40 minutes prior to HFS tetanus (arrow). Results expressed as mean±sem.

3.5.2 Tianeptine effects in stressed animals.

Having established a dose that did not produce baseline effects (see Section 3.4.1) tianeptine (1mg/kg i.p.) was used in stressed animals. The stress protocol was successful in blocking potentiation in all animals studied (101.3±2.8% 10 minutes prior to tetanus (HFS1) compared to 101.2±3.2% 10 minutes post tetanus, n=6). Tianeptine produced no changes in baseline at this dose in stressed animals as in the non-stressed controls (109.0±2.9% immediately prior to second tetanus (HFS2). The second tetanus, given 40 minutes after tianeptine injection, produced an immediate increase in EPSP amplitude (170.6±16.1%, $P<0.01$ compared to post-injection baseline, paired t-test). Although the resulting LTP was larger than that produced in control, non-stressed animals both with and without tianeptine, the difference was not statistically significant. The tianeptine-enabled LTP remained stable for at least 2 hours following the tetanus (164.1±12.0% and 159.5±17.2% at 30 and 120 minutes post HFS, $P<0.05$ compared to post injection baseline, paired t-test. $P<0.01$ at each time point after HFS2 unpaired t-test compared to vehicle control). See Fig 3.10.

To ensure that the results observed with tianeptine in stressed animals were not due to vehicle (water) effects experiments were conducted using the same protocol but substituting tianeptine for water. Although there had been no observed effects of vehicle in non-stressed animals the effects of stress upon an animal could have resulted in changes in response to the vehicle. Water was injected in equivalent volumes as that where drug was dissolved and at an equivalent corresponding time.

In vehicle animals stable baseline values in field EPSPs were established and there was a successful block of EPSP potentiation in all animals in response to the first HFS (100.5±0.6% immediately before tetanus and 101.2±2.7% following tetanus, n=5). Injection of vehicle 60 minutes after the first tetanus had no effect on EPSP amplitude (101.4±2.0% immediately prior to second tetanus compared to

101.8±2.4% immediately prior to injection). Exposure to the second tetanus produced no immediate change in amplitude nor was there any significant deviation for the remainder of the recording period (101.4±2.5% immediately following tetanus and 102.1±3.0% and 98.7±2.1% at 60 and 120 minutes post tetanus respectively).

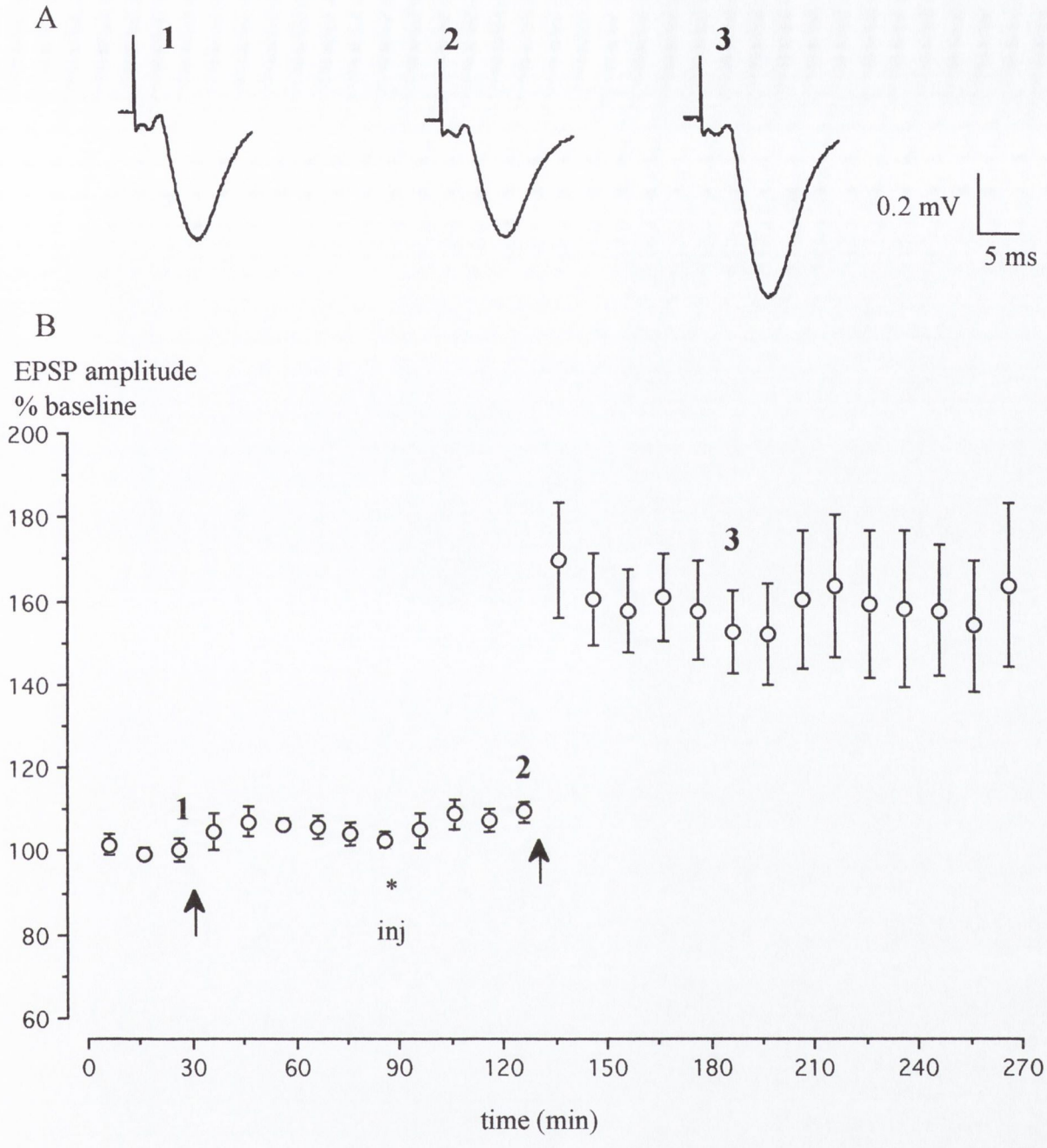

Fig 3.10. Tianeptine reverses the block of LTP induction by stress. A) Typical trace of baseline field EPSP prior to the first tetanus (1), 30 minutes following tianeptine injection (2) and 60 minutes following second tetanus (3). B) Graphical representation showing the effect of tianeptine (1mg/kg, i.p. injection, *) on the stress-induced block of LTP (HFS tetani are shown by filled arrows). Results are mean±sem , n=7.

3.5.3 Effect of individual tianeptine stereo-isomers.

The racemic form of tianeptine had been used in the experiments in Sections 3.5.1 and 3.5.2. In order to elucidate the degree to which each of the stereoisomers was responsible for the effects seen with the racemic form, experiments using the same protocol but with each separate isomer were conducted. This particular trial was conducted blind, the properties of each respective isomer being revealed after experiments were conducted. Each isomer was used at a dose equivalent to that which would be present in the racemic form. The (-) isomer is reported to contain 5-HT uptake enhancing activity, while the (+) isomer has been reported to have a much reduced capacity to affect the 5-HT transporter.

There was no enhancement in EPSP amplitude in response to the first tetanus from baseline (103.5±2.5% and 98.7±4.8%, n=5). The (-) isomer (0.5mg/kg i.p), had no visible effect on baseline transmission (103.0±4.5%). A second tetanus 40 minutes after isomer injection caused a rapid enhancement in EPSP amplitude (149.4±27.2% $P<0.01$, unpaired t-test vehicle control) that was similar in magnitude to that seen with the racemic form of tianeptine. All animals showed facilitation of EPSP amplitude although in one animals there was an STP of >250%. The EPSP amplitude was maintained at levels greater than baseline at all times although there was a gradual decline in the degree of facilitation over time in all animals (140.5±9.8% and 127.9±8.9% at 60 and 120 minutes post HFS respectively, $P<0.01$ and $P<0.05$ unpaired t-test to vehicle). See Fig 3.11

The (+) isomer (0.5 mg/kg), also produced no change in baseline EPSP amplitude and there was no LTP seen after the first tetanus (98.5±8.0% and 101.3±4.2%). Unlike the (-) isomer there was no corresponding reversal of the stress-induced block of LTP (101.2±10.3%) with no further changes to be seen for the remainder of the experiment (102.2±7.3% and 101.2±7.4% at 60 and 120 minute post HFS, n=5). However, in two animals there was a small increase in EPSP amplitude of

10% in response to the second tetanus. This was not statistically significant. See table 3.3.

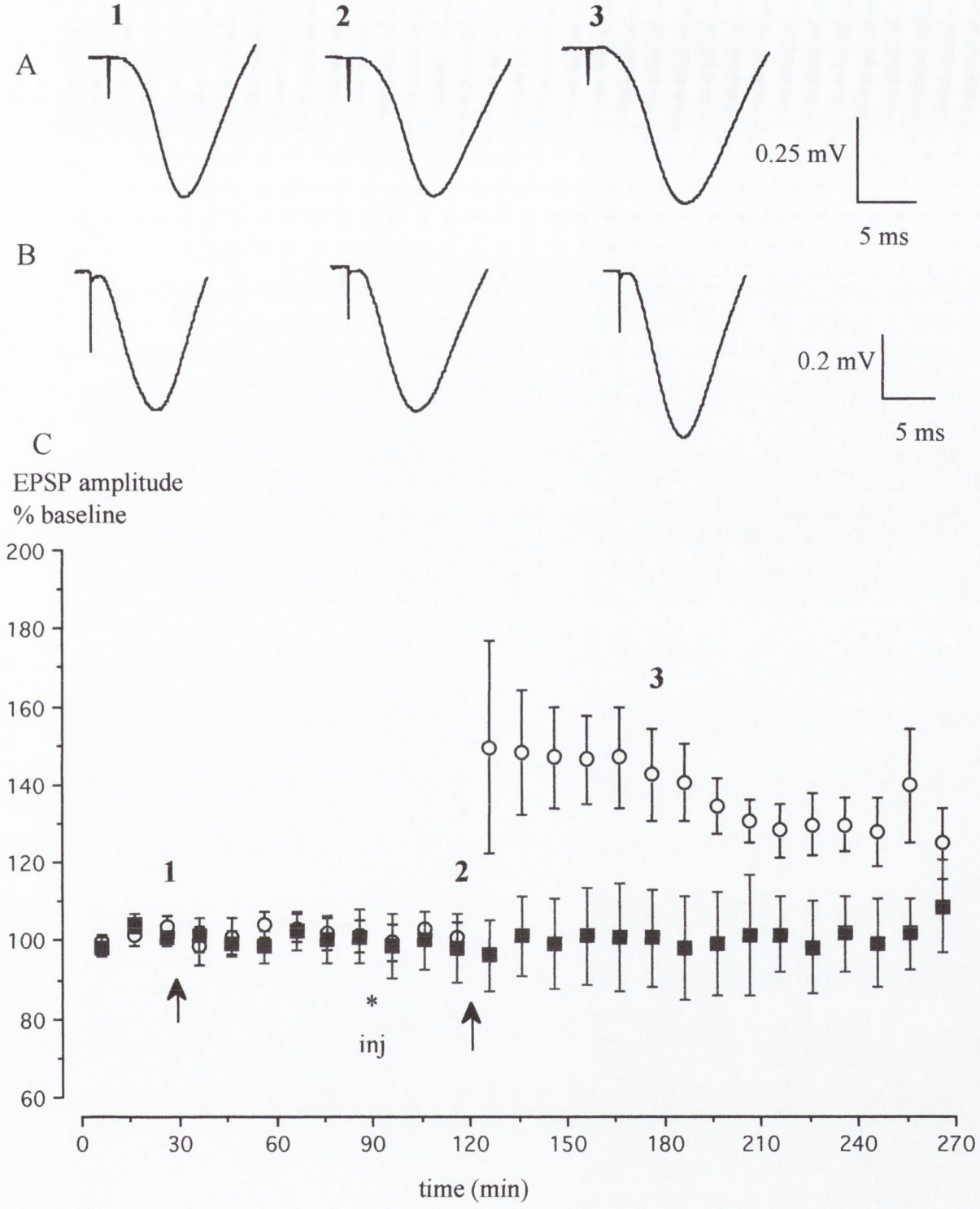

Fig 3.11 Only one stereo-isomer of tianeptine reverses the stress-induced block of LTP. Typical traces of field EPSPs. Baseline 10 minutes prior to tetanus (1), post isomer injection 10 minutes prior to second tetanus (2) and 60 minutes post tetanus (3) (A) (+) tianeptine and (B) (-) tianeptine. C) Graphical representation where HFS is given at t=30 and t=130 minutes (arrows). Tianeptine isomers (filled squares; (+) tianeptine and open circles; (-) tianeptine) were given 40 minutes prior to the second HFS (*), n=5 for both. Results expressed as mean±sem.

Table 3.3.

	(+/-) tianeptine	(-) tianeptine	(+) tianeptine	vehicle
Baseline	100.1±3.1	103.5±2.6	100.6±1.9	100.5±0.6
Post HFS1	101.2±3.2	98.6.3±4.8	101.3±4.2	101.2±2.7
Pre HFS2	105.5±4.8	100.6±3.9	98.5±8.0	101.4±2.0
Post HFS2	170.6±16.2 **	149.4.6±27.1 **	101.2±10.3	101.4±2.5
+60 minutes	155.9±13.6 **	140.5±9.7 **	102.2±7.3	102.1±3.0
+120 minutes	159.5±17.2 **	127.9±8.9 *	101.2±7.4	98.7±2.1

Table 3.3

Comparison of the effect of (±) tianeptine (1mg/kg i.p, n=6) to the effects of the individual stereoisomers of tianeptine (0.5mg/kg i.p, n=5 for both isomers). Values shown are mean ± sem percentage of baseline EPSP amplitude. The time column gives the 10-minute time point at which the values were taken. Statistical significance: ** $P<0.01$, * $P<0.05$, unpaired t-test compared to vehicle treated stressed animals at equivalent time points.

3.6 The effect of fluoxetine on synaptic plasticity.

Fluoxetine (10mg/kg i.p) is believed to have a mechanism of action that is in direct contrast to tianeptine (Section 3.4). It was used in the same experimental protocol as tianeptine and injected at the equivalent time within the experimental framework. The results using tianeptine had suggested that the ability of tianeptine to reverse the stress-induced block of LTP lay in its ability to enhance 5-HT uptake. Fluoxetine should enhance 5-HT levels both in stressed and non-stressed animals thereby providing a contrasting effect within the hippocampus.

