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SYNAPTIC PLASTICITY IN ANIMAL MODELS OF STRESS: SEROTONERGIC MECHANISMS

By

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This research was conducted in the Department of Pharmacology & Therapeutics in the Faculty of Health Sciences.

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Declaration

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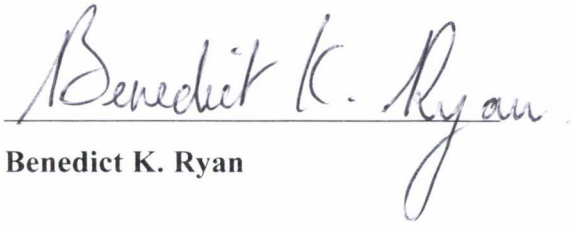

Benedict K. Ryan

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Abbreviations

ACTH	Adrenocorticotrophic hormone
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate
BDNF	Brain-derived neurotrophic factor
BLA	Basolateral amygdala
CaMKII	Calcium/calmodulin-dependent protein kinase II
cAMP	Cyclic adensoine monophosphate
cLH	Congenital learned helpless
cNLH	Congenital non-learned helpless
CNS	Central nervous system
CREB	cAMP response element binding protein
CRF	corticotrophin-releasing factor
DA	Dopamine
DAG	Diacyl glycerol
D-AP-5	2-Amino-5-phosphopentanoic acid
DARPP-32	Dopamine and cAMP regulated phosphoprotein
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
DRN	Dorsal raphe nucleus
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
ERK	Extracellular regulated kinase
FRL	Flinders resistant Line
FSL	Flinders sensitive Line
GABA	γ-Amino-butyric Acid
GAD	Generalized anxiety disorder
GR	Glucocorticoid receptor
GSK-3β	Glycogen synthase kinase-3β
HFS	High frequency stimulation
HPA	Hypothalamic-pituitary-adrenal axis
5-HT	5-Hydroxytryptamine (serotonin)

i.c.v.	Intra-cerebroventricular
I/O	Input/Output
IP3	Inositol 1,4,5-triphosphate
LFS	Low frequency stimulation
LH	Learned Helpless
LTD	Long term depression
LTP	Long term potentiation
MAO	Monoamine oxidase
mPFC	Medial pre-frontal cortex
MR	Mineralocorticoid receptor
NA	Noradrenalin
nLH	non-learned helpless
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide Y
PAG	Periaqueductal gray
PBP	Primed burst potentiation
PCPA	p-chlorophenylalanine
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PND	Postnatal day
PNS	Peripheral nervous system
PPF	Paired pulse facilitation
REM	Rapid eye movement
SD	Sprague Dawley
SERT	Serotonin transporter
SNRI	Serotonin noradrenalin reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
STP	Short term potentiation
TBS	Theta burst stimulation
VDCC	Voltage operated calcium channel

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Summary

The inhibitory effect of acute elevated platform stress exposure on synaptic plasticity in the CA1 region of the hippocampus *in vivo* was investigated. Such stress exposure was confirmed to inhibit subsequent long term potentiation (LTP) induction in urethane anaesthetised rats. We investigated the effect of modulation of the serotonergic system on this stress-induced inhibition of LTP induction. 5-hydroxytryptamine (5-HT) levels are reported to be raised following acute stress exposure and is thought to contribute to stress-induced inhibition of LTP in CA1. We found that injection of the 5-HT releasing agent (±)fenfluramine ((±)-N-Ethyl-α-methyl-m-[trifluoromethyl]phenethylamine) post-stress exposure enabled the induction of LTP. We also investigated distinct 5-HT receptors with respect to their participation in the (±)fenfluramine mediated abrogation of the inhibitory effect of stress on the induction of LTP. We identified 5-HT_2 receptor activation as being of importance in mediating the enablement of the induction of LTP in (±)fenfluramine treated animals which had been previously exposed to acute elevated platform stress. We also investigated agonists of 5-HT_2 receptors and found that activators of 5-$HT_{2B/2C}$ (mCPP:1-(3-Chlorophenyl)piperazine hydrochloride), 5-HT_{2B} (BW723c86: α-Methyl-5-(2-thienylmethoxy)- 1H-indole-3-ethanamine hydrochloride) or $5HT_{2C}$ (MK-212: 6-Chloro-2-(1-piperazinyl) pyrazine hydrochloride) receptors enabled the induction of LTP in animals exposed previously to acute elevated platform stress.

Using the 5-HT_4 receptor partial agonist RS67333 (1-(4-Amino-5-chloro-2-methoxy phenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride) we failed to find experimental evidence to suggest that activation of 5-HT_4 receptors had the ability to relieve the stress-induced inhibition of LTP induction.

We also investigated whether the inhibition of LTP induction due to the NMDA receptor antagonist D-AP5 (D-2-Amino-5-phosphononpentanoic acid) could be affected by (±)fenfluramine administration. We found that administration of (±)fenfluramine enabled the induction of LTP in non-stressed animals treated with D-AP5.

As hippocampal plasticity is disrupted by acute and chronic stress and is hypothesised to be negatively affected in disorders such as depression and anxiety we investigated two

separate animal models of depression: the Flinders Depression Model and the congenital Learned Helplessness Model.

Flinders Sensitive Line (FSL) rats showed impaired LTP in CA1 when compared to their control animals the Flinders Resistant Line. Flinders resistant line animals had significantly greater synaptic excitability and short term plasticity (paired pulse facilitation) which may account for the difference in LTP induction. There was no significant effect of maternal separation in FRL animals, although maternal separation resulted in the failure to induce a statistically significant LTP in FSL animals, the level of potentiation was not significantly different from non-maternally separated FSL animals. Chronic escitalopram ((s)-1-[3-(dimethyl)amino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-5-isobenzofurancarbo nitrile hydrobromide) treatment, a selective serotonin reuptake inhibitor failed to enhance LTP induction in either FRL and FSL treated animals or in FRL and FSL maternally separated animals.

High frequency stimulation induced LTP in congenital learned helpless rats expressing either a learned helpless (cLH-lh) or a non-learned helpless phenotype (cLH-nlh). Surprisingly congenital non-learned helpless rats expressing a non-learned helpless phenotype (cNLH-nlh) had much reduced LTP in CA1 compared to cLH(lh/nlh) animals ($p=0.057$). Outbred Sprague Dawley rats exposed to uncontrollable shock showed significantly less LTP than control non-shocked Sprague Dawley animals ($p=0.05$). This suggests that exposure of cNLH-nlh animals to uncontrollable shock may account for their reduced LTP induction. There was no difference between the baseline synaptic properties of cNLH and cLH groups.

The work presented here suggests that serotonergic modulation may overcome stress-induced inhibition of LTP induction. We suggest that both depression models warrant further scientific investigation. Clinically, these results suggest that 5-HT_2 receptor agonists may prove useful in the treatment of acute stress disorders. Also the models appear to represent two clinically distinct type of depression. The Flinders depression model may be useful in understanding impaired synaptic plasticity associated with unipolar depression while congenital Learned Helplessness may prove effective in modelling synaptic plasticity in refractive depression.

I. Introduction

1.1 The Hippocampus.

The hippocampus, the dentate gyrus and most of the hippocampal gyrus make up the hippocampal formation. The size of the hippocampal formation is species dependent (Stephan et al. 1981). The hippocampus has three sectors, CA1, adjacent to the subiculum, CA2, and CA3 which receives a major input from the dentate gyrus. Within the hippocampal cortex there are three layers, the molecular layer consisting of interacting axons and dendrites, the pyramidal cell body layer through which branches called Schaffer collaterals pass before synapsing with other pyramidal neurons in the molecular layer, and the polymorphic layer containing axons, dendrites and interneurons. The cerebral neocortex, the septal area, the contralateral hippocampus and the nuclei in the reticular formation of the brain stem are the four main sources of afferents to the hippocampal formation. The fornix is the largest efferent pathway of the hippocampal formation.

1.2 The Hippocampus is a centre of Learning and Memory.

It has long been accepted that the hippocampus plays a crucial role in memory formation in particular in relation to declarative memory. The original evidence obtained to support this notion was provided by Scoville & Milner (1957) in which they described a patient after bilateral hippocampal removal which resulted in anterograde amnesia. The hippocampus has also been shown to be critical for spatial learning. Rats having hippocampal/parahippocampal lesions perform poorly in the Morris Water Maze (Morris et al. 1982), a learning paradigm in which an animal is required to use spatial cues to find a hidden underwater platform. However, despite its pivotal role in memory formation, the hippocampus does not function in isolation with respect to memory processes. Memory impairment was less severe in animals having lesions confined to the hippocampus when compared to animals which had additional lesions to the perirhinal, entorhinal, and parahippocampal cortical regions (Bachevalier et al. 1999; Zola-Morgan et al. 2000; Zola-Morgan et al. 1989; Zola-Morgan et al. 1982). Also it is thought that most areas of the cortex can support some form of memory e.g.

visual sensory memory and tactile memory. Although the hippocampus is widely accepted as having the primary role in memory formation some groups have suggested that it may be the neocortex which is responsible for the recall of recent memories (Squire 1992). However, neuroimaging techniques have shown that hippocampal circuits are activated even when very remote memories are recalled (Ryan et al. 2001).

1.3 Long term potentiation.

1.3.1 Transmission at Glutamatergic synapses.

Fast transmission at hippocampal glutamatergic synapses is mediated by three types of ionotropic receptor (1) AMPA receptor (2) NMDA receptor and (3) Kainate receptor. The AMPA receptor is responsible for basal synaptic transmission (Collingridge et al. 1983a; Collingridge et al. 1983b; Monaghan et al. 1983). The AMPA receptor is permeable to Na^+ and K^+ ions which accounts for most of the inward current in postsynaptic spines while at resting potential. The NMDA receptor plays little role in basal transmission although it is critical for long term potentiation (LTP) induction. LTP is a stable, relatively long lasting increase in the magnitude of a postsynaptic response to a constant afferent volley following a brief tetanic stimulation of the same afferent (Bliss & Collingridge 1993).

1.3.2 LTP is a physiological model of memory formation.

The ability of the mammalian nervous system to process, store and retrieve large amounts of information on an ongoing basis has long intrigued scientists. Activity - dependent persistent increases and decreases in the strength of communication between nerve cells (synaptic plasticity) provides an attractive biologically plausible means of information storage in the brain. One such plasticity is called long term potentiation (LTP) (Bliss & Gardner-Medwin 1973; Schwartzkroin & Wester 1975). A number of factors point to LTP as a viable mechanism by which memories are formed and stored

(Doyere et al. 1992; Morris et al. 1990). LTP, like memories can be generated rapidly. It shows a high degree of synapse specificity which allows for a huge storage capacity given the number of synapses in the mammalian brain. In addition LTP appears associative in that activity at one point in the dendritic tree can influence the probability of a change in synaptic strength at another (Wigstrom et al. 1986b; Malinow & Miller 1986). Furthermore LTP is most reliably induced in brain areas thought to be involved in learning and memory while normal neuronal activity seen in learning and memory appears able to induce LTP (Power et al. 1997). LTP in the hippocampus may take two distinct forms (a) NMDA receptor dependent (b) NMDA receptor independent (Grover & Tyler 1990; Nicoll & Malenka 1995; Wang et al. 1997).

1.3.3 NMDA receptor dependent LTP.

Most experimental work has focused on NMDA receptor dependent LTP.

The NMDA receptor is a voltage dependent ion channel. Its channel is blocked by extra-cellular Mg^{2+}. Following high frequency stimulation (HFS) resulting in postsynaptic membrane depolarization, the Mg^{2+} is removed from its binding site on the NMDA receptor allowing an influx of Ca^{2+} and Na^{+} into the dendritic spine. If the influx of Ca^{2+} is sufficient in both duration and concentration LTP is induced. In situations where the elevation in calcium levels is not sufficient to induce LTP a short term potentiation (STP) may be induced lasting between 5–10mins or alternatively a long term depression (LTD), a long lasting decrease in synaptic activity may result. Experimental data demonstrating the critical role of Ca^{2+} in LTP induction was provided by Lynch et al. (1983). They showed that the use of calcium chelators to prevent a rise in postsynaptic Ca^{2+} resulted in a block of LTP induction. Furthermore, it was shown that experimentally raising postsynaptic Ca^{2+} concentration resulted in LTP. However, release of intracellular calcium in addition to the influx of extra-cellular calcium is required for LTP induction. Evidence pointing to this fact was provided by Bortolotto & Collingridge (1993). They showed that use of the intracellular calcium depleting agent thapsigargin prevented induction of LTP.

1.3.4 CaMKII plays a key role in LTP.

Research has suggested a role for many different molecules in the induction and maintenance of LTP. It has however been difficult to disentangle key mediators from modulators of the process. Following Ca^{2+} influx through NMDA receptors various kinases/phosphatates are activated, suggested to be the molecular basis for LTP. One such molecule is CaMKII (Fukunaga et al. 1993; Ouyang et al. 1997). CaMKII is thought to act as the bridge linking a transient increase in Ca^{2+} concentration to neuronal plasticity. It is well placed to play such a role as it makes up 1-2% of total neuronal protein content with its expression being particularly high postsynaptically (Kennedy 1997). Much experimental evidence has been provided supporting the notion of CaMKII as a key mediator of LTP. Following Ca^{2+} induced activation CaMKII remains active due to autophosphorylation at Thr-286. Mutation of this residue prevents autophosphorylation impairing both LTP and spatial learning (Giese et al. 1998). Also transgenic mice in which the CaMKII gene has been deleted have impaired LTP and memory (Silva et al. 1992). In CA1 pyramidal neurons, a constitutively active CaMKII when infused resulted in an increase in size of the AMPAR mediated EPSC concomitantly occluding LTP induction (Lledo et al. 1995; McGlade-McCulloh et al. 1993). Furthermore postsynaptic injection of inhibitors of CaMKII or genetic deletion of a crucial CaMKII subunit results in a block of LTP induction (Malenka et al. 1989). Finally, the GluR1 subunit of the AMPA receptor is phosphorylated during LTP. CaMKII can directly phosphorylate this subunit (Barria et al. 1997). Taken together CaMKII activation appears sufficient for and critical to induction of LTP.

1.3.5 LTP maintenance.

Once induced LTP may last for several hours *in vitro* or even days *in vivo* (Bliss & Gardner-Medwin 1973). Such long-term maintenance must obviously involve mechanisms other than those employed simply for induction. Maintenance of LTP may be divided into 2 phases: early phase LTP (lasting up to a few hours) requires post-

translation modification while maintenance of late phase LTP is dependent on *de novo* gene expression and protein synthesis (Krug et al. 1984; Otani et al. 1989; Nguyen et al. 1994). While LTP in the dentate gyrus has been shown to be decremental in nature (Abraham et al. 1994; Abraham 2000) LTP in CA1 has been reported to be both decremental (Buzsaki 1980; Leung & Shen 1995) and non-decremental (Staubli & Lynch 1987). Phosphorylation of the transcription factor CREB has been implicated as an essential component of the translational machinery necessary for maintenance of LTP in dentate (Bourtchouladze et al. 1994). A second transcription factor important for LTP maintenance is zif268 (Jones et al. 2001). Interestingly in CA1, late phase LTP appears dependent on activation of D1/D5 dopamine receptors (Frey et al. 1990; Swanson-Park et al. 1999). Furthermore it has been shown that LTP maintenance is not a passive process. It is an active process with late-phase LTP decay being prevented by the NMDA receptor antagonist CPP (Villarreal et al. 2001). Evidence was provided by Duffy et al. (1981) that linked LTP induction protocols with a concomitant increase in protein synthesis. Other theories put forward to explain LTP maintenance include: increase in the size of the dendritic spine (Fifkova &Van Harreveld 1977), an increase in the amount of transmitter release (implying the existence of a retrograde messenger) (Dolphin et al. 1982; Williams et al. 1989) and an increase in glutamate receptor density (Shi et al. 1999). Transgenic mice expressing an inhibitory form of the regulatory subunit of PKA do not maintain the late phase of LTP show however no disruption to early phase LTP (Abel et al. 1997). Moreover mice lacking a PKA subunit show LTP following HFS but also fail to maintain it. Mice lacking both types 1 and 8 calcium activated adenylyl cyclases show deficits in late phase LTP (Wong et al. 1999). Therefore cAMP and PKA can be said to be critical mediators of late phase LTP.

1.3.6 AMPA receptors and LTP

Two distinct mechanisms modulate the AMPA receptor function during LTP/LTD induction.

(1) Modulation of the channel itself

(2) Targeting of the receptor to silent synapses

AMPA receptor subunits are targeted by kinases/phosphatases following HFS (Lee et al. 1999; Soderling & Derkach 2000). They are phosphorylated on at least 12 distinct sites (Roche et al. 1996). Hippocampal neurons exposed to peptide inhibitors of PKA showed inhibited AMPAR mediated currents suggesting PKA to be important in regulating basal AMPAR function (Greengard et al. 1991). Furthermore maintenance of increased AMPA responses seems to require expression of newly synthesised AMPA receptors, a process which requires PKA (Nayak et al. 1998).

Silent synapses are synapses which contain NMDARs but no AMPARs and therefore do not normally react to synaptically released glutamate (Dingledine et al. 1999). LTP induced by NMDAR activation increases the amount of AMPAR clusters (Shi et al. 1999). Furthermore this process was blocked by tetanus toxin suggesting membrane fusion as the mechanism by which AMPARs are inserted into silent synapses during LTP (Lu et al. 2001; Liao et al. 2001). Additional evidence supporting a role for AMPARs involvement in synaptic plasticity was the finding that low frequency stimulation (LFS) that induced long term depression (LTD) was occluded by compounds which resulted in the internalization of the AMPA receptor (Luthi et al. 1999). A rapid redistribution of glutamate receptors has been shown to contribute to LTD in hippocampal cultures (Carroll et al. 1999). This results in a decrease in the number of synaptic AMPA receptors.

1.4 Stress.

1.4.1 The concept of stress.

Stress as a psychophysiological concept is as yet poorly defined. Traditionally 'stress' is defined as any variable which disturbs physiological and psychological homeostasis resulting in release of catecholamines by the sympathetic nervous system and corticosteroids from the adrenal glands. However, problems arise with such a broad definition for a number of reasons. Firstly, whether or not a stimulus is perceived as stressful is often subject dependent i.e. what may be perceived as stressful by one individual may not be so perceived by another. Secondly, while stressful events result in elevated glucocorticoid levels so too does exposure to pleasurable events such as feeding, exercise and sex (Phoenix et al. 1977; Bronson & Desjardins 1982; Kanaley et al. 2001; Rosmond et al. 2000). Furthermore, glucocorticoid levels are indifferent to controllability, yet controllability has a major influence on the impact of an aversive event on physiology and behaviour (Maier & Seligman 1976; Maier & Watkins 1998). A more recent and specific definition has been proposed by Kim & Diamond (2002). They define stress as " a condition in which an individual is aroused by an aversive situation with the magnitude of the stress and its physiological consequences being greatly influenced by the individuals perception of its ability to control the presence or intensity of the stimulation". Interestingly stress can precipitate psychiatric illnesses, in particular depression. Exposure to chronic stress has been shown to induce depression. Indeed depression is often preceded by stressful situations. Moreover changes observed in the 5-HT and stress hormone systems in depressed individuals are mimicked by exposure to chronic stress.

1.4.2 Biochemical effects of stress exposure.

The stress response involves an initial wave of catecholamine release followed by activation of their associated 2^{nd} messenger systems. This process occurs within

seconds of initial stress exposure. A second wave involves release of glucocorticoids from the adrenal glands. The hippocampus is involved in the neuroendocrine regulation of stress hormones (McEwen BS et al. 1995). The hippocampus is rich in both mineralocorticoid (Type 1) and glucocorticoid (Type 2) corticosteroid binding receptors (Reul & de Kloet 1985). Glucocorticoid mediated negative feedback in the hippocampus which inhibits the hypothalamus-pituitary-adrenal (HPA) axis is important in terminating the stress response (Miller & O'Callaghan 2002).

1.4.3 Anatomical impact of stress exposure on hippocampal structures.

Following daily restraint stress or chronic corticosterone injections atrophy of CA3 apical dendrites of pyramidal neurons occurs (McEwen et al. 2000). However, stress induced atrophy is not confined to CA3 but is also found in CA1 and dentate gyrus (Sousa et al. 2000). Interestingly, stress/corticosteroid induced atrophy may be prevented by agents which reduce extra-cellular 5-HT levels (tianeptine), reduce excitatory-amino-acid neurotransmission (phenytoin) or through an enhancement of GABA tone reduce general excitability (benzodiapines) (Brown et al. 1999). Intriguingly, while activation of NMDARs accompanied by the necessary influx of Ca^{2+} is required for LTP induction it seems that stress's adverse effects on hippocampal morphology may at least in part be NMDAR activation dependent. Evidence for this linkage was provided by McEwen (2000). They showed that corticosterone enhanced cell death which is linked to elevated Ca^{2+} levels can be reduced in the presence of NMDAR antagonists.

Hippocampal-dependent learning is associated with production of granule cells in the adult dentate gyrus (Gould & Gross 1999). Stress exposure decreases the proliferation of granule cells in adult rats (Gould et al. 1999). Since increased cortisol levels is linked to a decrease in granule cell production so the removal of endogenous corticosteroids enhances new granule cell synthesis (Gould E et al. 1997). As granule cell precursors lack both MRs and GRs, glucocorticoids effect on granule cell synthesis is therefore indirect.

1.4.4 Brief stress exposure enhances memory.

While most experimental work has focused on the deleterious impact of stress exposure on learning/memory and LTP it has also been shown that brief periods of stress can potentiate memory formation at least for the emotional event itself (Cahill et al. 1994). Animal studies have shown enhanced memory for emotional events. This process is thought to be mediated by enhanced catecholamine release (Cahill et al 1994; McGaugh et al. 1989). Catecholamines act indirectly to improve memory by increasing glucose utilization in the brain (Cahill et al. 1994; McGaugh et al. 1989) resulting in improved cognition. Elevation of glucose concentrations potentiates both anterograde and retrograde memory formation in both laboratory animals and humans (Parsons & Gold 1992). Furthermore, patients with Alzheimer's disease showed enhanced performance in memory tasks following glucose administration (Manning et al. 1993), while severe hypoglycemia is known to impair memory.

1.4.5 Stress inhibits memory formation.

Activation of the sympathetic nervous system and the rise in glucocorticoids contribute to the deleterious effect of stressors on memory formation and LTP induction. Exposure to elevated levels of catecholamines and supraphysiological concentrations of glucose as well as elevated corticosterone levels have been shown to be deleterious to memory (McGaugh et al. 1989). Healthy human subjects when administered stress levels of cortisol exhibit selective impairment of verbal declarative memory (Newcomer et al. 1994). Also individuals presenting with hypercortisolaemia and depression display hippocampus-dependent memory impairment. Furthermore, Cushing's disease sufferers (excessive glucocorticoid secretion) show deficits in declarative memory (Starkman et al. 1999) and hippocampal atrophy (Sapolsky et al. 1999) which is reversible upon treatment to reduce cortisol levels (Starkman et al. 1999). Similarly, in animal models, rats exposed to stress or treated with corticosterone show deficits in spatial memory (Luine et al. 1993; Diamond 1999; Diamond 1996; de Quervain 1998). Transgenic

mice having elevated corticosterone levels due to central over-expression of CRF also show impaired spatial memory (Heinrichs et al. 1996).

1.4.6 Stress inhibits LTP.

LTP in the hippocampus, a putative memory model is inhibited by psychological stressors which induce high levels of circulating catecholamines and supraphysiological levels of glucocorticoids. The inhibitory effect of stress on LTP induction was first described in 1987 (Foy et al. 1987). It was discovered that rats exposed to unpredictable and inescapable restraint tailshock showed reduced LTP in hippocampal CA1. Further studies showed that the stress induced impairment lasted for up to 48 hours (Shors et al. 1997). Also it was shown that stress induced LTP impairment was not limited just to CA1 but was also found in dentate gyrus (Shors & Dryver 1994). Interestingly, in parallel to behavioural studies (Maier & Seligman 1976) it was discovered that controllability was the key factor in the stress induced impairment of LTP with the intensity of the negative stimulus being of secondary importance (Shors et al. 1989). Other forms of psychological stressor such as inescapable elevated platform stress also has been shown to block CA1 LTP (Xu et al. 1997). Exposure to predator stress while blocking PBP (primed burst potentiation) (Mesches et al. 1999) and impairing hippocampal-dependent spatial memory (Diamond et al. 1999) did not impair LTP (Mesches et al. 1999) suggesting that PBP has a greater sensitivity to modulation by stress than LTP. Glucocorticoids have a biphasic effect on LTP and PBP (Diamond et al. 1992; Bennett et al. 1991). High levels of glucocorticoids evident following stress exposure inhibit LTP while lower concentrations seen during the diurinal rise enhance LTP. The concentration dependent differential effects of glucocorticoids is accounted for by the existence of 2 distinct types of glucocorticoid receptor. Activation of hippocampal high affinity mineralocorticoid receptors enhances LTP while inhibition of LTP is associated with high concentrations of endogenous glucocorticoids activating low affinity glucocorticoid receptors (Pavlides et al. 1995). Furthermore, the GR antagonist RU38486 prevented stress induced impairment of hippocampal LTP (Xu et al. 1998). Interestingly stress exposure and application of glucocorticoid agonists

facilitates the induction of LTD while concomitantly inhibiting LTP (Xu et al. 1997; Kim et al. 1996; Pavlides et al. 1995). Such a result indicates that stress may alter the physiological range of plasticity or may induce a metaplasticity favouring subsequent depression (Kim & Yoon 1998).

1.4.7 Raised corticosterone levels are not sufficient nor necessarily required to block hippocampal LTP or memory processes.

Many lines of evidence now suggest that a simple rise in corticosterone level is not sufficient of itself to block LTP. Furthermore evidence suggests that not all inhibition of LTP following stress is due to raised corticosterone concentrations. Rats having undergone adrenalectomy still show impaired LTP following stress (Shors et al. 1990). Non-adrenalectomised rats following treatment with dexamethasone still show LTP impairment following stress (Foy et al. 1990). Also although showing raised corticosterone levels, rats in which the amygdala has been lesioned display no stress induced LTP impairment (Kim et al. 2001). Exogenous administration of stress levels of corticosterone failed to induce spatial memory impairments (Diamond et al. 2002). In addition male rats with access to receptive females show elevated corticosterone levels however this is not accompanied by spatial memory impairment (Woodson et al. 2003).

1.4.8 Alternative neuromodulatory candidates that may mediate stress induced LTP blockade.

Antagonists of the NMDAR have been shown to prevent the effect of stress on learning (Shors & Servatius 1995), LTP (Kim et al. 1996) and the GR-mediated impairment of LTP (Coussens et al. 1997). Treatment with the opioid antagonist naltrexone prevents the negative impact of stress on learning (Maier 1990) and LTP (Shors et al. 1990). Furthermore, 5-HT, an indolamine released following stress exposure prevents CA1 LTP induction *in vivo* (Staubli & Xu 1995) and *in vitro* (Corradetti et al. 1992).