3.6.1 Fluoxetine effects in non-stressed animals.

The experiments were repeated with non-stressed, control animals. Fluoxetine produced no observable change in EPSP amplitude in any animal following injection (10mg/kg, i.p.) to the time of HFS (100.7±1.0% and 99.5±2.0% pre-injection, baseline EPSP amplitude and immediately pre-HFS, n=5). The tetanus 40 minutes after fluoxetine injection did not produce any significant change in EPSP amplitude from baseline values (115.6±6.21% in fluoxetine animals, n=5, and 140.6±4.5% in tianeptine animals, n=8 immediately following tetanus). EPSP amplitude during the remainder of the experiment remained stable (93.9±1.2%, 92.9±2.9% at 60 and 120 minutes post HFS, $P>0.05$ paired t-test compared to baseline values). See Fig 3.12.

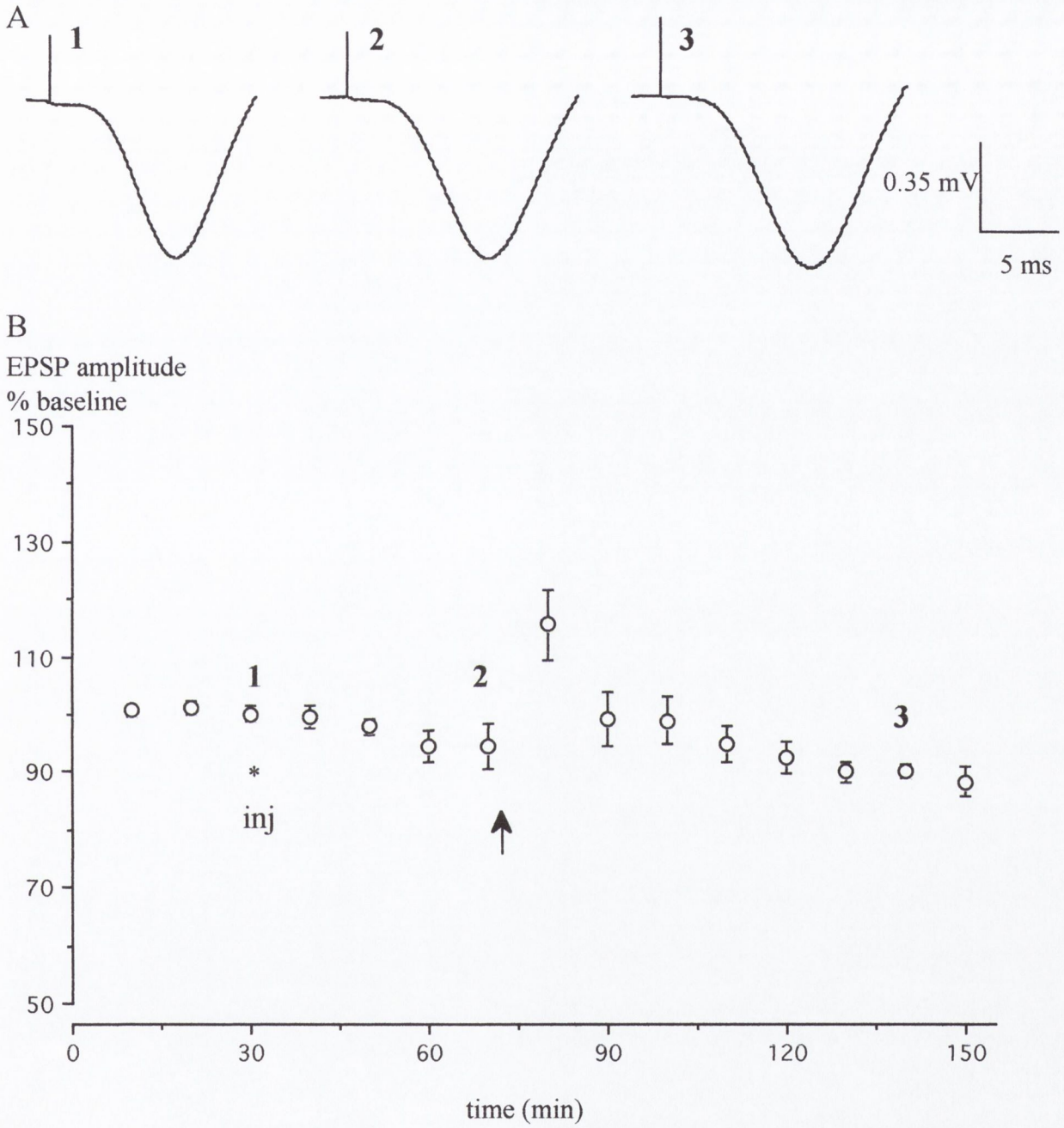

Fig 3.12 The effect of fluoxetine on synaptic transmission in non-stressed rats. A) Typical traces of field EPSP in the 10 minutes prior to fluoxetine (10mg/kg, i.p.) (1), the 10 minute period before HFS (2), the 60 minutes period HFS (3), all traces are a representation of 20 sweep average. B) A graphical representation of averaged EPSP amplitude for unstressed rats injected with fluoxetine(10mg/kg, i.p.). Fluoxetine injection is shown by *, HFS (filled arrow) followed 40 minutes later. Data is mean±sem , n=5.

3.6.2 Fluoxetine effects in stressed animals

In stressed animals there was no lasting effect of the first high frequency tetanus on EPSP amplitude except a small STP lasting for less than 10 minutes (100.0±1.3% pre-tetanus baseline and 111.1±3.0% for the 10 minutes period following tetanus, n=5, $P>0.05$, paired t-test). Fluoxetine injection caused no change in baseline transmission over the 40 minute period leading up to the second tetanus (93.4±6.0% pre-injection and 98.3±1.4% post-injection). Application of a second tetanus produced an immediate and rapid rise in EPSP amplitude (155.5±23.0% 10 minutes following tetanus, $P>0.05$). The LTP remained stable over the remainder of the recording period (145.7±22.1% and 131.2±12.4% at 60 and 120 minutes post HFS respectively, $P>0.05$ at 60 minutes and $P<0.05$ at 120 minutes). See Fig 3.13.

For vehicle effects see section 3.5.2

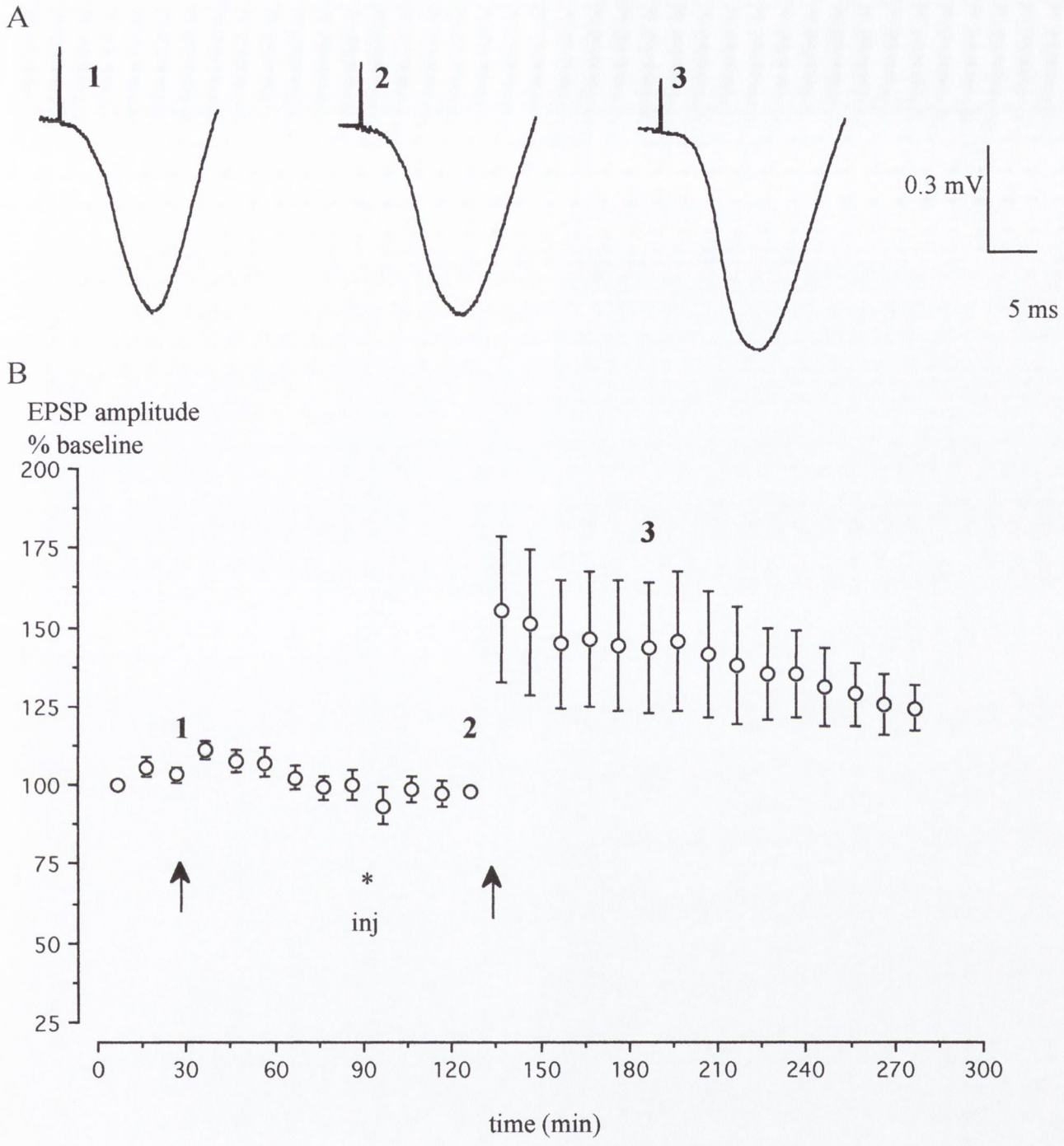

Fig 3.13 The effect of fluoxetine on synaptic transmission in stressed rats. A) Trace of average field EPSPs 10 minutes prior to the first tetanus (1), immediately prior to tetanus following injection (2) and 60 minutes following the second tetanus (3). B) Graphical representation experiments with fluoxetine (10mg/kg) in stressed animals, illustrating a stress-induced block of LTP in response to HFS (filled arrow) and subsequent recovery after fluoxetine (injection shown by *). Results are expressed as mean±sem, n=5.

3.7 The effect of dual administration of both fluoxetine and tianeptine in stressed animals.

While both compounds were found to enable LTP induction in stressed animals, there was still the apparent paradox of each mediating opposing effects on extracellular 5-HT levels. Therefore, utilising the same experimental protocol and doses of each drug that had been effective alone, a study was carried out administering both drugs together in stressed animals.

The stress protocol was shown to be effective in blocking LTP induction to HFS (100.6±1.4% 10 minutes prior to tetanus to 100.6±4.1% 10 minutes post HFS, n=4). There was no effect of the injection of the two drugs combined on baseline response as with each individually (104.8±5.0% immediately prior to tetanus). Unlike individual use of each compound, the combination failed to enable LTP induction. There was a small and short lasting increase in EPSP amplitude but values returned to baseline levels (111.5±4.0%, 104.5±3.5 and 99.04±2.5% immediately following HFS application and at 60 and 120 minutes after, $P>0.05$ paired t-test compared to baseline). See Fig. 3.14.

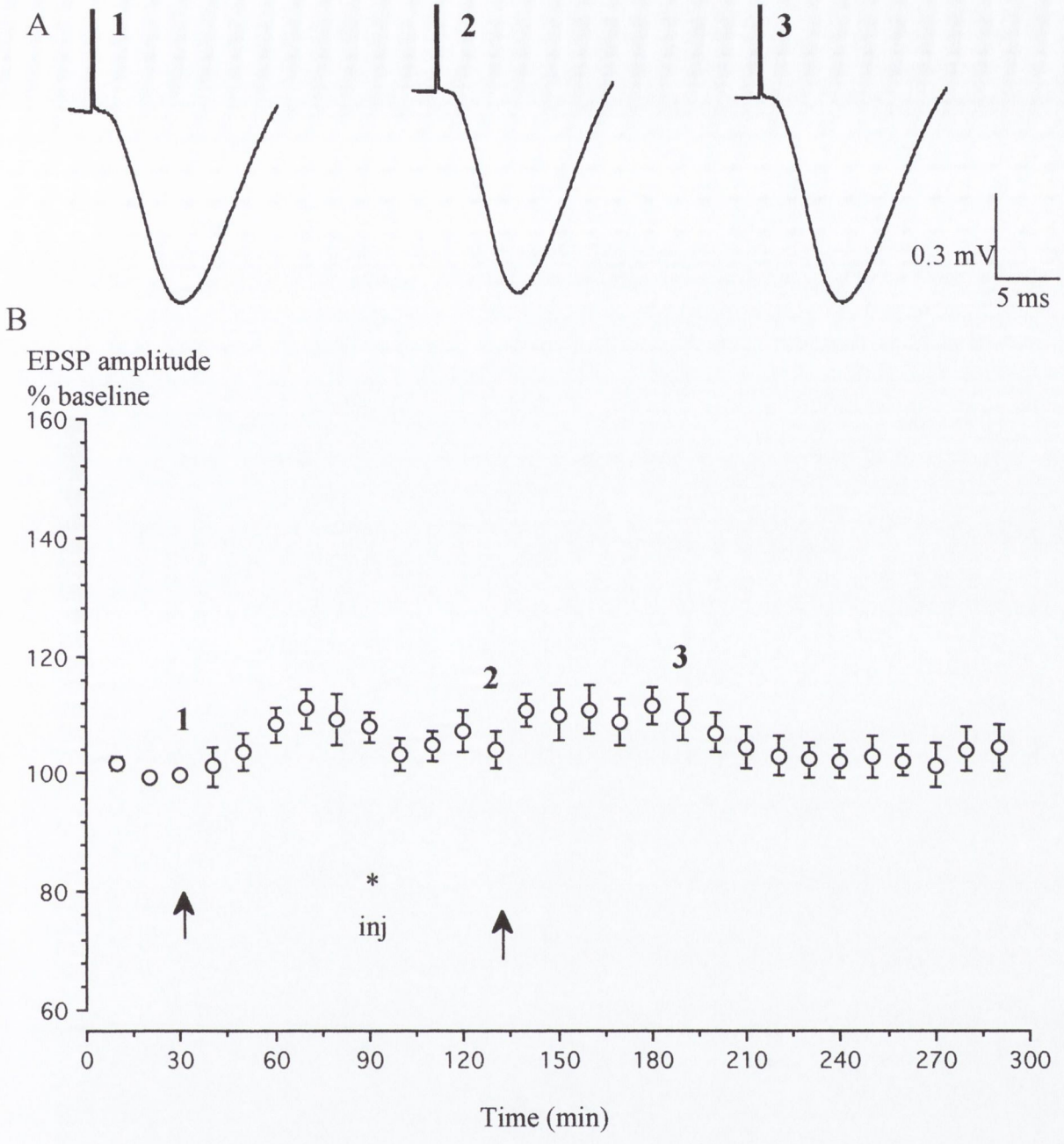

Fig 3.14 Co-administration of tianeptine and fluoxetine fails to reverse the effects of stress on synaptic plasticity. A) Sample traces of field EPSPs at baseline 10 minutes prior to tetanus (1), following drug injection 10 minutes prior to second tetanus (2) and 60 minutes immediately after the second tetanus (3). B) Injections (*) of tianeptine (1mg/kg) and fluoxetine (10mg/kg) occurred 40 minutes prior to the second tetanus (both tetani are shown by black arrows). Results are expressed as mean±sem, n=6.