1.5 Serotonin.

1.5.1 General Introduction.

Serotonin is a biogenic amine synthesised from the amino acid tryptophan and later metabolised by monoamine oxidase (MAO). It has numerous effects (regulation of appetite, control of sleep patterns and regulation of sexual activity) owing to the existence of 7 distinct 5-HT receptor families (Barnes & Sharp 1999). Apart from the 5-HT_3 receptor family which is a ligand gated ion channel the remaining members of the 5-HT super family are G-protein linked receptors. In the central nervous system 5-HT acts as a neurotransmitter and neuromodulator. It acts unilaterally as well as in concert with other neurotransmitter systems (Lieberman et al. 1998). Abnormalities in the serotonergic system have been implicated in the pathology of many different CNS disorders such as depression, schizophrenia and generalised anxiety disorder (GAD) (Jones & Blackburn 2002). This has led to intense interest in the 5-HT system in order to identify therapeutic agents to treat such debilitating disorders.

1.5.2 Serotonin Receptors.

All the native 5-HT receptors are found postsynaptically, with respect to 5-HT terminals, and some are located presynaptically where they regulate the firing rate of 5-HT neurons and/or release of transmitter from their terminals. 5-HT_{1A} receptors are localised as inhibitory autoreceptors on the dendrites of serotonergic cell bodies in the raphe nuclei. They are also localised postsynaptically to serotonergic neurones in the hippocampus, septum, amygdala, periaqueductal gray, entorhinal cortex and frontal cortex. In the hippocampus they are located on both pyramidal and granular neurones (Pompeiano et al. 1992; Burnet et al. 1995; Francis et al. 1992). 5-HT_{1B} receptors are located postsynaptically in the frontal cortex, hippocampus, amygdala as well as other corticolimbic structures (Bruinvels et al. 1994; Bonaventure et al. 1998). Inhibitory 5-HT_{1B} autoreceptors have also been located on the terminals of serotonergic neurons

(Bruinvels et al. 1994; Hopwood & Stamford 2001a; De Groote et al. 2002). It is also suggested that 5-HT_{1B} receptors may also be located pre-synaptically on both 5-HT and non 5-HT neurones (Barnes et al. 1999). They may act as terminal heteroreceptors, controlling the release of other neurotransmitters, such as acetylcholine, glutamate, dopamine, noradrenaline and γ-aminobutyric acid (Pauwels et al. 1997). 5-HT_{1D} receptors are located in low concentrations in the basal ganglia, nucleus accumbens, hippocampus, frontal cortex and raphe nuclei. It is suggested to be located presynaptically on both the 5-HT neuronal cell bodys and terminals. 5-HT_{2A} receptors are found in high concentrations in the entorhinal cortex, amygdala, nucleus accumbens and hippocampus (Lopez-Giménez et al. 1997; Cornea-Hebert et al. 1999; Xu & Pandey 2000; Vaidya et al. 2001, Millan et al. 2002a). Activation of 5-HT_{2A} receptors stimulates hormone secretion, e.g. ACTH, corticosterone, oxytocin, rennin and prolactin (Van de Kar et al. 2001). 5-HT_{2B} receptors have been found in low concentrations in the CNS when compared to the PNS. However, they have been detected in the amygdala, lateral septum and in the hypothalamus (Duxon et al. 1997a; Russel et al. 2002). 5-HT_{2C} receptors are found in the frontal cortex, hippocampus, amygdala, nucleus accumbens, hypothalamus, periaqueductal gray and septum (Wright et al. 1995; Sharma et al. 1997; Clemett et al. 2000). Activation of 5-HT_{2C} receptors exert a tonic, inhibitory influence upon frontocortical dopaminergic and adrenergic, but not serotonergic transmission. It also plays a part in neuroendocrine function (Millan et al. 1998; Jorgensen et al. 1999). 5-HT_3 receptors are expressed in high densities in the hippocampus (Waeber et al. 1994). Activation of 5-HT_3 receptors results in rapid depolarisation due to a transient inward current. A functional 5-HT_3 receptor requires a heteromeric combination of 5-HT_{3A} and 5-HT_{3B} subunits (Dubin et al. 1999; Hanna et al. 2000). Its activation results in dopamine release. 5-HT_4 receptors are located in the hippocampus, septum and amygdala (Waeber et al. 1994; Jakeman et al. 1994, Bonaventure et al 2000). 5-HT_4 receptor activation affects GABAergic transmission in frontocortical pyramidal neurones in a bi-directional fashion (Cai et al. 2002a; Yan, Z. 2002). In addition activation of 5-HT_4 receptors is reported to facilitate firing in DRN serotonergic neurones (Jakeman et al. 1994; Ge & Barnes 1996; Lucas & Debonnel; 2002). The 5-HT_5 receptor family has 2 members (5-HT_{5A} and 5-HT_{5B}). Human brain

contains only 5-HT_{5A} receptors (Grailhe et al. 2001). 5-HT_5 receptors are present in high densities in the hippocampus, cortex, amygdala and in the locus coeruleus and raphe nuclei (Pasqualetti et al. 1998; Oliver et al. 2000; Kinsey et al. 2001). 5-HT_6 receptors are located in high densities in the hippocampus, nucleus accumbens, amygdala, frontal cortex and entorhinal cortex (Yoshioka et al. 1998; Hamon et al. 1999; Kinsey et al. 2001; Millan et al. 2002a; Roberts et al. 2002b). 5-HT_6 receptors located in the hippocampus and striatum are predominantly post-synaptic to 5-HT neurons. They are located on the dendrites of both excitatory pyramidal and granule cell neurons in the hippocampus. 5-HT_7 receptors are found in the hippocampus, cortex, lateral septum, hypothalamus and amygdala (Hagan et al. 2000; Kinsey et al. 2001; Neumaier et al., 2001). ERK, a mitogen-activated protein kinase is activated in primary neuronal cultures by 5-HT_7 receptor activation (Errico et al. 2001).

Receptor	Subtype	Effector	G protein
5-HT_1	5-HT_{1A} 5-HT_{1B} 5-HT_{1D} 5-HT_{1E} 5-HT_{1F}	↓ Adenylyl cyclase	G_i/G_O
5-HT_2	5-HT_{2A} 5-HT_{2B} 5-HT_{2C}	↑ PLC	$G_q/_{11}$
5-HT_3	5-HT_{3A} 5-HT_{3B}	Ion channel $(Na^+/K^+/Ca^{2+})$	—
5-HT_4		↑ Adenylyl cyclase	
5-HT_5	5-HT_{5A} 5-HT_{5B}	↓ Adenylyl cyclase ?	G_i/G_0 ?
5-HT_6		↑ Adenylyl cyclase	G_s
5-HT_7		↑ Adenylyl cyclase	G_s

Table 1.1 Classification of Serotonin Receptors.

The above table shows the different 5-HT receptors as well as their respective signal transductions mechanisms.

1.5.3 Serotonin and the Hippocampus.

The serotonergic innervation of the hippocampus is derived from the 5-HT neurons of the median and dorsal raphe (Conrad et al. 1974). These neurons have a slow regular firing rate (Wang & Aghajanian 1977). This regular firing rate is attributed to a pacemaker which involves a calcium dependent potassium current (Aghajanian et al. 1987). Alterations to this regular firing pattern occur during the sleep wake cycle (Jacobs, B.L. 1990). 5-HT neurons are silent during REM sleep and discharge at 3Hz during quiet periods and 6Hz during arousal (Trulson & Jacobs 1979).

Reduction in firing rate may be achieved by activation of 5-HT_{1A} autoreceptors which leads to potassium channel opening and membrane hyperpolarization (Williams 1988; Sprouse & Aghajanian 1987). Activation of 5-HT_{1A} receptors in the raphe area also results in a decrease in serotonin release in the hippocampus (Blier et al. 1990; Sharp & Hjorth 1990). The 5-HT autoreceptors of the hippocampus are of the 5-HT_{1B} subtype (1D in non-rodents). They control the release of 5-HT from terminals without effecting the propagation of action potentials (Starke et al. 1989). Synaptically released 5-HT upon electrical stimulation of the 5-HT neuron has been shown to reduce the firing rate of postsynaptic pyramidal neurons (Blier & de Montigny 1983; Blier & de Montigny 1985). Also 5-HT autoreceptors are thought to decrease 5-HT release by reducing calcium influx by voltage –dependent calcium channels. Conversely activation of presynaptic 5-HT_3 receptors results in further 5-HT release (Blier & Bouchard 1993; Galzin & Langer 1992; Martin et al. 1992).

Postsynaptically 5-HT has a dual action. It is both inhibitory and excitatory of hippocampal neurons. Application of 5-HT to pyramidal neurons results in an increase in potassium conductance leading to membrane hyperpolarization (Colino & Halliwell 1987; Jahnsen 1980; Segal 1980).This phenomenon has been shown to be mediated by 5-HT_{1A} receptors. 5-HT_{1A} receptor activation achieves this hyperpolarisation in the absence of adenylate cyclase inhibition. The excitatory effect of 5-HT appears to be mediated via 5-HT_4 receptor activation leading to an increase in cAMP levels and a decrease in potassium conductances (Andrade & Chaput 1991; Chaput et al. 1990; Roychowdhury et al. 1994).

1.5.4 Serotonin and the HPA axis.

A two way interaction exists between the serotonergic system and corticosteroids in the brain. For instance the serotonergic system is known to regulate ACTH, cortisol and CRF release (Spinedi & Negro-Vilar 1983; Calogero et al. 1989; Plotsky et al. 1989; Van de Kar 1991; Jorgensen et al. 1998). Moreover, inhibitors of 5-HT synthesis or lesioning of central serotonergic neurons reduces fluctuations in ACTH and corticosterone concentrations due to the circadian rhythm (Tuomisto & Mannisto 1985; Chauoloff 1993). In addition activation of 5-HT_1, 5-HT_2 and 5-HT_4 receptors results in the release of ACTH (Bagdy et al. 1989; Gartside et al. 1992; Li et al. 1992). In contrast, Souza and De Loon (1986) reported a relationship between corticosterone level and 5-HT turnover in the CNS. Acute increases in plasma cortisol levels is reported to result in an increase in CNS 5-HT concentrations (Davis et al. 1995) while chronic stress exposure or chronic increases in plasma cortisol levels result in a decrease in both 5-HT turnover (Weiss et al. 1981) and release (Maes & Meltzer 1995). Indeed adrenalectomy has been shown to decrease serotonin turnover in rat brain. Furthermore the serotonin receptors themselves change in response to differential cortisol levels. 5-HT_{1A} receptor responds to increased cortisol levels by initially becoming more sensitive and later becoming desensitized upon more prolonged exposure to elevated cortisol levels (Young et al. 1994; Crayton et al. 1996). 5-HT_{1A} receptor mRNA is also reduced (Meijer & de Kloet 1994). Thus corticosterone facilitates 5-HT release. Similarly an acute increase in plasma cortisol level results in desensitization of the 5-HT_2 receptor (Yamada et al. 1995) while more prolonged exposure to raised cortisol leads to receptor upregulation (Fernandes et al. 1997).

1.5.5 Serotonin and LTP.

Hippocampal LTP has been shown to be affected by alterations in 5-HT levels (induced for example by stress exposure or by pharmacological manipulation of the 5-HT system). Studies have shown that the administration of selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Shakesby et al. 2002) or fluvoxamine (Kojima et al. 2003) inhibit the induction of hippocampal LTP. So too does the 5-HT_{1A} receptor agonist tandospirone (Mori et al. 2001). Villani & Johnston (1993) hypothesised that the reduction in LTP by 5-HT was due to the hyperpolarisation of postsynaptic neurones. Other authors have also provided evidence for the inhibitory effects of serotonin on hippocampal LTP induction (Corradetti et al. 1992; Staubli & Otaky 1994; Staubli & Xu 1995). However the effect of serotonin on hippocampal LTP remains controversial with other evidence suggesting that serotonin may facilitate hippocampal LTP induction (Bliss et al. 1983; Klancnik & Philips 1991; Markevich et al. 1994).

1.6 Animal models of depression.

1.6.1 Learned Helplessness.

Learned helplessness (Overmier & Seligman, 1967) is a stress model of depression in which a subset of animals following exposure to uncontrollable stress exhibit a deficit in learning to escape controllable stress. The behavioural condition is induced through application of uncontrollable and unpredictable aversive stimuli. The condition can be induced in 10-20% of rats with footshock, in a single training session of between 40 and 60 min depending on the sensitivity of the rat strain and intensity of the footshock. Helpless animals have face validity as a model of depression. Helpless animals have weight loss, agitated locomotor behaviour, sleep changes, decreased libido, decreased learning in some tests but not in spatial learning tasks and alterations in the HPA axis with elevated corticosterone which is not suppressible by dexamethasone (Adrien et al. 1991; Dess et al. 1988; Dess et al. 1989; Greenberg et al. 1989) Helpless animals also show less preference for sucrose than control animals indicative of an anhedoniac state (Gambarana et al. 2001; Minor et al. 1994; Vollmayr & Henn 2003). Helpless animals respond to antidepressant treatment. Intra-peritoneal administration of antidepressants was found to reverse helplessness (i.e. induced escape behaviours) at day 5 of treatment (Sherman et al. 1982). Interestingly this replicates the lag phase in antidepressant treatment seen in humans and lends predictive validity to helplessness as a model of depression. Furthermore BDNF a molecule thought to mediate anti-depressant activity (Coyle & Duman 2003) reverses learned helplessness behaviour (Shirayama et al. 2002; Siuciak et al. 1997). 5-HT_{1B} receptors are up-regulated in the cortex, hippocampus and septum of learned helpless animals (Edwards et al. 1991). Compared to nLH (non-learned helpless) hippocampal slices, slices from LH (learned helpless) rats show a significant increase in endogenous and K^+-stimulated 5-HT release (Edwards & Henn 1992). Moreover PCPA lesions that depleted 5-HT protect against the development of helplessness (Brown et al. 1982; Edwards et al. 1986). Both beta-adrenergic and mu opioid receptors in the hippocampus and cortex are upregulated in

LH animals (Henn 1993; Martin et al. 1990). Helplessness behaviour is also associated with dysregulation of the HPA-axis. Following inescapable shock corticosterone levels are raised but are not suppressed by dexamethasone (Greenberg et al. 1989). Furthermore adrenalectomised rats appear more sensitive to the effects of uncontrollable shock (Edwards et al. 1990).