Table 3.4

	Tianeptine		Fluoxetine	
	Stressed	**Non-stressed**	**Stressed**	**Non-stressed**
Baseline	100.1±3.1	101.1±3.1	100.0±1.3	100.7±1.0
Post HFS1	101.2±3.2	----	111.±3.0	----
Post injection	109.6±2.5	107.8±2.3	98.3±1.	99.5±2.0
Post HFS2	170.6±16.2 **	140.6±4.5	155.5±23.0	115.6±6.2
60 mins	155.9±13.6 **	142.9±12.8	145.7±22.1	90.5±1.3 *
120 mins	159.5±17.2 **	141.1±13.1	131.2±12.4 *	89.5±4.0 **

Table 3.4

A comparison between the effects of tianeptine and fluoxetine in both stressed and non-stressed animals over time. Both tianeptine (1mg/kg, i.p., n=6 stressed group and n=5 non-stressed group) and fluoxetine (10mg/kg, i.p., n=5 for both stressed and non-stressed groups) reversed the stress-induced block of LTP. In non-stressed animals fluoxetine blocked LTP tianeptine had no effect (only one tetanus was given in non-stressed animals and for comparison with stressed groups were placed in the HFS2 column). Results are expressed as mean±sem as a percentage of baseline amplitude. * P=0.05, **P<0.01 unpaired t-test compared to respective vehicle group.

Table 3.5.

	Tianeptine	**Fluoxetine**	**Tianeptine plus Fluoxetine**
Baseline	100.1±3.1	100.0±1.3	100.6±1.4
Post HFS1	101.2±3.2	111.±3.0	100.6±4.1
Post injection	109.6±2.5	98.3±1.	104.8±5.0
Post HFS2	170.6±16.2	155.5±23.0	111.5±4.0
+60 mins	155.9±13.6	145.7±22	104.5±3.5
+120 mins	159.5±17.2	131.2±12.4	99.04±2.5

Table 3.5.

Co-administration of tianeptine and fluoxetine cancels out the effects of single administration of each on the stress-induced block of LTP. Tianeptine (1mg/kg) and fluoxetine (10mg/kg) were given 40 minutes prior to second tetanus either singularly (n=6 for tianeptine and n=5 for fluoxetine) or together (n=5). Results are expressed as mean±sem as a percentage of baseline.

3.8 The effect of fenfluramine on synaptic plasticity.

Fenfluramine is a compound that has been observed to be primarily a releaser of 5-HT from nerve terminals thus raising levels extracellularly. It has also been reported however, to block re-uptake of 5-HT back into the terminals which would also result in raised extracellular 5-HT concentrations. It was used here to help elucidate whether the effects that had been observed with fluoxetine were likely to have been due to increased 5-HT rather than a direct action of the compound itself.

3.8.1 Effects of fenfluramine in non-stressed animals.

Fluoxetine in non-stressed animals had been shown to block the induction of LTP to a HFS tetanus. A further study in non-stressed animals was conducted using fenfluramine to clarify the potential involvement of 5-HT in this effect.

Stable baselines were obtained in all animals (102.2±1.5% prior to injection, n=5). Injection of fenfluramine (5mg/kg i.p.) produced a small but insignificant rise in EPSP amplitude in four of five animals the remaining one showed no in amplitude from baseline values (108.7±2.9% immediately prior to HFS). The response of the EPSP to HFS was small and not significant although one animal showed an STP (<20 minutes) of 40% (118.6±6.8% 10 minutes following HFS). EPSP values remained slightly elevated from baseline values for the duration of the experiment in all animals. The degree of elevation was not significant (107.0±2.1% and 105.5±3.5% at 60 and 120 minutes post HFS respectively). See Fig. 3.15.

For a summary of the effects of tianeptine, fluoxetine and fenfluramine compared to vehicle (water) controls in non-stressed animals see Fig. 3.16.

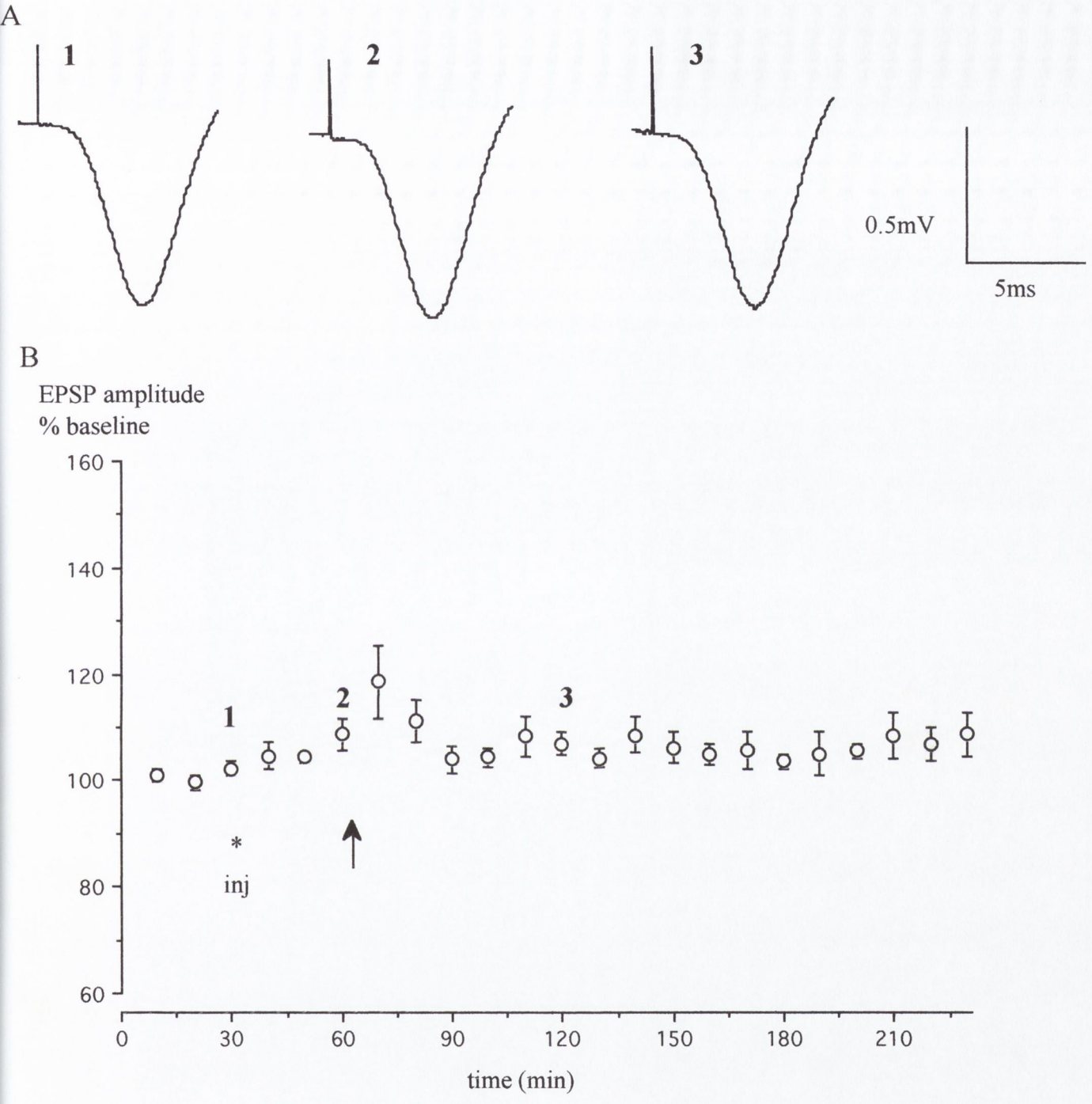

Fig. 3.15 Fenfluramine blocks LTP induction in non-stressed animals.
A) Typical traces of field EPSPs before fenfluramine injection (1), before HFS(2) and 60 minutes following HFS(3). B) Shows EPSP amplitude (n=5) for animals injected with fenfluramine (5mg/kg, i.p. *) 30 minutes prior to HFS (arrow). Results are mean±sem.

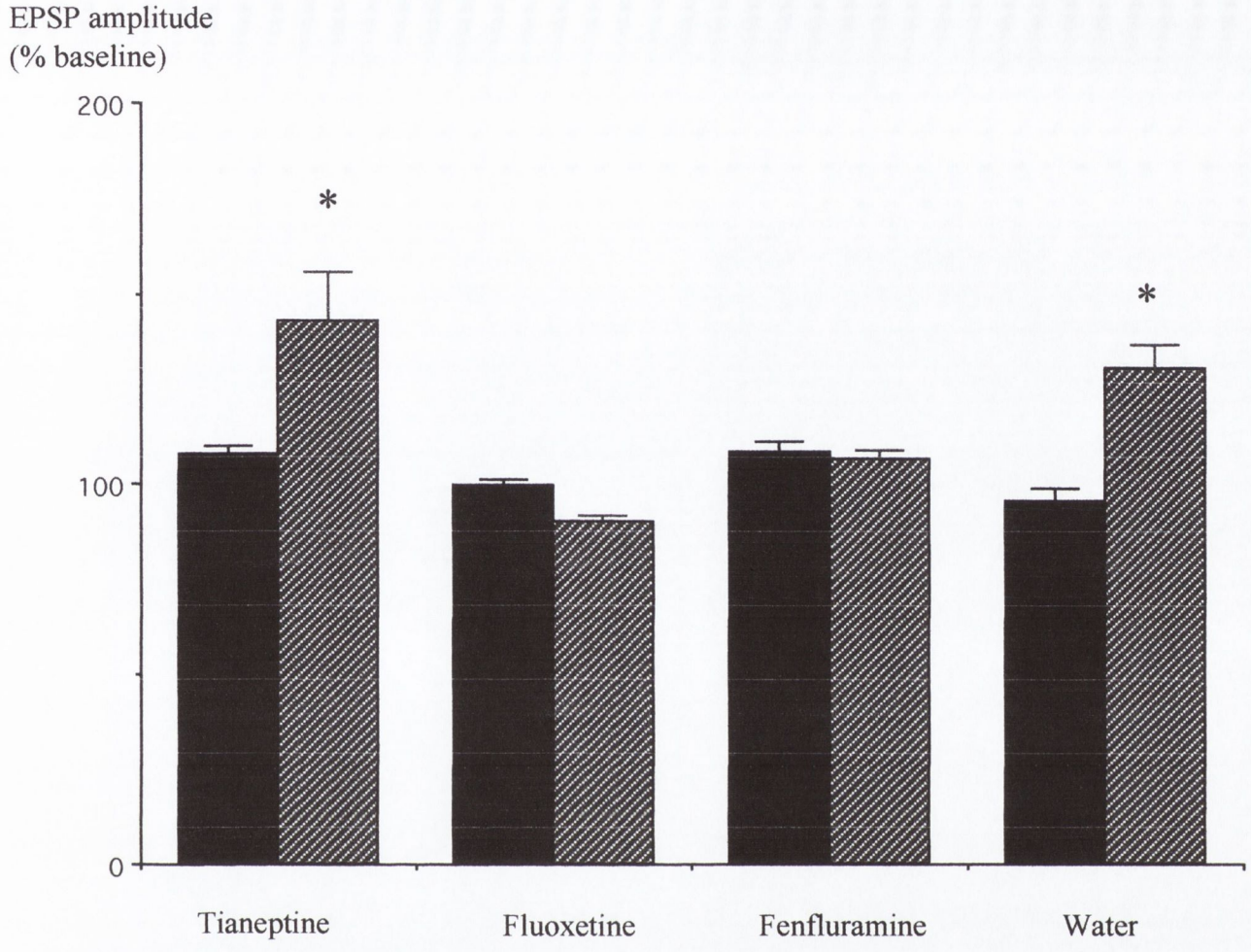

Fig. 3.16 Fluoxetine and fenfluramine block induction of LTP in non-stressed animals. Summary of data in non-stressed animals with tianeptine (1mg/kg, i.p.) n=6, fluoxetine (10mg/kg, i.p.) n=5, fenfluramine (5mg/kg, i.p.) n=5 and water (n=5). Time points represent post-drug injection baseline (solid bars) and 60 minutes following HFS tetanus (striped bars). Results are mean±sem, * $P<0.05$, paired t-test.

3.8.2 The effect of fenfluramine in stressed animals.

The effects of the same dose of fenfluramine that was injected in non-stressed animals was examined in the stress model of LTP blockade. A stable baseline of 101.5±1.8%, n=5 was established prior to the first tetanus. There was no LTP induced in response to the tetanus (111.5±2.8% immediately following tetanus). Injection of fenfluramine (5mg/kg, i.p.) 30 minutes before the second tetanus caused a slight increase in baseline EPSP in all animals though this was not significant (109.7±4.3% immediately prior to tetanus). Following the second tetanus there was a rapid and immediate increase in EPSP amplitude that lasted for over 60 minutes (157.1±17.4%, $P=0.051$, and 162.0±15.9%, $P<0.05$, paired t-test compared to baseline at 60 and 120 minutes respectively). See Fig. 3.17.

To assess whether there were any dose-dependent effects of fenfluramine on the recovery of LTP in stressed animals, the original dose of 5mg/kg was increased to 10mg/kg and the effects observed. There was no change in EPSP values from baseline following the primary tetanus establishing the success of applied stress in blocking LTP induction (99.7±3.6% to 106.5±2.3%, n=4). Fenfluramine (10mg/kg, i.p.) produced an increase in baseline EPSP values in stressed animals over a 30 minute period up to application of the tetanus which was not statistically significant (104.3±6.1% pre-injection to 125.8±0.6% 10 minutes pre-tetanus). Application of the second tetanus produced a further increase in the EPSP amplitude (125.8±0.6% to 155.7±6.9%, $P<0.05$), that was maintained for the duration of the recording period, (151.3±6.9% at 60 minutes post-HFS). See Fig 3.18.

Due to the observation of baseline increases at 10mg/kg, a further short study was conducted to compare both doses to assess the effects on baseline transmission in stressed animals. 10mg/kg fenfluramine administered i.p. caused an increase in the baseline EPSP value of 16% (n=1) within 30 minutes of injection. After 60 minutes this value had risen to 149.1% and rose slightly further to 163.6% after 120

minutes. This value then stabilised for the remainder of the recording period. 5mg/kg (n=1), had produced no effect within the first 30 minutes following injection (103.6%), although after 60 minutes there was a slight increase in baseline to 112.5% and then a further increase to 126.8% after 120 minutes.

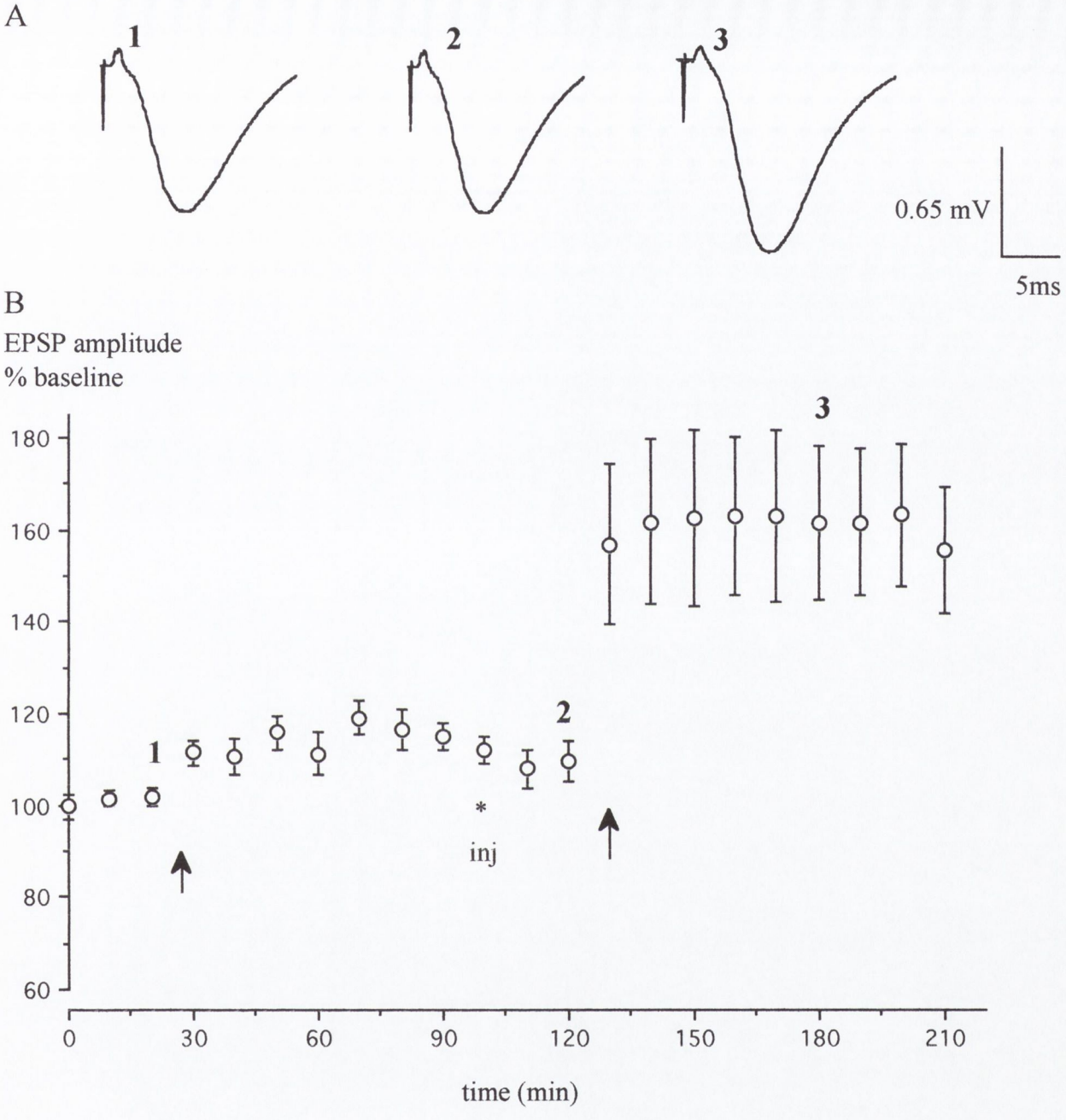

Fig 3.17 The effect of fenfluramine (5mg/kg i.p) on the stress-induced block of LTP. A) Typical traces of field EPSPs at baseline (1) 20 minutes after fenfluramine injection (2) and 60 minutes after tetanus (3). B) Tetani were given at times 30 and 130 minutes (solid black arrows). Fenfluramine injection (*) was given 30 minutes prior to the second tetanus, n=5. Results are mean±sem as a percentage of pretreatment baseline.

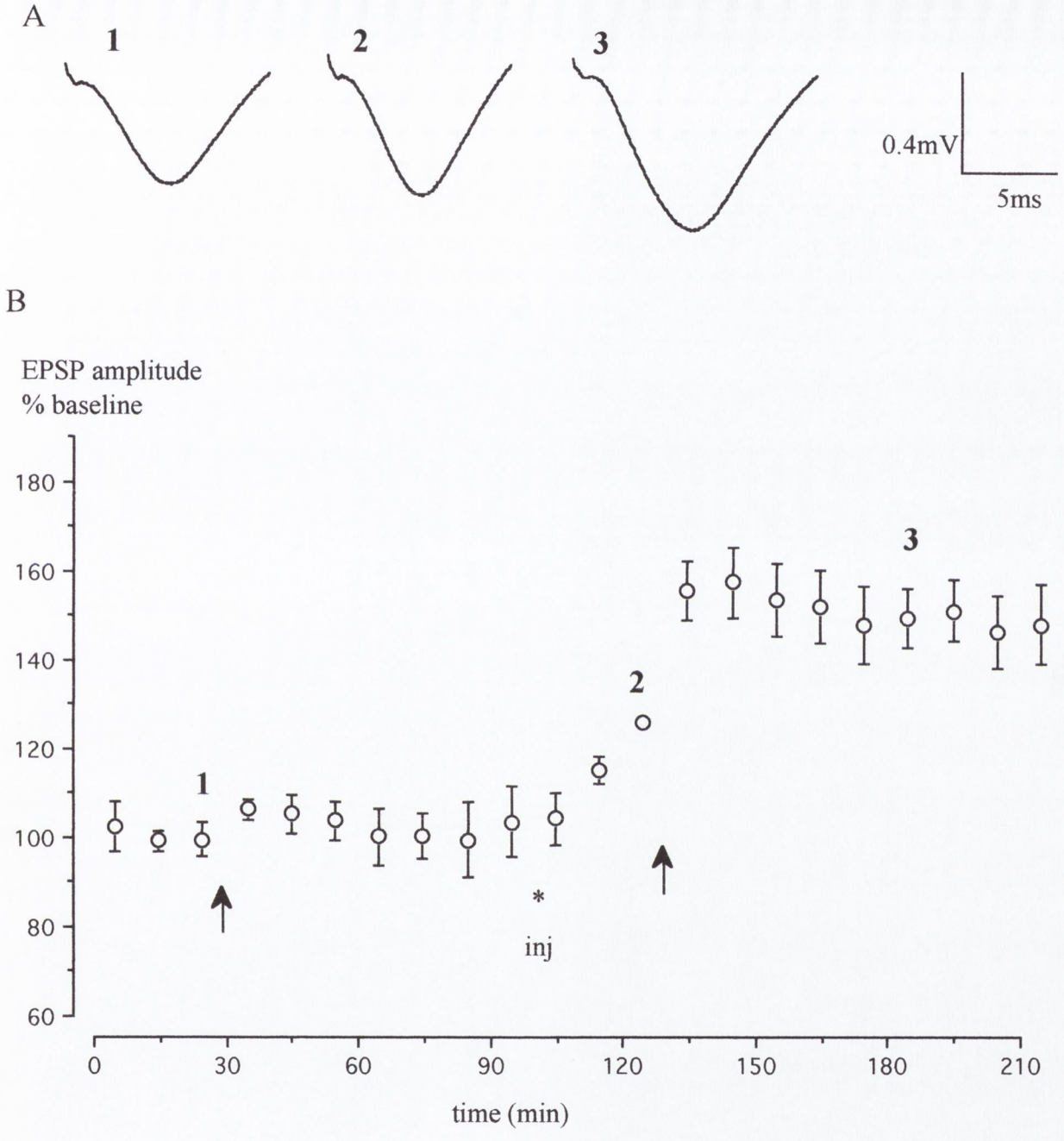

Fig 3.18. The effect of fenfluramine (10mg/kg i.p,) on the stress-induced block of LTP. A) Representative traces of field EPSPs at baseline (1) prior to fenfluramine injection (2), and 60 minutes after application of tetanus (3). B) Graphical representation of fenfluramine 10mg/kg (open circles, n=3). Tetani are shown by solid black arrows, fenfluramine injection *.

Table 3.6.

	Fenfluramine 10mg/kg	**Fenfluramine 5mg/kg**
Baseline	102.5±5.5	101.6±2.0
Post HFS1	105.3±4.8	111.5±2.7
Post injection	125.8±0.6 **	109.7±4.3
Post HFS2	155.7±6.8 **	157.1±17.5 *
+60 mins	151.3±6.9 **	162.0±15.9 **

Table 3.6.

The effect of different doses of fenfluramine on both baseline EPSP transmission and the stress-induced block of LTP over time. Both doses, 10mg/kg (n=4) and 5mg/kg (n=5), were administered 30 minutes prior to the second tetanus. Results expressed as mean±sem. * $P<0.05$ and **$P<0.01$ unpaired t-test compared to vehicle injected stress group.

3.9 The effect of NA in stressed animals.

Nisoxetine is a selective noradrenaline (NA) re-uptake inhibitor. NA is also implicated as being involved in the mediation of the effects of stress. Desipramine is a non-selective NA re-uptake inhibitor. The selectivity of the previous study results for 5-HT were examined by ascertaining whether agents that can raise hippocampal NA levels can also reverse the block of LTP by stress.

3.9.1 The effect of nisoxetine in stressed animals.

Stable baseline measurements were obtained for all experiments before the first tetanus was given which failed to produce any appreciable increase in the EPSP amplitude (99.9±1.1%immediately before tetanus to 100.7±3.1%, immediately following tetanus, n=5). Injection of nisoxetine (10mg/kg, i.p) 30 minutes prior to a second tetanus did not lead to any baseline changes in the EPSP (104.7±1.3% immediately prior to the tetanus). As with the first tetanus, there was no significant change in EPSP amplitude immediately following the second tetanus or for the remaining 2-hour recording period (111.2±4.7%, 107.8±2.3 and 107.2±6.2%, immediately following tetanus, 60 and 120 minutes post tetanus respectively $P>0.05$ paired t-test to post-injection baseline and unpaired t-test to vehicle injected group). See Fig. 3.19

For a summary of the effects of tianeptine, fluoxetine, fenfluramine and nisoxetine in stressed animals see Fig. 3.20.

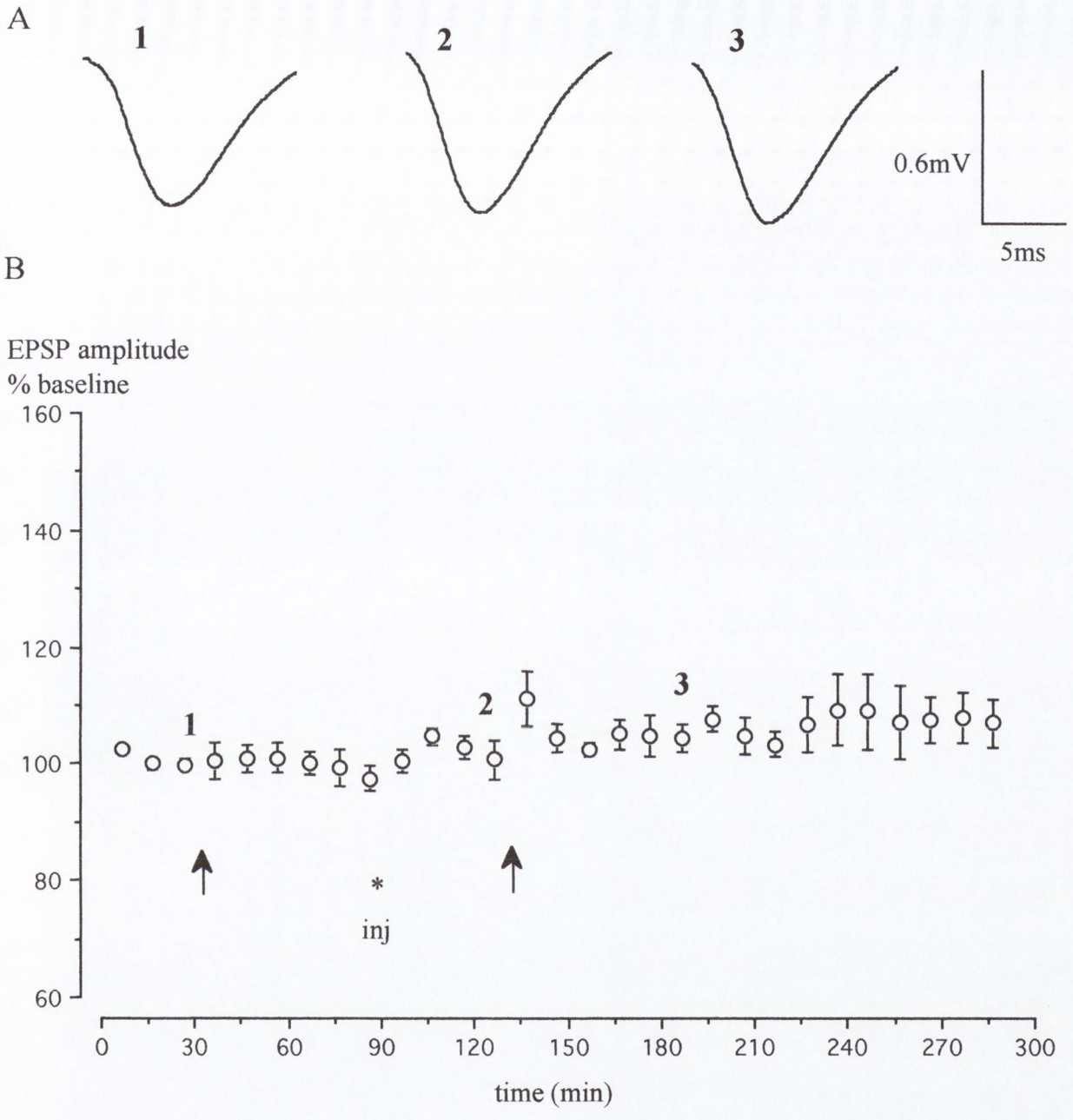

Fig. 3.19. The effect of the NA re-uptake inhibitor, nisoxetine, on the stress-inuced block of LTP. A) are typical traces of field EPSPs in animals from this study. A) baseline (1), post drug (2) and 60 minutes after application of the second tetanus of HFS (3). B) Graphical representation of the results (n=5). Nisoxetine (10mg/kg i.p , *) was given 30 minutes before the second HFS. Stress-induced block of LTP was shown by the first tetanus (both shown by filled black arrow). Results expressed as mean±sem as a % of baseline.