1.6.2 Congenital Learned Helplessness.

In an effort to increase the yield of helpless animals following shock training research groups have developed a congenital learned helplessness model in Sprague-Dawley rats. In this model selective breeding takes place, mating animals susceptible to learned helplessness following shock exposure. In this way genes are selected which predispose for a helpless phenotype. As a control, animals resistant to the learned helplessness phenotype following training are mated. After multiple generations two distinct phenotypes have evolved. Firstly a congenital Learned Helpless line (cLH) which exhibit a helpless phenotype in the absence of uncontrollable shock. A second group termed congenital non-Learned Helpless (cNLH) are a strain of animals resistant to the effects of uncontrollable shock exposure. Unlike learned helpless outbred Sprague Dawley rats that become learned helpless following uncontrollable shock exposure cLH rats do not respond to antidepressant treatment. cLH and cNLH animals show similar acquisition of the Morris water maze task (Vollmayr et al. 2004) They also show similar memory retrieval in the probe trial. Both cLH and cNLH animals acquire the FR1 response for sucrose. SERT is upregulated in the raphe nuclei of cLH rats and $5HT_{1B}$ mRNA is up regulated in the raphe dorsalis of cNLH rats (Neumaier et al. 2002). Mu opioid receptors of cLH rats are up regulated in the hippocampus and cortex but down regulated in the hypothalamus (Henn et al. 1993). There are no differences in the levels of corticosterone between nonstressed and stressed cLH and cNLH rats (Vollmayr et al. 2001). cLH rats have an altered responsiveness to early stress which result in changes to the HPA axis and the renin-angiotensin system (Edwards 1999; King et al. 1993; King & Edwards 1999). Moreover cLH animals have an impaired BDNF response to stress (Vollmayr et al. 2001). Interestingly cNLH animals show a

decrease in hippocampal neuropeptide Y (NPY) mRNA of the order of 30-35% when compared to SD or cLH animals (Lachman et al. 1992). In addition, cLH rats show altered gene expression including a decrease in hippocampal CREB, PKA, PKCα and GSK-3β mRNA (Kohen et al. 2003). Finally, in the hypothalamus of cLH rats expression of bcl-2 mRNA is increased.

1.6.3 Flinders Sensitive Line.

The Flinders Sensitive Line (FSL) is a widely accepted animal model of depression developed originally at Flinders University, Australia. It is a Sprague-Dawley line bred selectively for individuals supersensitive to cholinergic agents (Overstreet & Russell 1982; Overstreet 1986; Overstreet et al. 1986). The logic behind the development of such a model came from the cholinergic hypothesis of depression (Janowski et al. 1972). Depressed humans are known to show exaggerated responses to cholinergic agonists (Janowsky & Risch 1984; Janowsky & Risch 1987). Also treatment with cholinomimetics results in depressed mood, shortened REM latency and elevation of HPA function all of which are symptoms of depression (Janowsky et al. 1980). The Flinders sensitive line has face, construct and predictive validity as a model of depression. Face validity is confirmed by the observations that when compared to control animals FSL animals weigh less (Overstreet 1993) are less active in an open field (Bushnell et al. 1995), have elevated amounts of REM sleep (Shiromani et al. 1988; Benca et al. 1996) and have a reduced intake of saccharin following exposure to a chronic mild stress paradigm. (Pucilowski et al. 1993). Construct validity has also been satisfied. Numerous biochemical/pharmacological hypotheses have been put forward to explain depression. Amongst the most important among them is the monoamine hypothesis of depression in which depression in humans is linked to aberrant monoamine activity (Schildkrautet 1995; Maes & Meltzer 1995; Janowsky et al. 1995). FSL animals have altered monoaminergic metabolism consistent with such a hypothesis suggesting construct validity of the model (Zangen et al. 1997; Serova et al. 1998., Zangen et al. 1999a; Zangen et al. 1999b). Finally predictive validity was confirmed when it was shown that, as in humans, chronic but not acute treatment with

known anti-depressants was required to alleviate symptoms of depression in this model (Overstreet et al. 1995).

1.6.4 Maternal Separation

Maternal separation is an animal model for early life stress. It has been suggested that exposure to adverse early life events may precipitate the development of psychiatric disorders in adulthood (Heim & Nemeroff, 1999). Maternal separation has been shown to result in changes in neurochemical, neuroendocrine and behavioural changes in the adult animal (Cirulli et al. 2003). As these changes were reported to be long-lasting it was hypothesised that maternal separation may alter LTP in the Flinders depression model.

1.7 Aims.

Stress/depression is a debilitating disorder which affects ~ 10% of the population at any given time. It is a disorder which has serious implications for both the individual concerned as well as for the economy of the Western World. Much effort has been spent on developing stress/depression models. It is hoped that from the study of these models effective pharmaceutical interventions for the treatment of stress/depression may be developed. Currently, most pharmacological agents used for the treatment of stress/depression and anxiety involves manipulation of the serotonergic system.

It is widely accepted that stress affects synaptic plasticity. Our laboratory has developed an acute mild stress model which is of use in the study of how synaptic plasticity may be affected by stress exposure. Xu et al. (1997) showed that exposure to a mild elevated platform stress prevented the induction of LTP in CA1. Further work by Shakesby et al. (2002) has shown that pharmacological manipulation of the serotonergic system subsequent to stress exposure may recover LTP. The work presented in this thesis develops further the experimental work presented by Shakesby et al. (2002).

The specific aims of this thesis were as follows:

(1) To examine the effect of elevated platform stress on the induction of LTP *in vivo*.

(2) To investigate how drugs that alter extracellular 5-HT concentration may affect the induction of LTP in the acutely stressed animal.

(3) To investigate which members of the 5-HT receptor family are important in mediating recovery of LTP, if any, following stress exposure.

(4) To investigate LTP induction and other electrophysiological parameters in 2 widely recognised and accepted depression models: Learned helplessness and Flinders Sensitive Line.

It is hoped that this work will contribute to our understanding of the effects of stress/depression on synaptic plasticity, and may in turn lead to improved pharmacological interventions to treat these psychiatric illnesses.

II. Materials & Methods

2.1 Animals.

Male Wistar rats (in–bred strain, Bioresources Unit, Trinity College Dublin) weighing between 240–360 g and were used for the acute stress experiments. This weight range corresponds to an age of 7–11 weeks. Wistar rats were housed 2 per cage (unless otherwise stated) in a Scantainer (Denmark) with food (standard rodent chow) and water available ad libitum.

Male Congenital Learned Helpless (cLH, cNLH) and control animals (outbred Sprague-Dawley rats) weighing between 300-400g were obtained from B. Vollmayr, Mannheim, Germany. Male Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) rats (Sprague Dawley strain) weighing between 200-300g were obtained from A. Mathé, Karolinska Institute, Stockholm, Sweden. Sprague Dawley rats were housed 4 per cage in a Scantainer with food and water available ad libitum.

Imported animals were rested following arrival at the laboratory for a period of at least one week. The animals were housed under a twelve hour light–dark cycle with the room temperature being maintained between 19–22 degrees Celcius. Animals were weighed before use to determine dose of anaesthetic required.

2.2 Anaesthesia.

Prior to surgery animals were anaesthetised with urethane (ethyl carbamate; 2.1g/kg i.p.). The animal usually remained stably anaesthetized for at least 4–5 hours without requiring further anaesthetic. A heating blanket (Harvard Apparatus Homeothermic Blanket Control Unit) was used to monitor and maintain a temperature of between 36–38°C in the anaesthetised rat. Following completion of the experimental protocol the animal was killed by cervical dislocation. The experimental protocols were licensed by the Department of Health & Children, Ireland.

2.3 Surgery.

After 10 minutes had lapsed following delivery of anaesthetic the animals paw was pinched to test for any muscle response to the stimulus. This was used to assess the depth of anaesthesia in the animal and to see if anaesthetic supplementation was necessary. Local anaesthetic (Norocaine) (2ml) containing lidocaine 20mg/ml and adrenaline 0.0125mg/ml was injected subcutaneously to the skull region where electrodes were to be later implanted. A scalpel incision was made from between the ears to between the eyes. The periosteum was removed using a curved scissors exposing the skull surface. The skull was wiped clean and allowed to dry.

2.4 Position of Electrodes.

Monoploar recording electrodes and bipolar stimulating electrodes were used in each experiment. The electrodes were made in the laboratory using two lengths of teflon coated tungsten wire (625μm tungsten diameter, 750μm total external diameter, Advent Research Materials Ltd.) the ends of which having had the teflon removed. Each of the two wires were soldered to an individual pin of a two pin connector and then twisted together. The wires were then glued together with cyanoacrylate to ensure strength and stability, allowed to dry and fixed in place by dental (acrylic) cement. The electrical continuity of each wire was checked before use to ensure that the electrode was working. The ends of the wires were cut at an angle exposing the tips so that one was marginally below the other. The reference and ground electrodes consisted of small stainless steel screws (Bilaney, Germany) to which single pins had been soldered to the screw.

The coordinates for both the reference and earth screws and the recording and stimulating electrodes were noted on the skull surface using a waterproof marker. The recording electrode was positioned 3.4 mm posterior to bregma and 2.5mm lateral to the midline. The stimulating electrode was positioned 4.2 mm posterior to bregma and 3.8 mm lateral to the midline. A screw which acted as a reference electrode was

positioned 8.0 mm anterior to bregma and lateral on the opposite hemisphere (left) to that used for electrode implantation. The earth screw which acted as ground electrode was positioned 7.0 mm posterior to bregma and 5 mm lateral to the midline. Coordinates for the electrodes position was obtained by referring to a rat brain atlas (Pelligrino et al. 1979) and work carried out previously in our laboratory.

A dental drill using drill bits (1.5 mm and 1 mm) made burr holes over the electrode implantation sites. The drill was not allowed to penetrate the skull in such a manner as to disturb the underlying dura matter or cortical hemispheres. The screws were then implanted and secured in place with a single drop of glue. The dura was then burst using a sterile syringe needle. This was done to allow easy insertion of the electrodes. The rat was then placed in the stereotaxic recording apparatus (ASI Instruments) in preparation for the implantation of electrodes into the CA1 area of the dorsal hippocampus. Following satisfactory localisation of the electrodes (Figure 2.1) the electrode assembly was fixed in place for the duration of the experiment using glue and dental cement.

2.5 Cannula Implantation.

Drugs that were not able to pass the blood brain barrier were delivered intracerebroventricularly using a cannula. Cannulae were made from a stainless steel hypodermic needle (22 gauge, 0.7 mm outer diameter) which was cut to 13mm in length. The bevelled end of the needle was ground down to a length of 1.5 mm to reduce the angle of the exposed tip. When the cannula was not in use an internal plug consisting of stainless steel wire (28 gauge, 0.36 mm diameter) was used to prevent blockages from occurring. The cannula was inserted 1.5 mm anterior to bregma, 0.5 mm lateral to the midline and 3.55 mm below the dura's surface. These coordinates had been shown previously by our laboratory to be effective for injecting into the ventricles. Verification of the location of the cannula was carried out post-mortem by checking the spread of dye (Indian ink) after i.c.v. injection. Following implantation in the right lateral cerebral ventricle the cannula was sealed with glue and dental cement. A 10μl Hamilton syringe to which an internal cannula (28 gauge, 0.36 mm outer diameter) was

attached was used to deliver drug solutions i.c.v. The internal cannula was fixed so as to protrude 1.8 mm below the end of the cannula assembly. The drugs were administered in a 5μl volume over a 5 minute period. Following injection the injector was slowly removed and the stainless steel plug replaced.

2.6 Recording Apparatus.

The electrophysiological equipment was surrounded by a Faraday cage to remove environmental electrical interference. In addition all electrical equipment was grounded to a central point to eliminate 50 Hertz noise. The electrophysiological equipment comprised of a constant current isolation unit (Grass Instrument Co. photoelectric stimulus isolation unit) linked to the stimulating electrode. The evoked response was transmitted via a pre-amplifier (gain 11) to an analogue-to-digital coverter (MacLab 4S, Analog Digital Instruments) operated by Scope Program versions 3.28 and 3.5 using an Apple Machintosh Power PC G3.

2.7 Location of Recording and Stimulating Electrodes during Surgery.

The rat was placed in the stereotaxic and the electrodes lowered to the surface of the brain. This was taken as point '0'. The position of the electrodes moving through the cortical and hippocampal layers to the dendrites of stratum radiatum was constantly monitored as they were lowered through the tissue. Evoked responses (0.1 ms duration, 2 ms delay, 4V pulse through the stimulating electrode at a frequency of 0.1 Hz) were displayed on the computer screen as the electrodes were lowered into place in the CA1 area. Both the cerebral cortex and the hippocampal formation possess laminar structures. When a local depolarisation such as an excitatory post-synaptic potential (EPSP) was created, a current was set up along a vertical superficial-deep axis. A phase reversal was encountered when this dipole was crossed, indicating that this was the area generating electromotive forces and the response recorded was not from a distal site by voltage conduction. By this method it was possible to determine which layer the

electrodes were in by referring to the electrophysiological criteria determined for each region of the hippocampus as defined by Leung (1980).