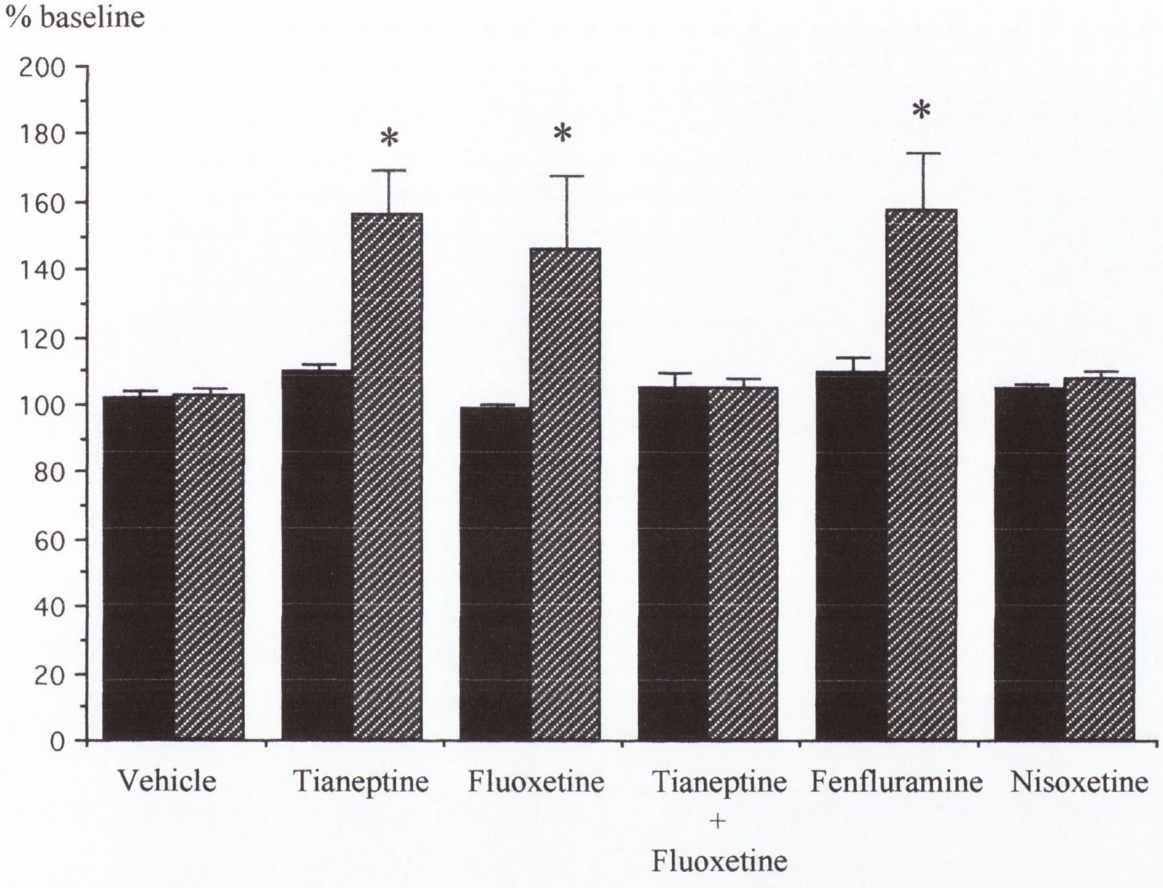

Fig. 3.20 Tianeptine, fluoxetine and fenfluramine reversed the stress-induced block of LTP. Summary of the effects of tianeptine, a 5-HT uptake enhancer (1mg/kg, n=6), fluoxetine, a 5-HT uptake inhibitor (10mg/kg, n=5) and the effect of both administered together, n=5. Each was given i.p. 40 minutes prior to second HFS tetanus as with vehicle (water, n=5). Fenfluramine (5mg/kg, n=5) and nisoxetine (10mg/kg, n=5) were injected i.p. 30 minutes prior to tetanus. Results are expressed as mean±sem and show EPSP amplitudes 10 minutes prior to second tetanus (solid bar) and 60 minutes follwing tetanus (striped bars). * $P<0.05$ unpaired t-test compared to vehicle.

Table 3.7.

	Fluoxetine	**Fenfluramine**	**Nisoxetine**	**Desipramine**
Baseline	100.0±1.3	101.6±2.0	99.9±1.1	100.1±1.7
Post HFS1	111.1±3.0	111.5±2.7	100.7±3.1	98.3±0.8
Post injection	98.3±1.4	109.7±4.3	104.7±1.3	102.7±2.1
Post HFS2	155.5±23.0	157.1±17.5 **	111.2±4.7	104.1±0.2
+60 mins	145.7±22.2	162.0±15.9 **	107.8±2.3	115.1±5.7
+120 mins	131.2±12.4 *	----	107.2±6.2	110.7±5.8

Table 3.7.

A comparison of 5-HT uptake inhibitors fluoxetine (10mg/kg, n=5) and fenfluramine (5mg/kg, n=5) to the NA uptake inhibitors, nisoxetine (10mg/kg, n=5) and desipramine (5mg/kg, n=5) in their ability to reverse the stress-induced block of LTP. Only the 5-HT uptake blockers were able to enable LTP in stressed animals. All drugs were administered i.p and given 30 minutes prior to second tetanus. Results are expressed as mean±sem percentage of baseline values. ** $P<0.01$ *$P<0.05$ unpaired t-test compared to non-stressed vehicle injected group.

3.9.2 The effect of desipramine in stressed animals.

All animals involved in the study with desipramine exhibited a stable baseline (100.1±1.7% immediately prior to first tetanus, n=5). Stress was effective in preventing the induction of LTP following HFS (98.3±0.8% following tetanus). Injection of desipramine (5mg/kg, i.p.) did not affect EPSP amplitude from baseline values (102.7±2.1% immediately prior to tetanus). Exposure to the second tetanus following desipramine showed a small but insignificant increase in amplitude (104.1±0.2% immediately following tetanus and 115.1±5.7% and 110.7±5.8% at 60 and 120 minutes post tetanus respectively, $P>0.05$ paired t-test to post-injection baseline and unpaired t-test compared to vehicle injected group). See Fig 3.21.

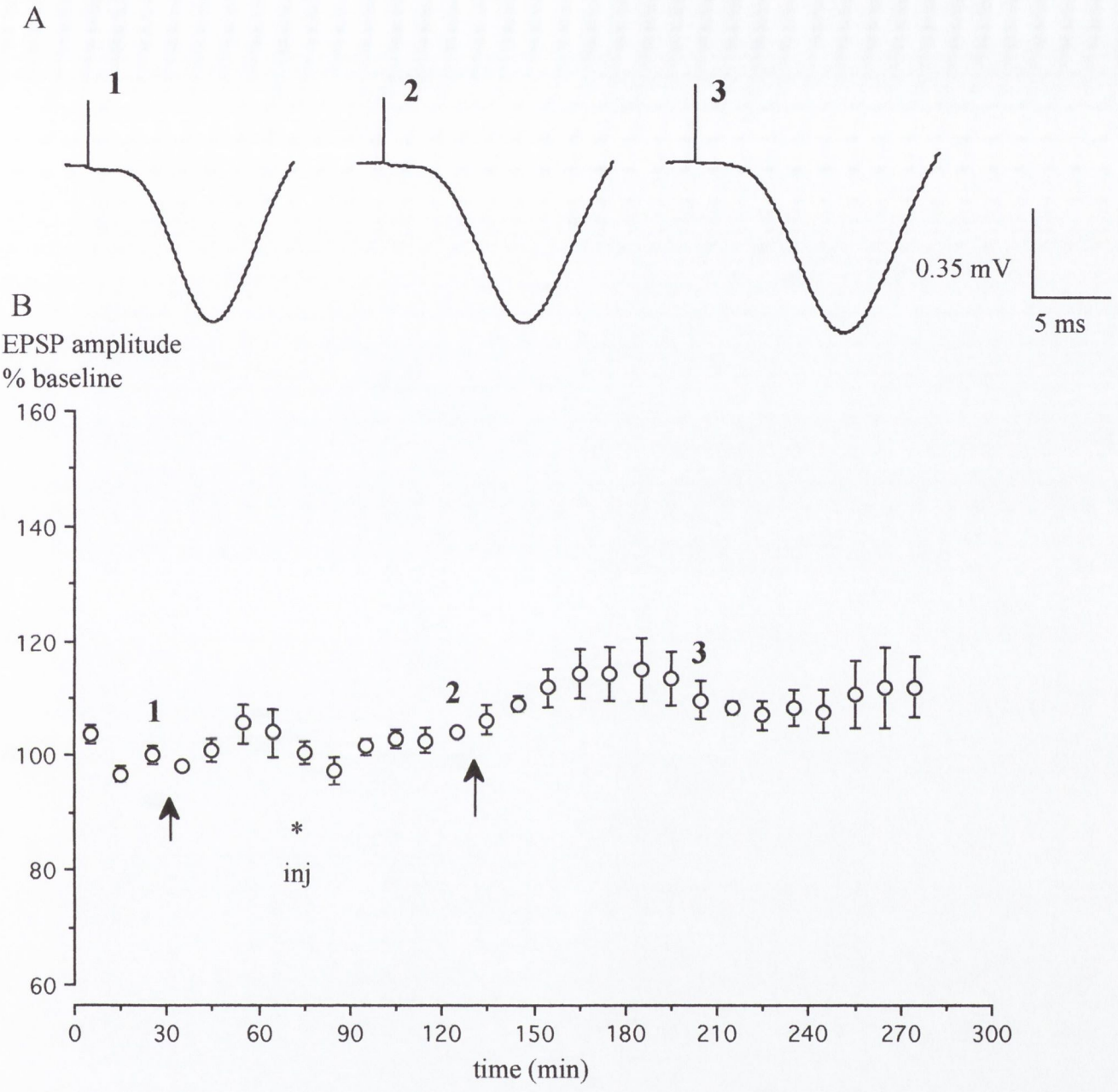

Fig. 3.21 Desipramine does not reverse the stress-induced block of LTP. A) shows typical field EPSPs before first tetanus (1), following desipramine injection (2) and 60 minutes after second tetanus (3). B) Stress successfully blocked LTP induction to HFS (arrows) which was not altered by desipramine (*) injection. Results are mean±sem, n=5.

3.10 The effect of 5-HT receptor antagonists on the stress-induced block of LTP.

3.10.1 The effect of the 5-HT_3 receptor antagonist ondansetron in stressed animals.

Ondansetron has been reported to increase the degree of LTP that can be induced in non-stressed animals in the CA1 area of the hippocampus in response to theta stimulation (Staubli *et al*, 1995), thus implicating the involvement of 5-HT_3 receptors in the control of LTP. Here, ondansetron was used in stressed animals to assess whether 5-HT_3 receptor blockade had a potential role in the block of LTP induced by stress exposure.

A stable baseline was established in all animals followed by the first tetanus showing that LTP induction had been blocked by stress exposure (100.3±1.0% immediately prior to tetanus and 101.0±1.9% 10 minutes following the tetanus, n=5). Ondansetron (100μg/kg i.p.) was administered 20 minutes prior to a second tetanus and produced no observable changes in EPSP amplitude (103.1±5.0% 20 minutes after injection). The second tetanus failed to induce any potentiation in the EPSP (101.9±4.1% immediately following tetanus and 109.2±8.2% and 104.9±6.7% at 60 and 120 minutes post tetanus respectively, $P>0.05$ paired t-test compared to post-injection baseline and unpaired t-test to vehicle injected group). See Fig 3.22.