The monopolar recording electrode was positioned initially to a depth of 2.5 mm below the surface of the dura. The bipolar stimulating electrode was then positioned initially to a depth of 1.6 mm below the surface of the dura. The stimulating electrode was then lowered in increments of 0.05mm to approximately 2.5 mm below the dura. The first potential recorded was the evoked positive response as the stimulating electrode penetrated the alveus. A larger positive response was seen as the stimulating electrode penetrated the stratum oriens. Upon approaching the cell body layer the amplitude of the evoked response became smaller and reversed as the stimulating electrode penetrated through from the cell body layer of stratum radiatum into the dendritic layer. Further small adjustments (increments of 20μm) were made in the positioning of the stimulating electrode to maximize the EPSP amplitude. Once satisfied minor adjustments (10 μm) were made to the position of the recording electrode to further maximize EPSP amplitude. Electrode position were verified post-mortem by manual dissection.

2.8 Input/Output curves.

Once found, evoked responses (0.033 Hz) were recorded continuously for the duration of the experiment. The animal was allowed a recovery period of 1 hour post electrode implantation after which an I/O curve was recorded. The I/O curve was constructed by recording the average amplitude of four evoked responses induced by each of a series of stimulating intensities (2-10 V at 1 V intervals).

2.9 Excitatory Post-Synaptic Potential (EPSP) Recordings.

Test excitatory post-synaptic potentials (EPSPs) were evoked by a single square wave pulse of current at low frequency (0.033 Hz, 0.1 ms duration with a voltage of 4V) generated by a constant current isolation unit. The test stimuli were set to evoke

responses of 50-55% maximum EPSP amplitude. Baseline synaptic transmission was recorded for at least 1 hour. High frequency stimulation (HFS) was used to induce LTP. The HFS protocol comprised 10 trains of 20 pulses, interpulse interval of 5 ms (200 Hz) and intertrain interval of 2s. The intensity of stimulation was raised to give 75% of maximum EPSP amplitude for the HFS. For Wistar rats an increase in transmission of 15% or greater at 60min post HFS was regarded as LTP. For Sprague Dawley animals an increase in transmission of 10% or greater at 60min post HFS was regarded as LTP. Electrophysiological experiments carried out on acutely stressed animals involved the application of two sets of HFS trains. The first HFS was given after baseline recording. Stress was deemed to have blocked LTP (i.e. have been effective) if the level of potentiation at 60min post HFS was less than 15%. In the rare event when this was not the case the stress protocol was said to have failed and the experiment concluded. In this way each animal acted as its own control.

2.10 Inescapable Stress Protocol.

The stress protocol consisted of placing the animal on a clear platform (25cm x 25cm) at a height of approximately 150cm from ground level. The room was brightly lit. The animal was required to remain on the platform for 30 minutes. This protocol has been shown previously to reliably raise corticosterone levels and to block the induction of LTP in the CA1 region of the hippocampus (Shakesby et al. 2002). Physical parameters which indicate stress levels were recorded which included piloerection, faecal deposits, incidence of urination and time spent immobile (Table 2.1). Following exposure to the stress protocol the animal was anaesthetised immediately and placed back on the platform to allow the anaesthetic to take effect.

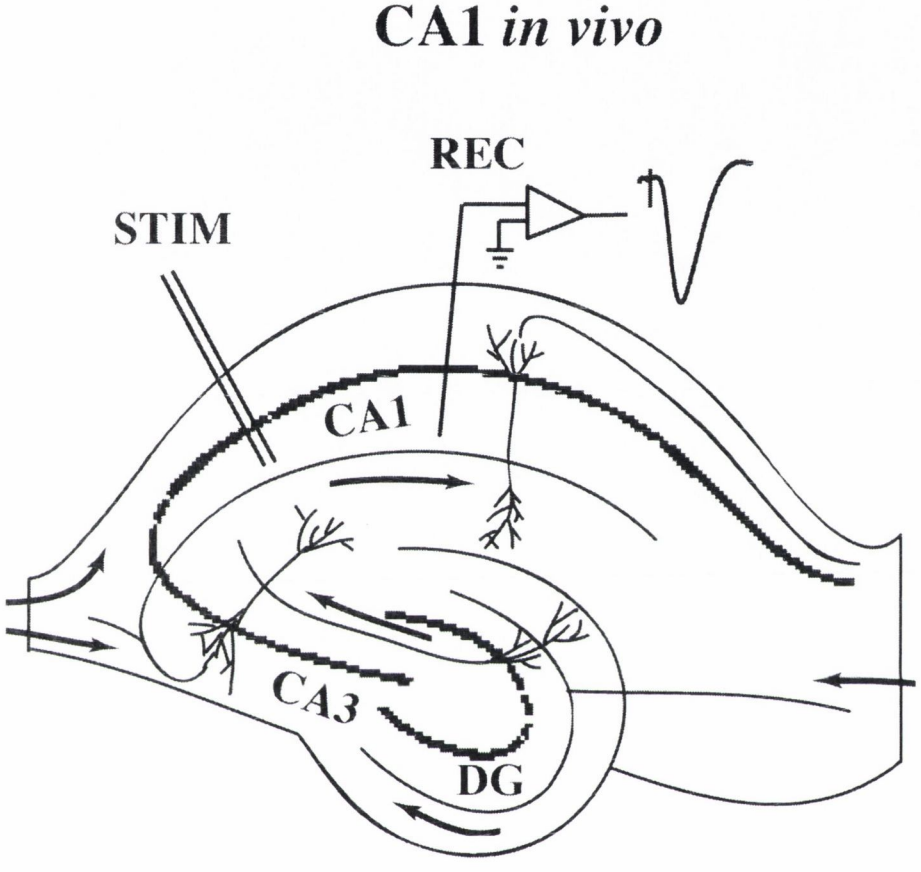

Figure 2.1 Schematic diagram of the rat hippocampus showing the stimulation and recording sites. The recording electrodes (REC) are placed in the dendritic layers of stratum radiatum in CA1 and stimulation (STIM) is applied to Schaffer collateral inputs from CA3 (Adapted from Rowan et al. 2004).

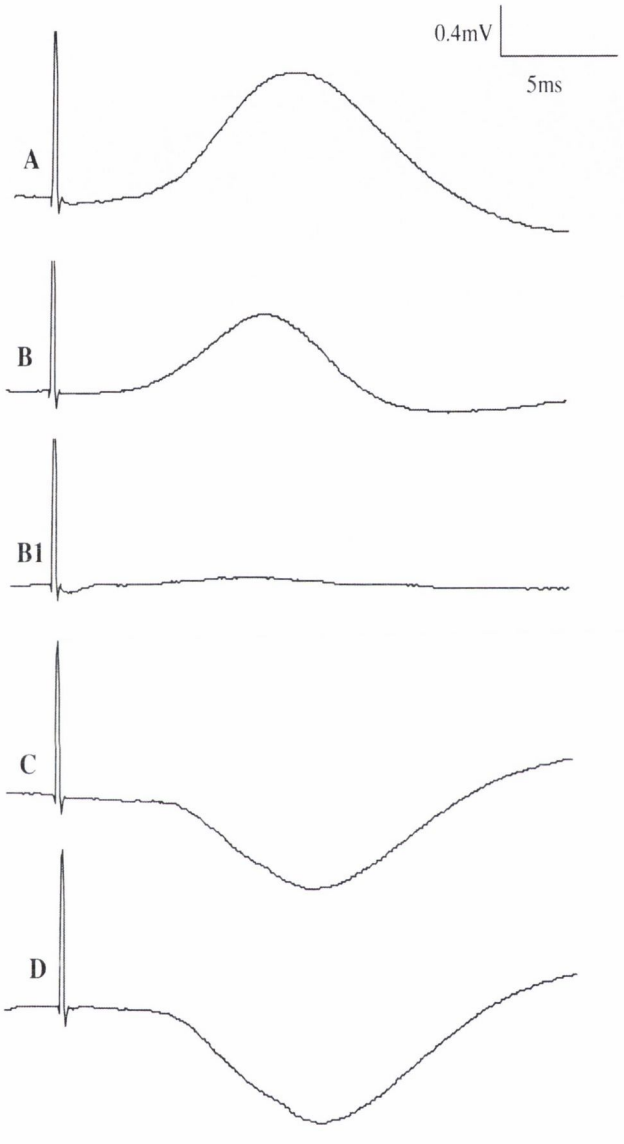

Figure 2.2 Representative EPSP from a single experiment during which the electrodes were lowered into place. Monitoring of the characteristic shape indicated position of electrodes in the hippocampus. (A) Potential recorded from the alveus. (B) Potential recorded from the cell body layer. (C) Reversal of potential as electrodes enters the CA1 region. (D) Adjustment of electrodes to obtain best possible EPSP.

	Number of faecal deposits	Incidence of urination	Exhibition of piloerection	Time spent immobile (min)
Rat 1	9	3	+	27
Rat 2	8	5	+	28
Rat 3	8	6	+	27
Rat 4	6	6	+	28
Rat 5	9	2	+	28
Rat 6	6	3	+	25
Average	7.7	4.2	+	27

Table 2.1 The effects of elevated platform stress on behavioural parameters from 6 randomly chosen male Wistar rats. A (+) indicates that piloerection was present for at least 15 minutes. The time that an animal was immobile was counted to the nearest whole minute. The data were collected only for the time spent on the elevated platform.

2.11 Learned Helplessness Protocol.

The Learned helplessness protocol was carried out by B. Vollmayr, Mannheim, Germany. The animals are placed individually in an operant conditioning chamber (48.5x30x21.5cm) consisting of a steel rod floor, steel walls, shock generator, white light and lever (Operant Behavior System Mannheim Type 259900).
The protocol was carried out over 2 consecutive days. On the shock (Training) day (Day 1) the lever was withdrawn and the animal exposed to a 40 minute session of 0.8mA inescapable shock consisting of single shocks with inter-shock intervals ranging from 5 to 15 seconds as determined randomly by a computer. In total each animal was exposed to 20 minutes of shock, then marked for identification purposes and returned to their home cage. On the test-day (Day 2) 15 shocks were applied (phase duration of 200ms and intensity 0.8 mA). Each shock lasted 60 seconds with an inter-trial interval of 24 seconds. The current was accompanied by a light cue to facilitate detection of the lever and discrimination to the inescapable shock session. Animals could stop the shock by pressing the lever but must release and depress lever again to terminate the next shock. If an animal failed to terminate the shock on more than 10 occasions it was classified as 'learned helpless' (lh). Animals with less than 5 failures were considered 'non-learned helpless' (nlh). Animals with between 5 and 10 failures were considered as having an intermediate phenotype. cNLH animals receive both shock and testing. cLH animals receive testing only.

2.12 Maternal Separation Protocol for Flinders Depression Model.

The Flinders rats were provided by A. Mathé, Karolinska Institute, Stolkholm, Sweden. Flinders Resistant Line and Flinders Sensitive Line rats pups were randomly selected for maternal separation. The maternal separation protocol was performed in Sweden and consisted of 180 minutes separation per day from PND 2-14. During this time the rat pup siblings were placed collectively on a heated blanket in order to maintain their temperature.

2.13 Drug Treatment.

All drugs required for the acute experiments were dissolved in water and administered i.p. or i.c.v. as indicated. For the Flinders study, escitalopram was administered as a constituent of the food pellet, available ad libitum for a period of two weeks. Two different concentrations of escitalopram pellet were provided (340mg/kg pellet administered up to PND 57 and 408mg/kg pellet administered post PND 57). This was to ensure that a constant dose was administered taking into account the rats increase in weight during treatment.

2.14 Data Analysis.

EPSP amplitude was taken as a measure of excitatory synaptic transmission. Data is expressed in epochs of 10 minutes. A stable baseline was recorded for 30 minutes before HFS or pharmacological intervention. LTP is expressed as a percentage of the average EPSP amplitude of the proceeding 30 min (baseline). Statistical comparisons are made between average baseline EPSP amplitude (10 minutes proceeding HFS) and post HFS (50-60minute epoch) unless otherwise stated. Data are expressed as averages ± S.E.M. Paired and unpaired Student's t-test are used as appropriate and $p<0.05$ is interpreted as significant. Care should be taken when interpreting significant results following multiple use of t-tests due to the increased risk of type 1 errors. Paired Pulse Facilitation (PPF) was expressed as the percentage of the second EPSP (EPSP2) minus the first EPSP (EPSP1) divided by the first. Data were analysed using JMP 3.2.1 Statistical software.

2.15 Compounds and Solutions.

D-AP5 (D-2-Amino-5-phosphononpentanoic acid)	Tocris Cookson Ltd. Northpoint, Fourth Way, Avonmouth BS118TA, UK.
BW723c86 (α-Methyl-5-(2-thienylmethoxy)-1 H-indole-3-ethanamine hydrochloride)	Tocris Cookson Ltd. Northpoint, Fourth Way, Avonmouth BS118TA, UK.
Cinanserin (N-[2-[[3-Dimethylamino)propyl] thio]phenyl]-3-phenyl-2-propenamide hydrochloride)	Tocris Cookson Ltd. Northpoint, Fourth Way, Avonmouth BS118TA, UK.
Dental Acrylic Liquid	Associated Dental Products Ltd., Kemdent Works, Purton, Swindon, SN5 9HT, UK.
Dental Acrylic Powder	Associated Dental Products Ltd., Kemdent Works, Purton, Swindon, SN5 9HT, UK.
Escitalopram pellet ((s)-1-[3-(dimethyl amino)propyl]-1-(4-fluorophenyl)-1,3-dihydro -5-isobenzofurancarbonitrile hydrobromide)	H. Lundbeck Ltd., Copenhagen, Netherlands.
(±)Fenfluramine ((±)-N-Ethyl-α-methyl-m-[trifluoromethyl]phenethylamine)	Sigma-Aldrich Corporation, St. Louis, Missouri 63178, USA.