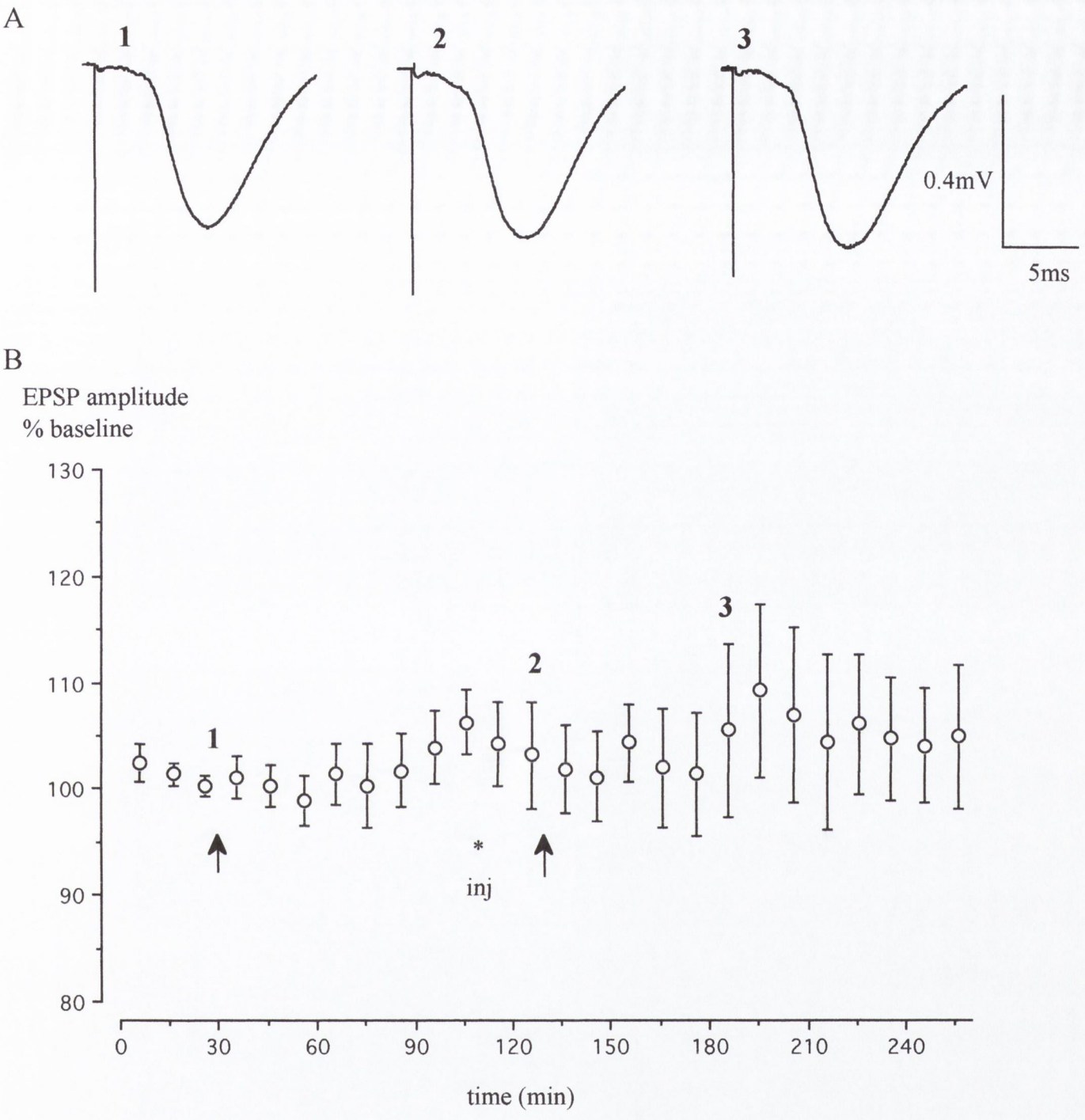

Fig. 3.22. The 5-HT$_3$ receptor antagonist, ondansetron, does not alter the stress-induced block of LTP. A) Typical traces of field EPSPs from left to right; baseline 10 minutes prior to the first tetanus (1), following ondansetron injection 10 minutes prior to second tetanus (2), and 60 minutes after second tetanus (3). B) Shows the lack of effect of ondansetron (100ug/kg i.p., injection shown by *) after stress exposure. HFS tetani are represented by arrows 100 minutes apart. Results are mean±sem, n=5.

3.10.2 The effect of the 5-HT_{1A} antagonist, WAY100635, in stressed animals.

5-HT_{1A} receptors are found throughout the CNS both pre- and post-synaptically they are found primarily post-synaptically in the hippocampal CA1 area where they act as auto-receptors to reduce the amount of 5-HT released. It was thought that by blocking these particular receptor subtypes the potential role of 5-HT_{1A} receptors could be investigated.

A stable baseline was established in all animals (100.5±1.2%, n=6). Stress effectively blocked the induction of LTP in all animals in response to HFS (101.9±1.5%). EPSP amplitude remained stable to injection of WAY100635 (0.5mg/kg, i.p.) and beyond up to the second tetanus 15 minutes following injection (100.9±3.3% pre-tetanus). The second HFS produced variable results with no response or a reduction in EPSP amplitude in 2 animals and increased EPSP amplitude in the other 4 (109.3±7.3% immediately following tetanus). Each individual response to the second tetanus remained stable in all animals (119.5±14.9% and 122.7±13.7% at 60 and 120 minutes post tetanus respectively, $P > 0.05$ paired t-test compared to post-injection baseline and unpaired t-test compared to vehicle injected group). See Fig.3.23.

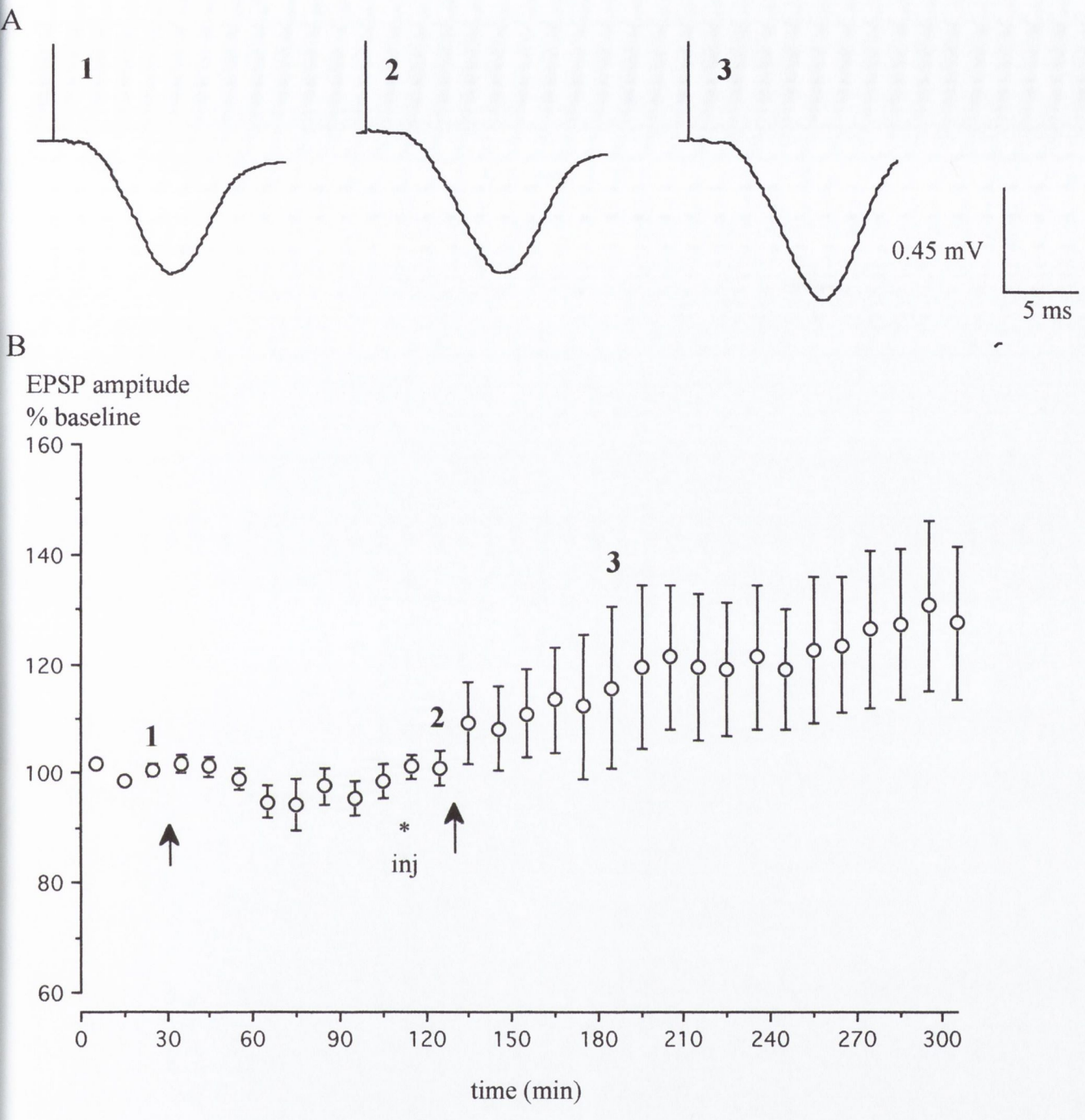

Fig. 3.23 The effect of the $5HT_{1A}$ anatagonist, WAY100635, on the stress-induced block of LTP. A) Typical examples of field EPSPs before first tetanus (1), following injection of WAY100635 (2) and 60 minutes after second tetanus (3). B) Injection of WAY100635 (0.5mg/kg i.p. *) 15 minutes prior to a second tetanus (arrows) induced EPSP amplitude facilitation in 4 of 6 animals. Results are mean±sem, n=6.

IV. Discussion

4.0 Discussion

4.1 The effect of acute stress exposure on synaptic plasticity in CA1.

The primary findings of the behavioural acute inescapable stress study were that exposure to stress, in the form of placement on a raised platform, is sufficient to prevent the induction of LTP in response to HFS, which is normally robust in this region. This is in agreement with several other studies that have been carried out in other laboratories *in-vitro* (Foy *et al*, 1987) and *in-vivo* (Shors *et al*, 1997). Mesches *et al*, (1999) observed no effect on LTP but a block of primed burst potentiation using a predator exposure stress protocol and then assessing the effects *in-vitro*. Here, the effects of stress were shown to outlast the actual exposure time to stress by several hours when the animals had been anaesthetised immediately following removal from the platform. This was shown by the continued inability of the hippocampus to express LTP in response to HFS.

Several groups have observed that there are differences between the effects that are seen with differing stress protocols, the type and duration of exposure to stress. Shors *et al*, 1989 observed that there was a greater degree of LTP inhibition in rats that had been exposed to an inescapable form of stress rather than those exposed to an escapable form. Stress has also been shown to block the acquisition of learning in spatial memory learning tasks (Diamond *et al*, 1996).

One hypothesis put forward by Kim *et al*, 1996, is that stress-induced impairment of LTP induction is due to elevation of baseline hippocampal synaptic transmission thus occluding further LTP. Such a hypothesis could not be assessed in the model used here as animals were anaesthetised hence a comparison of EPSP amplitude before and after stress could not be made in the same animal.

A number of neurotransmitters have been implicated in the effects of stress on synaptic plasticity. Many of these are discussed in more detail in other sections such as glutamate, GABA, 5-HT and NA. In addition to those, Shors *et al*, 1990, reported that there was evidence for the involvement of opioids in the stress-induced block of LTP in CA1 *in-vitro*. There was no effect of opioid antagonism on CA1 LTP induction in non-stressed animals but a marked effect following stress exposure. It was suggested that there was interaction between opioids and other transmitters present in the hippocampus.

In most of the studies where LTP has been blocked by behavioural stress paradigms, the emphasis has been on investigating the role of glucocorticoids in this effect. The actual alterations in levels of circulating corticosterone in the animals of this study are not known. However, the elevated platform (Xu *et al*, 1997) has been shown to cause levels to rise within the stress range of 21-93μg/dL (Diamond *et al*, 1992). It is evident that episodes of acute, inescapable stress exposure cause a dramatic effect on synaptic plasticity in CA1. It has been reported previously by Shors *et al*, 1990, that corticosterone is not solely responsible for the deleterious effects of stress upon LTP induction. Stress exposure did not cause a further reduction in the amount of LTP induced in animals without an adrenal medulla, but high plasma levels of corticosterone were still present in these animals. Exposure to escapable stress also produced high levels of corticosterone but failed to block LTP (Shors *et al*, 1989).

Further evidence that high levels of corticosterone may not be responsible for the block of LTP that is seen in stressed animals can be observed in the anaesthetic used. It has been widely reported that levels of corticosterone can increase to within the reported stress range in response to anaesthesia by urethane (Bennett *et al*, 1991, Dunn, 1987, Spriggs & Stockham, 1964). Within this laboratory and in others, LTP is clearly inducible in animals where urethane anaesthesia is used. This indicates either that urethane at the dose used here does not significantly alter corticosterone levels or that elevated corticosterone is not the factor involved in

stress-induced changes in synaptic plasticity. It can also be surmised from our study that the stress-induced changes, following immediate anaesthesia, outlast the actual exposure to the stress-inducing situation.

4.2 The role of glutamatergic receptors in the stress-induced block of LTP.

Two studies were conducted where pharmacological manipulation of hippocampal excitatory receptors was the target. Both AMPA and NMDA receptors, both receptors for the excitatory neurotransmitter glutamate, were assessed as potential mechanisms by which recovery of LTP could occur.

4.2.1 AMPA receptor involvement.

CX546 is reported to act as an ampakine and to increase post-synaptic responses by facilitation of post-synaptic AMPA receptor kinetics through a reduction in the rate of receptor desensitisation (Arai *et al*, 1996). It is possible that exposure to stress produced an alteration in the kinetics resulting in an attenuation of the post-synaptic response. However, as mentioned previously it was not possible in this particular model to assess whether there were any changes in EPSP amplitude or slope in the same animal following stress exposure.

The findings of the study seemed to indicate that pharmacological enhancement of AMPA function could at least partially recover LTP induction. This may simply be an effect of facilitated postsynaptic AMPA receptor function which was sufficient to overcome potential other stress-induced effects, such as increases in inhibition through elevated 5-HT levels. It does not support the idea that stress directly attenuates AMPA receptor function, certainly not as the only mechanism by which stress leads to the failure of LTP induction otherwise a more complete recovery of LTP should have been observed.

Ampakines have been reported (Larson *et al,* 1996) to increase the slope of field EPSPs recorded *in-vitro* in the hippocampus, though no such effect was observed in the *in-vivo* model used here. It may be possible that the dose used in the study here was not sufficiently high to cause a sufficient number of altered receptors to see full

recovery of LTP. It would be interesting to assess whether there were any dose-dependent effects of this particular compound and whether the effects of stress could be altered independently of the mechanism leading to the block of synaptic plasticity.

AMPA receptors have been implicated to be involved in the acute stress-induced block of LTP to theta-burst stimulation *in-vitro* (Shors and Thompson, 1992). The hypothesis claimed that stress exposure induced changes in post-synaptic glutamate receptors specifically, AMPA receptors. There was no impairment of paired-pulse facilitation indicating no alteration in the degree of pre-synaptic release. Foy *et al*, 1987, found that there was no effect of stress upon short-term potentiation and also suggested that therefore, changes that were induced by stress were due to postsynaptic rather than pre-synaptic changes. An increase in the degree of binding to AMPA receptors in the hippocampus was also observed upon exposure to acute stressful stimuli, while no change was observed in the degree of NMDA receptor binding further implicating that stress induces changes in AMPA but not NMDA receptors (Tocco *et al*, 1991). The results here are consistent with a possible role of postsynaptic AMPA receptors and that maybe the changes that are induced by stress may involve an alteration in the kinetics of the receptors.