(+)Fenfluramine ((+)-N-Ethyl-α-methyl-m-[trifluoromethyl]phenethylamine)	Sigma-Aldrich Corporation, St. Louis, Missouri 63178, USA.
(-)Fenfluramine ((-)-N-Ethyl-α-methyl-m-[trifluoromethyl]phenethylamine)	Sigma-Aldrich Corporation, St. Louis, Missouri 63178, USA.
Indian Ink	George T. Gurr Ltd., London, SW6, UK.
mCPP (1-(3-Chlorophenyl)piperazine hydrochloride)	Tocris Cookson Ltd., Northpoint, Fourth Way, Avonmouth BS118TA, UK.
MK-212 (6-Chloro-2-(1-piperazinyl) pyrazine hydrochloride)	Tocris Cookson Ltd., Northpoint, Fourth Way, Avonmouth BS118TA, UK.
Norocaine (lignocaine hydrochloride and aderenaline acid tartrate)	Norbrook Laboratories Ltd., Newry, BT35 6JP, UK.
RS67333 (1-(4-Amino-5-chloro-2-methoxy phenyl)-3-[1-butyl-4-piperidinyl]-1-propanone hydrochloride)	Tocris Cookson Ltd., Northpoint, Fourth Way, Avonmouth BS118TA, UK.
Urethane (Ethyl carbamate)	Sigma-Aldrich Corporation, St. Louis, Missouri 63178, USA.

III. Results

3.1 Effect of HFS in non-stressed control animals.

This initial experiment was designed to ascertain the level of LTP inducible *in vivo* in CA1 by HFS in urethane anaesthetised inbred male Wistar strain rats provided by the Bioresources Unit, TCD, under the conditions which pertained in our laboratory. Firstly a stable baseline was obtained for 30 minutes (101.3±1.3%, n=8 for the 10 minutes prior to HFS). A single HFS (parameters outlined in Section 2.9) was applied. There was an immediate and statistically significant increase in glutamatergic transmission, short term potentiation, at 10 minutes post HFS (144.4±13.7%, n=8, $p<0.05$ compared with baseline, paired student's t-test) which reduced to stable LTP (132.3±4.6% at 60 min post HFS, n=8, $p<0.001$, paired student's t-test). This experiment was repeated at regular intervals so as to ensure that the level of LTP inducible in CA1 remained relatively constant.

3.2 Effect of acute mild elevated platform stress on LTP induction in CA1.

This experiment was designed to assess the effect of stress on the ability of HFS to induce LTP. Each animal was exposed to elevated platform stress as outlined in Section 2.10. A stable baseline was obtained for at least 30 minutes (101.6±0.8%, n=5, for the 10 minutes prior to HFS1). A first high frequency stimulation (HFS1) was then applied. There was no significant increase in synaptic transmission following HFS1 (102.4±4.9% at 60 min post HFS1, n=5, $p>0.5$ compared with baseline, paired student's t-test; $p<0.01$ compared with 60 min post HFS in non-stressed control animals, unpaired student's t-test). At 90 minutes post HFS1 a second HFS (HFS2) was then applied. Again there was no significant increase in synaptic transmission (109.8±4.7% at 60 minutes post HFS2, n=5, $p>0.1$ compared with baseline, paired student's t-test). These data clearly show that exposure to elevated platform stress immediately prior to anaesthesia blocks the induction of LTP several hours later in CA1. Thus it appears that

the anaesthetic acts to "freeze" the brain in the emotional state of the animals last waking moments.

3.3 Effect of (±) fenfluramine on stress-induced inhibition of LTP.

This experiment was designed to assess the effect of raising extracellular 5-HT concentrations on stress-induced LTP blockade. First the animals were exposed to elevated platform stress and then anaesthetised. A stable baseline was obtained for 30 minutes (103.4±1.0%, n=7, for the 10 minutes prior to HFS1). HFS1 failed to induce LTP (108.8±7.0% at 60 minutes post HFS1, n=7, $p>0.1$ compared with baseline, paired student's t-test) confirming that the stress exposure had been successful in blocking LTP. At 60 minutes post HFS1 an i.p. injection of (±)fenfluramine (5mg/kg) was administered. (±)Fenfluramine had no effect on baseline transmission at this dose. Thirty minutes following (±)fenfluramine administration a second HFS (HFS2) was applied. HFS2 induced a statistically significant and stable LTP (140.0±9.1% at 60 minutes post HFS2, n=7, $p<0.01$ compared with baseline, paired student's t-test). The level of recovery of LTP was on average numerically greater than that seen in control non-stressed animals (140.0±9.1% vs 132.3±4.6%). This difference was not statistically significant ($p>0.1$, unpaired student's t-test). This suggests that raising extracellular 5-HT levels may mediate recovery of LTP previously blocked by mild elevated platform stress exposure.

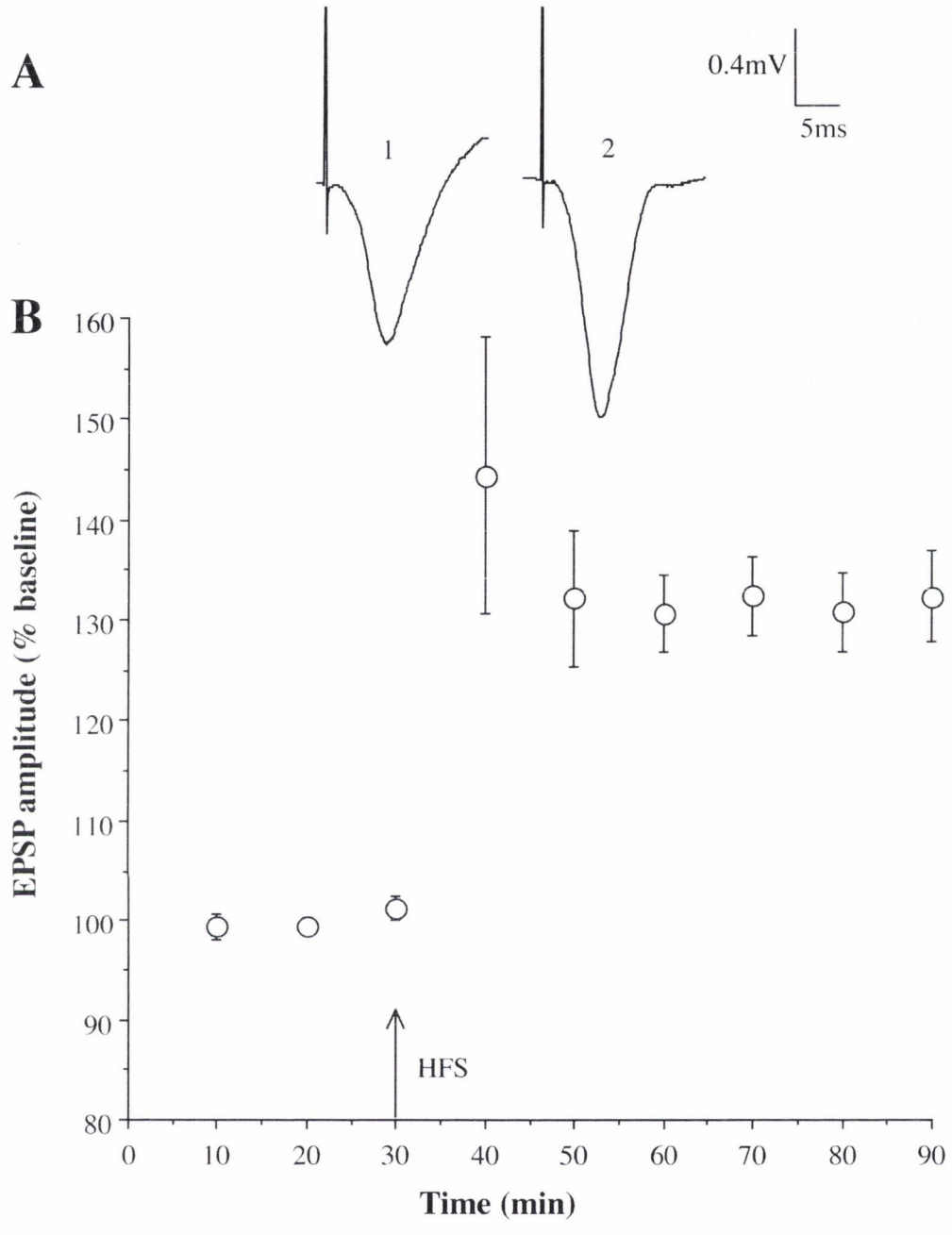

Figure 3.1 Effect of high frequency stimulation (HFS) on synaptic transmission.
(A) Insets show typical traces of fEPSPs recorded ~ 5 min before (1) and 60 min after HFS. (B) Application of HFS induced an immediate and enduring increase in synaptic transmission (132±5% at 60 min post HFS, n=8, $p<0.001$ compared with baseline, paired student's t-test). Data expressed as mean±s.e.m.

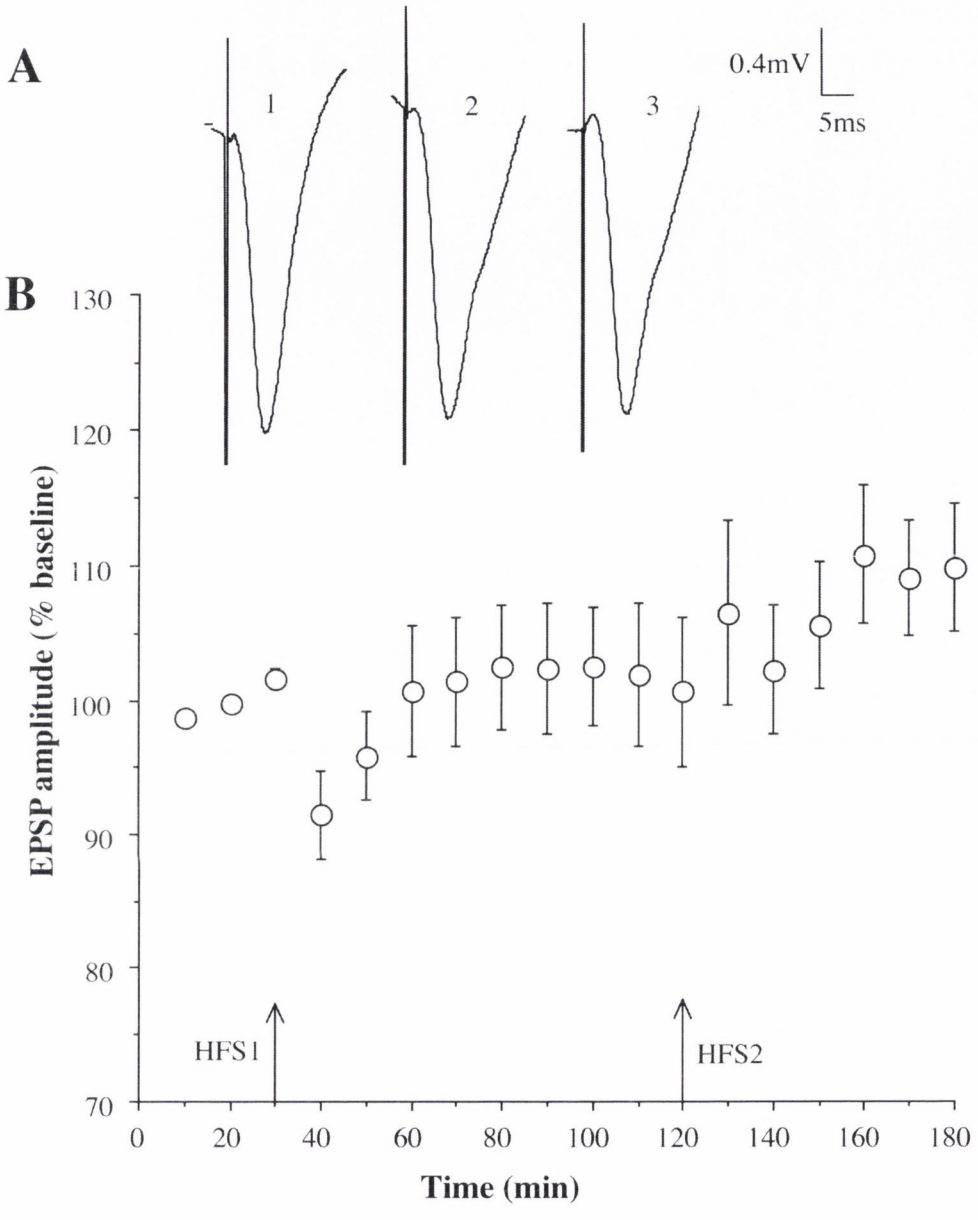

Figure 3.2 Effect of acute platform stress on the induction of LTP by HFS.

(A) Insets show typical traces of fEPSPs recorded ~ 5 min before (1) and 60 min after HFS1 (2) and 60 min after HFS2 (3). (B) Mild stress prevented the induction of LTP by HFS1 (102±5%, at 60 min post HFS1, n=5, $p>0.5$ compared with baseline). A second HFS (HFS2) also failed to induce LTP (110±5% at 60 min post HFS2, n=5, $p>0.1$ compared with baseline). Data expressed as mean±s.e.m.