4.2.2 NMDA receptor involvement.

The potential involvement of NMDA receptors in the stress-induced block of LTP was also assessed. The study involved block of NMDA receptor function using CPP, which is reported as a competitive NMDA receptor antagonist. The results of this study were inconclusive although the overall trend, which was not statistically significant, across the study was attenuation of the field EPSP amplitude in response to HFS following NMDA receptor blockade. There was no observable effect on EPSP baseline transmission as this is mostly mediated via AMPA receptors.

NMDA receptors are normally thought to be required for many forms of LTP induction in many areas of the CNS particularly in CA1 area of the hippocampus. The experimental design looked at the effect of NMDA receptor blockade following exposure to stress assessing whether stress initiated and maintained an alteration in NMDA receptor function that could have contributed to a block of LTP induction. Reducing NMDA receptor-mediated transmission after exposure to stress, followed by the application of an HFS tetanus did not recover the ability to induce LTP. The observation in 3 animals of EPSP amplitude attenuation following HFS may have been due to an incomplete block of NMDA receptors by the dose of CPP chosen such that moderate levels of postsynaptic Ca^{2+} were achieved rather than the high levels that are normally seen in response to HFS. Also, it has been reported that CPP does not have a high binding affinity for the NR2C/D subunits, which meant that CPP was unable to block LTD compared to another NMDAR antagonist, AP5 (Hrabetova *et al*, 2000). It may be that NMDA receptors containing these subunits are sufficient to induce the observed LTD.

An *in-vitro* study by Kim *et al*, 1996, found, in contrast to the results presented here, that blockade of NMDA receptors could prevent the stress-induced block of LTP. Unlike, this study, the NMDA antagonist, CGP39551, was administered prior

to exposure to stress thus preventing any NMDA receptor-dependent stress-induced changes. If that is the case then whatever changes are potentially incurred by stress via NMDA receptors, the effect cannot be reversed by NMDA receptor blockade after stress exposure.

However, from the two studies conducted, it would appear to indicate that the effects of stress on LTP of glutamatergic transmission are not mediated through direct alteration in either AMPA or NMDA receptor function, but maybe through an indirect mechanism of modulation of excitatory transmission.

4.3 The effect of manipulation of the GABA-ergic on stress-induced changes in synaptic plasticity in CA1.

In this study, the model used was that of acute stress-induced block of LTP in response to HFS. The administration of picrotoxin (PTX) before HFS in stressed animals was shown to consistently produce a delayed long-lasting attenuation of field EPSP amplitude. The two unusual characteristics of the observed LTD-like attenuation were that the inducing stimulation protocol was HFS rather than LFS, although Bramham & Srebro, (1987) have shown that HFS at 400Hz can induce LTD in the DG area of the hippocampus. Also, there was consistently a delay in all animals from stimulation to the actual appearance of LTD of around 30 minutes.

GABA-ergic neurones are believed to be very important in modulating the conditions for pyramidal neurones to undergo synaptic change (Paulsen & Moser, 1998). Evidence for the role of interneurones in synaptic plasticity remains contradictory although interneurones present throughout the hippocampus are heterogeneous and could mediate different actions. However, it would seem likely that the role of hippocampal interneurones should continue or even become more important under stressful conditions. One of the mechanisms by which stress exposure could have caused a block in synaptic plasticity is by enhancement of inhibitory transmission. The presence of PTX under these conditions, which should have led to a reduction in the level of inhibition from the GABA-ergic basket cells within the hippocampus, could restore neuronal excitability sufficiently to enable synaptic plasticity. The stimulation protocol given was HFS, which would normally be expected to induce LTP. Conditions could favour the induction of LTD by HFS if the reduction in inhibition caused by PTX was insufficient, there being some degree of inhibition remaining. In this case HFS might only be sufficient to allow moderate activation of AMPAR and NMDAR so that Ca^{2+} entry is only enough for LTD, which is thought to require lower intracellular levels of Ca^{2+} than LTP induction. Alternatively stress may alter conditions within neurones such that there

is a shift in the intracellular concentration of Ca^{2+} required to induce LTP hence a larger influx of Ca^{2+} is required. With this hypothesis it would be possible that the concentration of Ca^{2+} that enters during HFS in the presence of PTX, that would normally induce LTP is therefore only sufficient to induce LTD.

The above scenario does not explain the observed time delay in the expression of the LTD-like phenomenon seen in stressed animals. Such a time delay may indicate that the mechanisms underlying this particular form of EPSP attenuation are either induced by, or rely upon protein synthesis. Protein synthesis has been shown to underlie the induction of LTD in stressed animals (Xu *et al*, 1998). These mechanisms would have to require both the intervention of stress and reduced $GABA_A$-mediated inhibitory effect caused by the presence of PTX. Treatment of rats with diazepam before exposure to stress resulted in a delayed increase in hippocampal glutamate levels (Bagley & Moghadden, 1996). In that particular study there was no evidence to indicate that enhancement of GABA levels affected the stress-induced changes by enabling either LTP or LTD. If stress is causing a degree of increased inhibition within the hippocampus, and the addition of PTX is not sufficient to reverse this completely enough to allow LTP only LTD, then stress could be inducing additional inhibition via another system. However, Kim *et al*, 1996, found that LTD was more easily inducible in response to LFS and proposed that stress induces a change in the basal output of hippocampal cells such that they are in an LTP-like state. In our animals the combination of PTX and HFS may have produced similar conditions to enable LTD in stressed animals.

Another interesting observation in this study was the block of LTP induction in non-stressed animals injected with PTX. The presence of PTX cause reduced inhibition and therefore might be expected to increase LTP. The block of LTP was noted both where PTX was administered in awake and anaesthetised animals. In awake animals PTX injection was observed to produce behavioural effects that were similar in nature to those observed by raised platform exposure such as defaecation, immoblisation and urination. Therefore, in this particular group of

animals the block of LTP induction could have been caused by PTX-induced stress. The same hypothesis may be applied to anaesthetised animals but it was not possible to observe whether there were any behavioural signs of stress while it remains a possibility that physiological effects that are seen under stressed conditions still occur and account for block of LTP induction.

Alternatively, mild seizure-like activity has been shown to block LTP. It has been reported that seizure activity caused by removal of Mg^{2+} block from NMDA receptors caused a persistant block in the ability to induce LTP (Coan *et al*, 1989) in CA1 area (Hsu *et al*, 2000). This effect also involved the activation of VOCCs and was dependent upon PKC activation thought to be through Ca^{2+} entry via NMDA receptors and VOCCs. Decreased GABA-ergic inhibition produced by the presence of PTX could perhaps allow for a small degree of NMDA receptor activation leading to Ca^{2+} entry and may cause block of LTP by similar mechanisms proposed by Hsu *et al*, 2000. This, however, does not seem a viable hypothesis for the results presented here due to there being no noticeable increase in the EPSP amplitude following PTX administration, which could have indicated a potential increase in AMPA-mediated transmission.

4.4 The effect of pharmacological manipulation of the 5-HT system on stress-induced changes in synaptic plasticity in CA1.

4.4.1 The effect of tianeptine on stress-induced block of LTP.

There were several findings from this study. The primary finding was that tianeptine at a dose that produced no baseline effects completely reversed the effects induced by acute, inescapable stress, enabling LTP induction in response to HFS. This same dose of tianeptine when administered to non-stressed animals produced no effect on the inducibility or magnitude of LTP in response to the same HFS protocol.

Tianeptine is a selective uptake enhancer of 5-HT from the extracellular space (Fattaccini *et al.*, 1990; Kamoun *et al.*, 1989; Broqua *et al.*, 1992; Kato & Weitsch, 1988). Stress has been shown to raise extracellular hippocampal 5-HT concentrations (Amat *et al*, 1998, Kirby & Lucki, 1997), and also in 5-HT and 5-HIAA production (Emerson *et al*, 2000). Inescapable stress raised 5-HT extracellular levels in the basal amygdala whereas escapable stress failed to raise levels relative to control, non-stressed animals (Amat *et al*, 1998). From the results presented here, it is apparent that stress-elevated 5-HT levels caused a block of LTP induction that was long-lasting and complete but reversible in nature, as the 5-HT lowering actions of tianeptine presumably accounted for the complete recovery of the ability to induce LTP.

It has been shown *in-vitro* that tianeptine does not bind to any of the various receptor subtypes within the CNS including GABA, dopamine, 5-HT, muscarinic, histamine or glutamate (Kato & Weitsch, 1988). This apparent lack of pharmacological activity at any of these receptors, shows that the effect of tianeptine must be via another means, perhaps supporting the idea that a direct modulation in the levels of 5-HT produced the alteration in synaptic plasticity.

Anaesthetising the animals immediately after exposure to stress has been shown to maintain the effects of stress. 5-HT levels that have been observed to elevate from exposure to inescapable stress were also observed to still be elevated above basal levels 24 hours following stress (Amat *et al*,1998) in awake animals. Under anaesthetic, the block of LTP was shown to be effective for up to 5 hours after the initial exposure and therefore this may be due to a continued elevation of 5-HT levels.

Further evidence for the role of 5-HT in the stress-induced block of LTP was provided by the results from the studies where individual stereo-isomers of tianeptine were used. Of the two stereo-isomers available both are reported to exhibit a degree of activity in enhancing 5-HT uptake from the extracellular space. However, (-) tianeptine is considered to have a greater ability to enhance 5-HT uptake than (+) tianeptine. The findings from this study found that the (-) tianeptine was the only isomer that was capable of producing a significant reversal similar to that seen with racemic form. (+)Tianeptine produced a small, non-significant potentiation that supports the reports of it having some 5-HT enhancing activity (Spedding *et al*, 1998). This result indicates that the mechanism of action by which tianeptine reverses the effects of stress on synaptic plasticity was by reduction of the stress-elevated levels of 5-HT towards those seen under control, non-stressed conditions. Both isomers were used at a concentration that should have been present in the racemic form. However, the LTP that was seen in the presence of the (-) tianeptine appeared to be lower than that seen with (±) tianeptine. This was not statistically significant. It is also possible that the racemic tianeptine was not a pure 50:50 ratio of the two isomers. Also a slight synergistic effect of the two isomers together is a possibility making the overall effect greater as there was a small increase in field EPSP amplitudes in two of the stressed animals that received (+) tianeptine.

However, there are reports that there is no difference in the actions of the stereo-isomers in reversing some effects incurred by exposure to stress. Magarinos *et al*,

(1999) reported that there was no difference between the two isomers upon the stress-induced atrophy of CA3 neurones. The mechanism by which such atrophy occurs may be different from that which causes the block of LTP, but does imply that tianeptine could have more than one mechanism for reversing stress-induced changes. Given that tianeptine has a mechanism of action that is directly opposed to other clinically used anti-depressants such as fluoxetine, the 5-HT re-uptake inhibitor, it could be that the clinical effect of tianeptine is unrelated to its primary action on 5-HT transport.

The actual site of tianeptine's action in the CNS is difficult to assess. Systemic administration of the drug would have access to the entire area of the brain and potentially affect all 5-HT systems in the CNS. The feedback connections that form part of the 5-HT systems are complex. Initial changes in 5-HT levels could be in the MRN, which is known to have projections to the hippocampus. It remains more likely that any effects of tianeptine are due to a more direct effect on levels in the hippocampus.

Tianeptine injected into animals that had not been exposed to stress produced no alteration in LTP that was induced in response to HFS. This in agreement with finding by Stanton and Sarvey, 1985, that depletion of 5-HT levels did not affect the percentage of LTP induced in rat hippocampus. However, they did observe that while depletion of 5-HT failed to reduce LTP, a similar depletion in the levels of NA within the DG area, depletion of either 5-HT or NA failed to reduce the degree of LTP produced in the CA1 area.

Interestingly, most of the published data on tianeptine involves doses that are ten-fold higher (10mg/kg) than that used in this study. A dose of 5mg/kg tianeptine produced an enhancement in EPSP amplitude in non-stressed animals. This effect was not in evidence at 1mg/kg, the dose which produced such marked changes in synaptic plasticity in stressed animals. Perhaps this illustrated a tonic activity of serotonergic neurones within the hippocampus being partly responsible for

inhibition of pyramidal neurones as 5-HT_3 receptor activation can increase GABA release from interneurones (Kawa, 1994). Tianeptine, at this concentration, could be sufficient to lower the overall degree of inhibition by lowering of extracellular 5-HT, hence enhancing pyramidal excitability.

4.4.2 The effect of fluoxetine and fenfluramine on the stress-induced block of LTP.

The primary findings of this study were that fluoxetine, a 5-HT selective re-uptake inhibitor, when administered to anaesthetised previously stressed animals enabled the induction of LTP. A similar effect on LTP induction was observed when fenfluramine was administered. Both drugs are known to raise 5-HT levels within the hippocampus, although in the case of fenfluramine this may also be due to its additional action as a releaser of 5-HT from nerve terminals.

The results are similar to those observed in stressed animals treated with tianeptine. Interestingly, the unusual aspect of these findings is that the respective drugs exhibit opposite primary mechanisms of action on extracellular levels of 5-HT. However, it could be that, as with corticosterone, there are different effects of 5-HT within the hippocampus depending on the baseline extracellular concentration. It could be that stress-induced elevation of 5-HT is only an intermediate level. This level of 5-HT can be pharmacologically manipulated lower or further elevated, both of which allow for recovery of LTP induction. Therefore, at intermediate levels of 5-HT that are induced by stress exposure, the induction of LTP in response to HSF is reduced or blocked completely. Alterations in the hippocampal levels of 5-HT either by lowering them towards levels that are seen in non-stressed animals or by increasing them further leads to a recovery of LTP.

A study that combined administration of tianeptine and fluoxetine, both of which when given alone enabled recovery of LTP induction, showed that together there was no reversal of the effects of stress. This observation supports the idea that it is modulation of 5-HT levels induced by stress by both of these drugs that enables LTP induction when administered alone. The beneficial effect of each drug is achieved by opposite mechanisms leading to opposite effects on 5-HT levels but leading to the same overall effect on LTP induction. Co-administration inhibits this

effect from both drugs due to a net effect of no change in the extracellular levels or not sufficiently by either one to move out of the 5-HT range that inhibits LTP induction. The results would indicate that the relative doses used are approximately equivalent to one another. Very little work has been done combining the two anti-depressants. Nowakowski *et al*, (2000) observed that both tianeptine and fluoxetine used alone had mediated opposite effects in the forced swim test but both drugs showed anxiolytic activity. The anxiolytic effects of each drug were completely abolished in the immobility time test when the two drugs were given together supporting the results reported here.