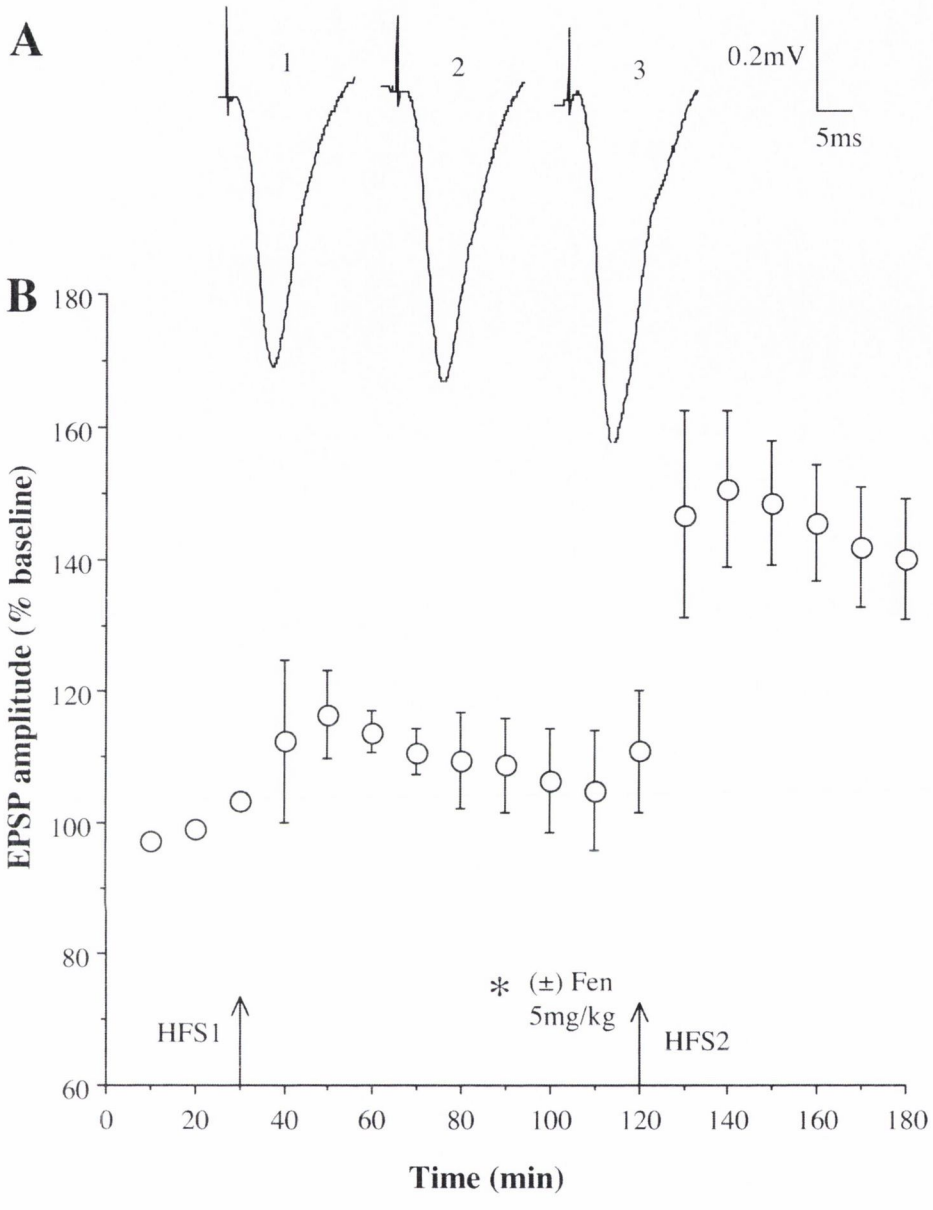

Figure 3.3 Effect of (±)fenfluramine on stress induced LTP blockade.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS1, and 60 min after HFS2 (3). (B) Mild stress prevented the induction of LTP by high frequency stimulation (HFS1) (109±7%, at 60 min post HFS1, n=7, $p>0.1$ compared with baseline). Administration of (±) fenfluramine (5mg/kg), a 5-HT releasing agent overcomes LTP inhibition, a second HFS (HFS2) inducing significant LTP (140±9%, at 60 min post HFS2, n=7, $p<0.01$ compared with baseline). Data expressed as mean±s.e.m.

3.4 Effect of (±)tianeptine pre-treatment on the (±)fenfluramine mediated recovery of LTP.

To determine if the recovery of LTP seen post (±)fenfluramine administration in stressed animals was mediated via an increase in extracellular serotonin concentration it was decided to investigate the effect of pre-treatment with (±)tianeptine on this recovery process. (±)Tianeptine is a 5-HT uptake enhancer (Fattaccini et al. 1990; Kamoun et al. 1989; Broqua et al. 1992; Kato & Weitsch 1988). The animals were exposed to acute mild elevated platform stress. A stable baseline was obtained for 30 minutes (100.3±1.1%, n=7 for the 10 minutes prior to HFS1). HFS1 failed to induce LTP (102.0±5.8% at 60 minutes post HFS1, n=7 $p>0.5$ compared with baseline, paired student's t-test). At 40 minutes post HFS1 (±)tianeptine (2mg/kg) was administered i.p. At 60 minutes post HFS1 (±)fenfluramine (5mg/kg) was administered i.p. Application of HFS2 at 90 minutes post HFS1 failed to induce LTP (109.3±9.9% at 60 minutes post HFS2, n=7, $p>0.1$ compared with baseline, paired student's t-test). This result implicates an increase in extracellular 5-HT levels as the most likely mediator of LTP recovery by treatment with (±)fenfluramine.

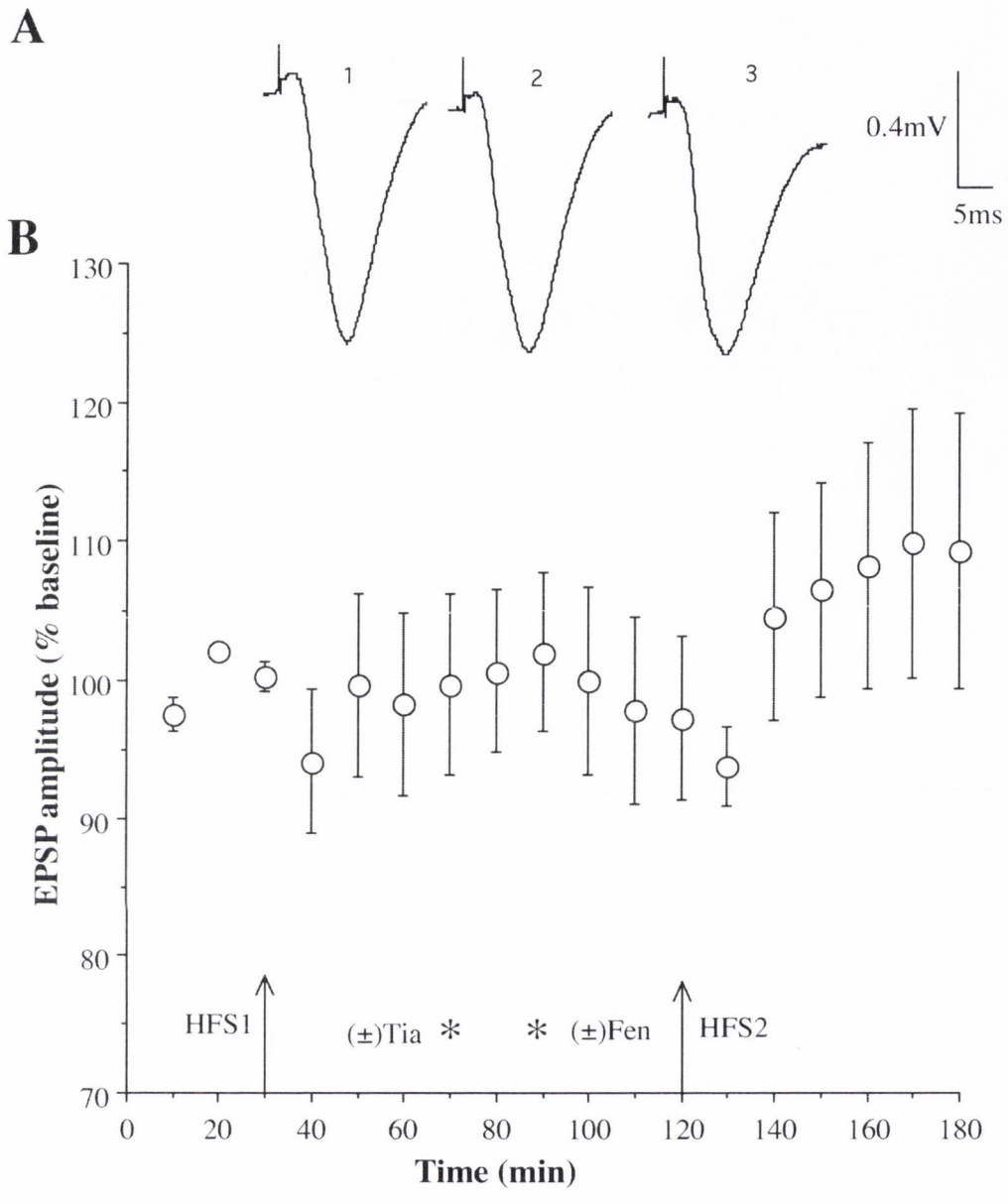

Figure 3.4 Effect of (±)tianeptine on (±)fenfluramine induced LTP recovery.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS1, and 60 min after HFS2 (3). Mild stress prevented the induction of LTP by HFS1 (102±6%, at 60 min post HFS1, n=7, $p>0.5$ compared with baseline). Administration of (±)tianeptine (2mg/kg, i.p.), a 5-HT uptake enhancer prevented (±)fenfluramine (5mg/kg, i.p.) overcoming LTP inhibition (109±10% at 60 min post HFS2, n=7, $p>0.1$ compared with baseline). Data expressed as mean±s.e.m.

3.5 Effect of (+)fenfluramine on LTP induction in non-stressed rats.

Previously it was shown by Shakesby et al. (2002) that (±)fenfluramine (5mg/kg) blocked LTP in non-stressed rats. As (+)fenfluramine is said to be a more active enantiomer than (-)fenfluramine it was decided to investigate the effect of (+)fenfluramine (2.5mg/kg) on LTP induction in non-stressed animals *in vivo*. A stable 30 minute baseline was obtained (100.2±1.4%, n=5 in the 10 minutes prior to (+)fenfluramine administration). Following baseline recordings an i.p. administration of (+)fenfluramine (2.5mg/kg) was administered. (+)Fenfluramine (2.5mg/kg) did not have any effect on baseline transmission (101.8±5.1%, n=5 for the 10 minutes prior to HFS). 30 minutes post (+)fenfluramine administration application of HFS induced a stable and robust LTP (142.1±14.2%, n=5 at 60 minutes post HFS, $p<0.05$ compared with baseline, paired student's t-test). The level of LTP was not statistically significantly greater than that seen in non-stressed control animals (142.1±14.2% vs 132.3±4.6%, $p>0.1$, unpaired student's t-test).

3.6 Effect of (-)fenfluramine on LTP induction in non-stressed rats.

The effect of (-)fenfluramine (2.5mg/kg) on LTP induction in non-stressed rats was also investigated. A stable baseline was obtained for 30 minutes (100.4±0.7%, n=5 for 10 minutes prior to (-)fenfluramine administration). Following baseline recordings an i.p. injection of (-) fenfluramine (2.5mg/kg) was administered. Injection of (-)fenfluramine did not affect baseline transmission (103.6±3.2%, n=5 for the 10 minutes prior to HFS). HFS was applied 30 minutes post (-)fenfluramine injection. HFS resulted in robust and stable LTP (147.5±7.9%, n=5 at 60 minutes post HFS, $p<0.01$, compared with baseline, paired student's t-test). The level of LTP was on average numerically greater but not statistically significantly different from that of control animals (147.5±7.9% vs 132.3±4.6%, $p>0.05$ unpaired student's t-test).

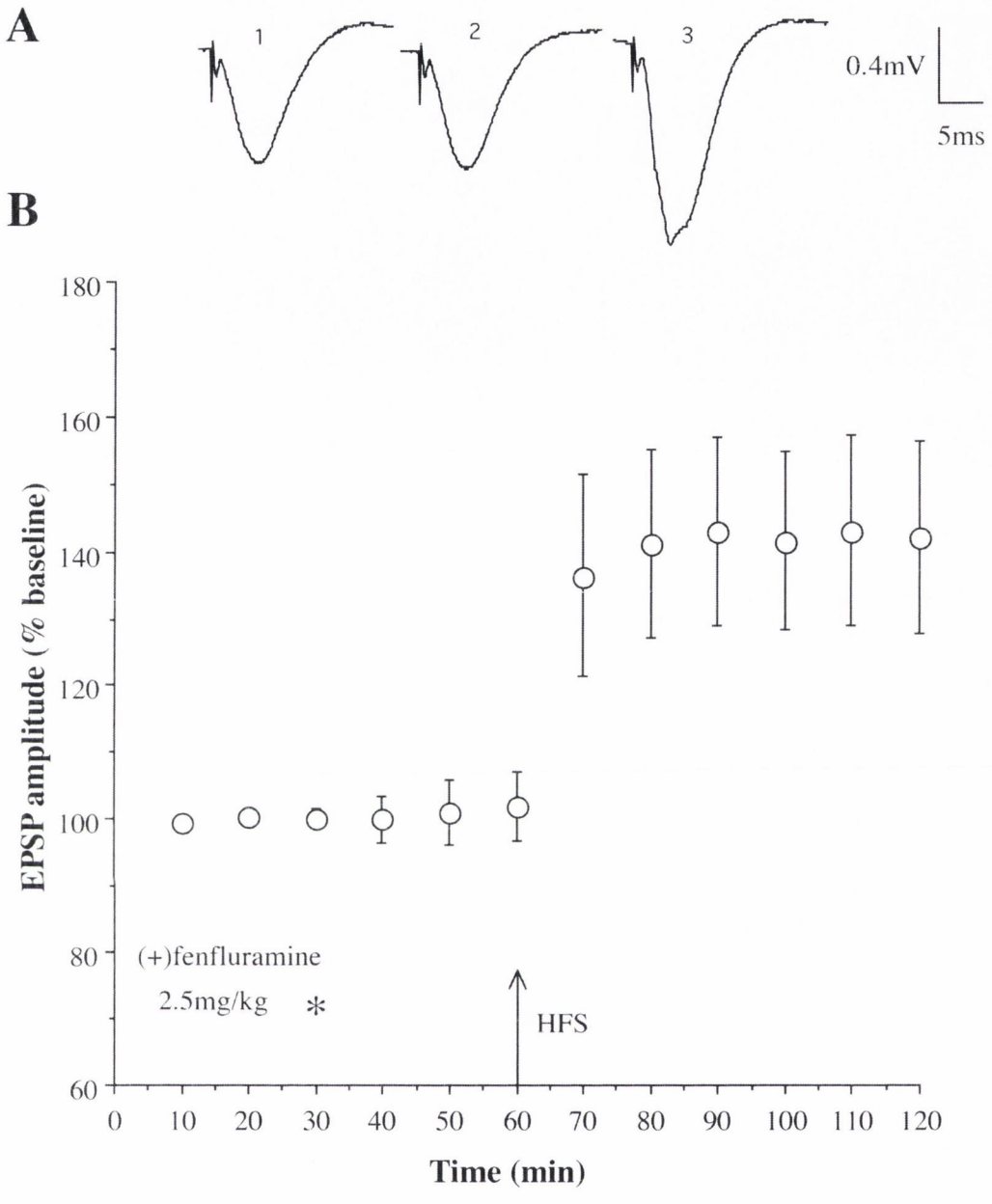

Figure 3.5 Effect of (+)fenfluramine on LTP induction in non-stressed rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 30 min after (2) (+)fenfluramine (2.5mg/kg, i.p.) administration, and 60 min after (3) HFS. (B) Application of HFS induced a statistically significant LTP (142±14% at 60 min post HFS, n=5, $p<0.05$ compared with baseline). Data expressed as mean±s.e.m.