The hypothesis is further supported by the observation that fluoxetine (10mg/kg) when administered to non-stressed animals caused a block of LTP. Acute fluoxetine (10mg/kg) administration is known to raise 5-HT levels (Scorza *et al*, 1999) by *in vivo* microdialysis studies (Hervas & Artigas, 1998). Fluoxetine could therefore raise 5-HT levels to within the range that is observed in stressed animals implicating moderately heightened 5-HT levels in the stress-induced block of LTP. Further support for this theory came from the results of the study using fenfluramine in non-stressed animals. Fenfluramine has also been shown to raise the extracellular levels of 5-HT (Series *et al.*, 1994). The observation that fenfluramine at the equivalent dose that produced recovery of LTP in stressed animals, also blocked the induction of LTP in non-stressed animals, not only further implicated the involvement of 5-HT but reduced the likelihood of some alternative action of fluoxetine.

Both fenfluramine and fluoxetine enabled the induction of LTP in stressed animals that was significantly greater than that produced in non-stressed animals. This indicated that LTP induced in non-stressed animals was not saturated. One idea is that stress induces changes such that the actions of fenfluramine and fluoxetine allow for greater potentiation when HFS is applied, or that the first HFS, while not causing LTP, primes the neurones to make them more susceptible to the fluoxetine/fenfluramine/HFS combination.

There was also a difference in the degree of LTP induced in the presence both of these agents. Fenfluramine induced a significantly ($P<0.05$) greater degree of LTP than fluoxetine 60 minutes following HFS. It is not known whether the respective doses of the two drugs are equivalent in their effect on 5-HT levels, but it has been shown that systemic administration of fluoxetine does not cause such a substantial increase in extracellular 5-HT as releasing agents such as fenfluramine (Rutter and Auerbach, 1993, Sabol *et al*, 1992) due to auto-inhibitory mechanisms. This may indicate that there could be a threshold level where the stress-induced detrimental effects of 5-HT are exceeded to facilitate LTP induction once again but that this effect is itself subject to modulation by the absolute levels of 5-HT, higher levels allowing for greater LTP induction. As it has been reported that co-administration of a 5-HT_{1A} antagonist with fluoxetine can potentiate the effects of fluoxetine (Trillat *et al*, 1998), such an approach could have been used here to try and investigate that theory.

4.5 Involvement of 5-HT receptors

4.5.1 5-HT_3 receptors.

5-HT receptors mediate differing effects on hippocampal function. There are a large number of subtypes that mediate both excitatory and inhibitory effects on their target neurones. The studies here that used receptor antagonists did not help to elucidate the potential roles 5-HT receptors in either the block or recovery of LTP. Ondansetron is a 5-HT_3 receptor antagonist and has been reported to increase LTP in non-stressed animals (Staubli *et al,* 1995). However, it would appear from the results presented here that 5-HT_3 receptor blockade following stress exposure by ondansetron, does not restore HFS-induced LTP.

5-HT_3 receptor activation has been shown to increase GABA release from interneurones (Kawa, 1994), which by blocking the effect of could have increased neuronal activity by reducing the overall amount of inhibition in the area. But whatever the changes that are initiated within the hippocampus by stress they do not appear to directly involve this particular group of 5-HT receptors following stress exposure. Whether these receptors are involved in the recovery of LTP when levels of 5-HT are changed from stress levels was not investigated also it was not assessed whether blockade of these receptors prior to stress exposure could affect the mediation of the effects of stress. From the evidence presented here and that is available at present it does not seem likely that there is a role for 5-HT_3 receptors in the stress-induced block of LTP.

4.5.2 5-HT_{1A} receptors.

WAY-100635, the $5HT_{1A}$ receptor antagonist, produced a variable response in stressed animals, with some animals showing a partial recovery of LTP, and others showing attenuation of field EPSPs following HFS. 5-HT_{1A} receptors are found both in the raphe and in the hippocampus. The drug was administered systemically allowing for global access to the CNS. Blockade of 5-HT_{1A} autoreceptors in the raphe nuclei increases 5-HT released at the terminals of serotonergic projections including those in the hippocampus. Conversely, blockade of hippocampal 5-HT_{1A} receptors, which lie post-synaptically, would prevent the increase in 5-HT activation and the subsequent attenuation of target neuronal activity. Therefore maybe the rise in 5-HT caused by WAY-100635 may be negated, at least in part, by the additional receptor blockade in the hippocampus, accounting for the variable response.

Jorgenson *et al*, 1998 observed that WAY-100635 partly inhibited ACTH release that was induced by restraint stress but not swim stress. This may indicate that the effects of 5-HT_{1A} blockade could be dependent upon the type of stress that is used and that effects that are mediated by 5-HT_{1A} receptors may account for only a small part of the serotonergic response to a particular type of stress. Any effects mediated by 5-HT_{1A} receptors does not appear to be attributable to a change in receptor number. Steciuk *et al*, 2000, reported that exposure of rats to acute stress did not alter 5-HT_{1A} receptor density, although density was not assessed in CA1 area of the hippocampus but in the DG area.

5-HT_{1A} receptors may not be directly involved in the stress-induced block of LTP but have been shown to have a possible role in potentiating the effects of 5-HT re-uptake inhibitors (Hashimoto *et al*,1997). Fluoxetine has been shown to produce 30-40% greater 5-HT levels in animals that had been treated with WAY100635 and also potentiated behavioural effects in food deprived animals (Trillat *et al*, 1998).

Similar potentiation of therapeutic effect with combined administration has been observed using pindolol to block 5-HT_{1A} receptors and with other re-uptake inhibitors such as fluvoxamine and paroxetine where the effect was an enhanced anti-depressant response (Blier *et al*, 1997).

4.6 5-HT and corticosterone.

Another aspect of serotonergic involvement in the response to stress exposure is the effect on corticosterone. The relationship between these two systems is a complex series of feedback mechanisms that are tightly linked for maintaining homeostasis (for reviews see Chauloff, 1993 and Raap & Van de Kar, 1999). Exposure to stress is known to cause an increase in peripherally circulating glucocorticoids via release of ACTH. However, different types of stress release ACTH by differing degrees. Stress exposure causes increases in extracellular 5-HT levels (Amat *et al*, 1998, Kirby & Lucki, 1997).

Stress-induced increases in 5-HT have been shown to be dependent upon corticosterone (Azmitia & McEwen, 1974) and these increases can be blocked by administration of the GR antagonist, RU 38486 (Singh *et al*, 1994). Conversely, stress-induced increases in 5-HT have been shown to cause increases in ACTH and corticosterone release. This effect is believed to be mediated by serotonergic afferents from the raphe nuclei to the hypothalamus via 5-$HT_{1A,}$ 5-HT_2 and 5-HT_4 receptors (Jorgenson *et al*, 1998). Interestingly, it has been reported that antidepressants, including tianeptine and fluoxetine, can inhibit gene transcription mediated by glucocorticoids (Budziszewska *et al*, 2000). These reports provide some links between these drugs, particularly tianeptine, and corticosterone. However, desipramine, which was shown not to be effective in reversing the stress-induced block of LTP, also produced the same effect on transcription which may indicate that inhibition of glucocorticoid transcription is not the mechanism by which tianeptine and fluoxetine enable LTP induction in stressed animals.

Acute administration of fluoxetine has been shown to elevate extracellular levels of corticosterone, which is probably from elevating 5-HT at terminals of raphe serotonergic projections. Fenfluramine (both DL and D- forms) also has been shown to raise plasma levels of stress hormones, although to a greater extent than

fluoxetine, again thought to be due to the autoreceptor effect being greater with fluoxetine (Raap & Van de Kar, 1999). Tianeptine has been shown to reduce HPA activity and thus corticosterone release from stress exposure (Delbende *et al*, 1991). It was this action of tianeptine that had been proposed to account for part of tianeptine's therapeutic action in depressed patients. However, it also provides another contrast between the effects of fluoxetine and tianeptine not only with extracellular 5-HT levels but also on corticosterone levels, yet still producing a similar clinical effect. Clinically there has been no observable difference in the response of patients with depressive disorders nor in safety between fluoxetine and tianeptine (Loo *et al*, 1999).

The block of LTP that is seen with exposure to stress, acute treatment of fluoxetine and fenfluramine in the results presented here could be due to a similar mechanism. Alterations caused by stress and pharmacologically raising extracellular 5-HT lead to an increase in the amount of corticosterone. Raised levels of corticosterone have been shown to cause a block of LTP and GR receptor occupation has been implicated to be involved from *in-vitro* studies (Pavlides *et al*, 1996). Perhaps total block of LTP can be achieved alone, but also by both agents acting synergistically and in concert with each other. Such a theory of 5-HT-mediated corticosterone increase explains the block of LTP by stress, fluoxetine, fenfluramine and corticosterone. It also explains the actions of tianeptine-induced recovery of LTP, whereby tianeptine reduces extracellular 5-HT, lowering output stimulation to the hypothalamus hence lowering ACTH and corticosterone (Delbende *et al*, 1991). contributing to the recovery of LTP in the hippocampus.

However, such an idea does not explain the recovery of LTP that was caused by fluoxetine and fenfluramine in stressed animals. Given that 5-HT is such a potent stimulator of ACTH release, raising 5-HT levels above those that are already existing under stress conditions should cause further increases in peripherally circulating corticosterone. The inverted-U relationship that is reported to exist between plasma levels of corticosterone and synaptic plasticity (Diamond *et al*,

1992) would indicate that LTP should continue to be blocked due to additionally elevated levels of corticosterone. Instead, a recovery of the ability to induce LTP is seen. This would indicate that 5-HT levels in themselves could play a large role in the control of synaptic plasticity separate from and above the effects of corticosterone.

4.7 The effect of NA on stress-induced block of LTP.

NA has also been implicated as a neurotransmitter involved in depression and also in the modulation of synaptic plasticity in both the DG and CA1 areas of the hippocampus (Chaulk *et al*, 1998; Sah and Bekkers, 1996). NA levels were raised by the use of both desipramine and nisoxetine, NA uptake inhibitors. In stressed urethane anaesthetised animals there was no effect of these agents on the stress-induced block of LTP in response to HFS. This would indicate that the role of NA is different from that of 5-HT. NA neurones in the locus coeruleus have been described to increase their rate of firing in response to exposure to stressful stimuli (Jacobs *et al*, 1991). Increases in the extracellular levels of NA have been reported to either have no effect on the ability to induce LTP (Katsuki *et al*, 1997), or to enhance the response of the hippocampal system to stimulation that induces LTP (Sah and Bekkers, 1996). Therefore it is difficult to hypothesise what effects stress-induced increases in NA levels might have or what the effect of further increases in NA due to treatment with re-uptake inhibitors would produce.

It has been observed that noradrenergic neurones are significantly more sensitive to stressful stimuli in their responses than 5-HT neurones (Wilkinson and Jacobs, 1988). The increase that is observed in firing rate has been reported to lead to an increase in the amount of extracellular NA in the hippocampus (Abercrombie *et al*, 1988). Modulation of hippocampal function may be more sensitive to changes in 5-HT under both stressed and non-stressed conditions. A reduction in NA levels, comparable to the effect of tianeptine for 5-HT, could have been used to assess whether stress-induced increases in extracellular NA concentration contributed to the block in synaptic plasticity, or by raising levels in non-stressed animals as with 5-HT. The potential modulatory role of NA as a mediator of the effect of stress itself, would not appear to be as powerful or as significant as that of 5-HT and in the recovery of LTP under stressed conditions there appears to be very limited or no involvement of NA at all.

V. Conclusion

5.0 Conclusion.

Exposure to acute episodes of stress blocks the induction of LTP in the CA1 area of the hippocampus in response to HFS. From the evidence presented here and from work done by other laboratories, it can be concluded that a number of changes occur when animals are exposed to stress that can lead to an inability to induce LTP. Reports of the direct involvement of various transmitters, modulators and hormones including glutamate (via NMDA receptors), corticosterone, 5-HT and opioids have been published. Results presented here have further implicated the involvement of 5-HT, glutamate (via AMPA receptors), and GABA, all of which appear to have a role in the stress-induced block of LTP.

A large body of research exists reporting upon the involvement of corticosterone and glucocorticoids in the response of the hippocampus to stress. However, there also exists a growing body of evidence that stress hormones, such as glucocorticoids, are only a part of the responses throughout the periphery and CNS to stress. These changes are all capable of contributing to the effects that are observed within the hippocampus, particularly the block of LTP induction. It was the effects of these other potential factors initiated by stress that were primarily looked at here. It has emerged that 5-HT has a significant role in the mediation of the effects of stress. Manipulation of 5-HT levels within the hippocampus can either aid the recovery of synaptic plasticity from a stress-induced blockade, or can cause blockade of LTP under non-stressed conditions. These effects of 5-HT appear to be dependent upon the degree of change from basal levels. Stress raises 5-HT levels above basal concentrations, treatment that either reduces or increases extracellular 5-HT levels further enables recovery of synaptic plasticity in the form of LTP. GABA has also been implicated from the work here to have a modulatory role in the response to stress. However, a reduction in the level of inhibition under stressed conditions seems to favour a shift in plasticity towards LTD induction rather than LTP. The noradrenergic system, as it was investigated, seems to have

little or no involvement in the effects of stress on hippocampal plasticity. A similar conclusion can be made with regard to the involvement of NMDA receptors. However, there may be a role for AMPA receptor although it remains unclear as to what exactly that may be or whether increasing AMPA conductance is sufficient in itself to overcome the effects of stress.

It remains unclear how each of these stress-induced factors may interact with one another and to what degree or what form any interactions may take. From the evidence available it would appear that all of these factors occur simultaneously or at least they all occur at some stage during and continue after, stress exposure. Extensive work will be required to understand which and when each factor played a role after stress was initiated. The initiation of these stress-induced factors seems to be independent of the stress protocol used, although there is some evidence that stress needed to be of an inescapable variety (Shors *et al*, 1989). However, interestingly, each factor seems to be active during stress exposure as intervention to prevent the actions of just one of them allows for recovery of LTP. In most cases of intervention LTP recovery was equal to or greater than that achieved in non-stressed animals. This means that while each factor has a separate deleterious effect upon LTP induction it only requires one of them to be removed for LTP induction to occur.

There are profound implications in the treatment of acute stress episodes. With the possibility that effective therapy would only require treatment of one stress-induced factor, a broad variety of options for treatment become available as any one of them would be effective in preventing the deleterious effects of stress upon synaptic plasticity.

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