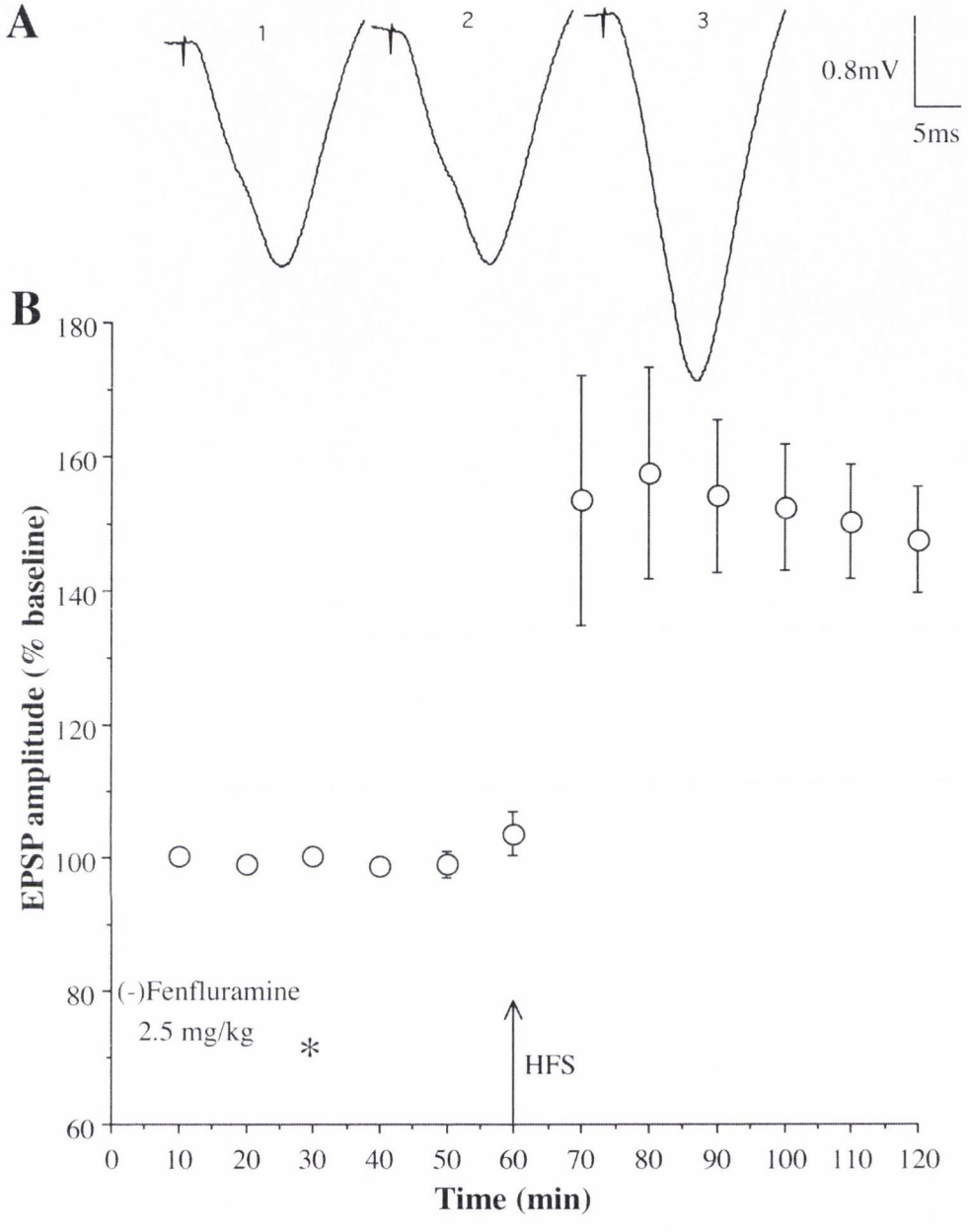

Figure 3.6 Effect of (-)fenfluramine on LTP induction in non-stressed rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 30 min after (2) (-)fenfluramine (2.5mg/kg, i.p.) administration, and 60 min after (3) HFS. (B) Application of HFS induced a statistically significant LTP (148±8% at 60 min post HFS, n=5, $p<0.01$ compared with baseline). Data expressed as mean±s.e.m.

Having shown a role for the serotonergic system in overcoming stress induced LTP blockade this next set of experiments set out to identify which member of the 5-HT receptor family may be involved in mediating LTP recovery. Shakesby et al. (2002) have shown how fluoxetine (an SSRI antidepressant) was able to prevent the inhibition of LTP following acute elevated platform stress. A further paper published by Svenningsson et al. (2002) suggested that fluoxetine was mediating its therapeutic effects via DARPP-32 phosphorylation and hypothesised that this process was linked to 5-HT_2,5-HT_4 or 5-HT_6 receptor activation. 5-HT_2 and 5-HT_4 receptors were therefore targeted for investigation into the receptor mechanism involved in the effect of (±)fenfluramine on stress-induced inhibition of LTP.

3.7 Cinanserin prevents (±)fenfluramine-induced recovery of LTP inhibited by acute elevated platform stress.

This set of experiments were designed to investigate whether 5-HT_2 receptor activation can mediate LTP recovery post stress exposure. Animals were exposed to acute mild elevated platform stress. A 30 minute stable baseline was recorded (101.9±0.5% for the 10 minutes prior to HFS1). Following HFS1 there was no significant increase in synaptic transmission (99.4%±11.7%, n=5, $p>0.5$ compared with baseline). At 40 minutes post HFS1 an i.p. injection of cinanserin (30mg/kg), a nonsubtype selective 5-HT_2 receptor antagonist (Leysen et al. 1981; Pierce et al. 1992) was administered. At 60 minutes post HFS1 an i.p. injection of (±)fenfluramine (5mg/kg) was administered. Thirty minutes later HFS2 was applied. This second HFS also failed to induce LTP (107.7±13.1%, n=5 at 60 min post HFS2, $p>0.5$ compared with baseline, paired student's t-test). However, due to the variability in this data set the difference between co-treated animals (cinanserin and (±)fenfluramine) when compared to (±)fenfluramine only treated animals was not quite statistically significant ($p=0.062$ at 60 min post HFS2, unpaired student's t-test).

Pretreatment with cinanserin (30mg/kg) prevented (±)fenfluramine (5mg/kg) mediating the recovery of LTP. This suggests that 5-HT_2 receptors may be important in mediating fenfluramine's ability to enable LTP induction in previously stressed animals.

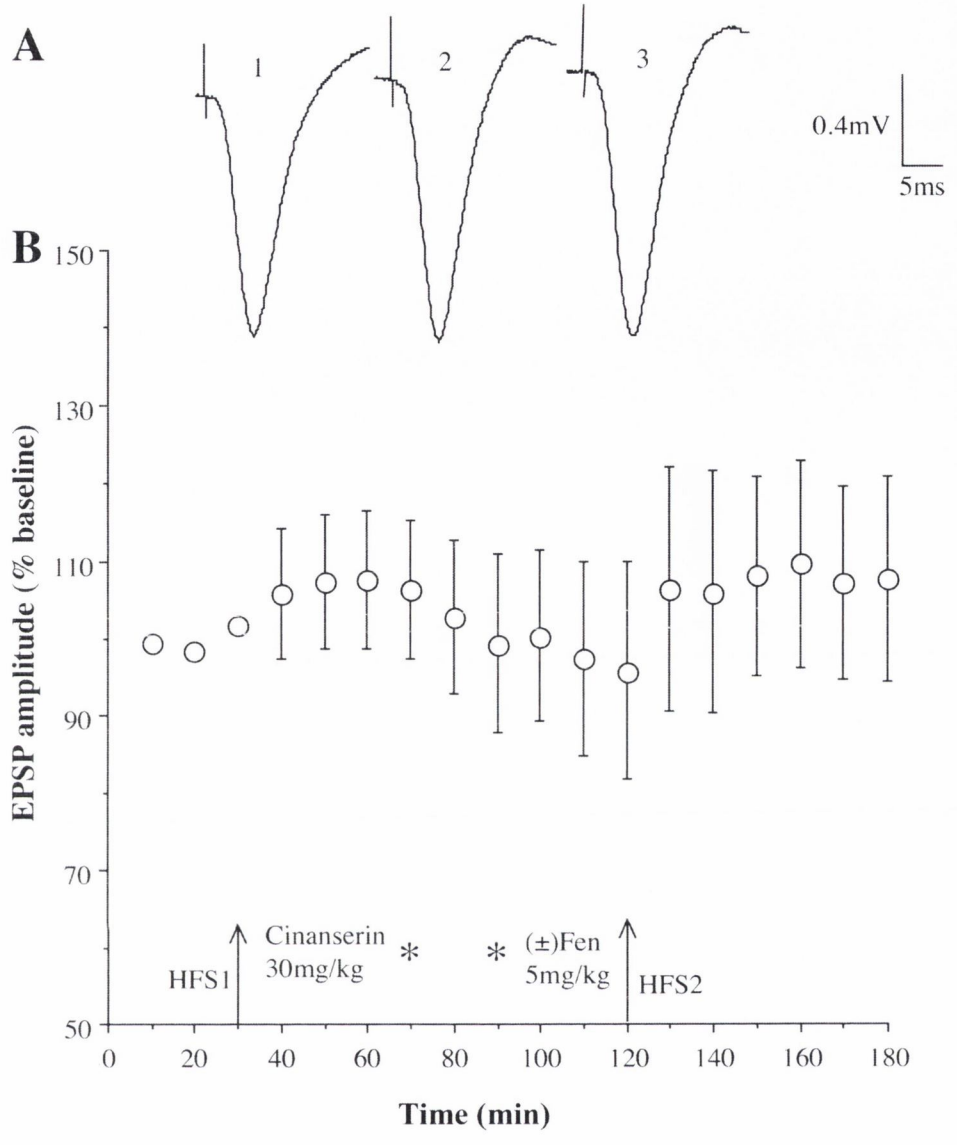

Figure 3.7 Effect of cinanserin on (±)fenfluramine-induced LTP recovery following acute stress exposure.

(A) Insets show typical fEPSPs recorded ~5 min before (1) and 60 min after (2) HFS1, and 60 min after HFS2 (3). (B) Mild stress prevented the induction on LTP by HFS1 (99±12% at 60 min post HFS1, n=5, $p>0.5$, compared with baseline). Cinanserin (30mg/kg, i.p.) a non-subtype selective 5-HT_2 receptor antagonist prevented (±)fenfluramine (5mg/kg, i.p.) overcoming LTP inhibition (107±13%, at 60 min post HFS2, n=5, $p>0.5$ compared with baseline). Data expressed as mean±s.e.m.

3.8 mCPP, a 5-$HT_{2B/2C}$ receptor agonist overcomes the inhibition of LTP by stress.

To further investigate the role that 5-HT_2 receptors play in regulating synaptic plasticity subsequent to stress exposure it was decided to investigate various 5-HT_2 receptor agonists.

The rats were exposed to acute mild elevated platform stress. A stable baseline was recorded for 30 minutes (100.4±1.1%, n=6 for the 10 minutes prior to HFS1). Application of HFS1 failed to induce LTP (94.8±6.0% at 60 minutes post HFS1, n=6, $p>0.1$ compared with baseline, paired student's t-test). Administration of mCPP (10mg/kg, i.p.), a 5-$HT_{2B/2C}$ receptor agonist (Curzon et al. 1990; Hoyer et al. 1994; Porter et al. 1999) at 60 minutes post HFS1 resulted in an initial small reduction in synaptic transmission which returned to baseline values within 30 minutes post injection (96.4±5.8%, n=6). At 90 minutes post HFS1 a second HFS was applied. HFS2 resulted in a stable induction of LTP (146.2±14.3%, n=6 at 60 minutes post HFS2, $p<0.05$ compared with baseline, paired student's t-test). There was no significant difference between the level of recovered LTP and control LTP in non-stressed rats (146.2±14.3% vs 132.3±4.6%, $p>0.1$, unpaired student's t-test). This suggests that the serotonergic mediated recovery of LTP following stress may be mediated, at least in part, by 5-$HT_{2B/2C}$ receptor activation.

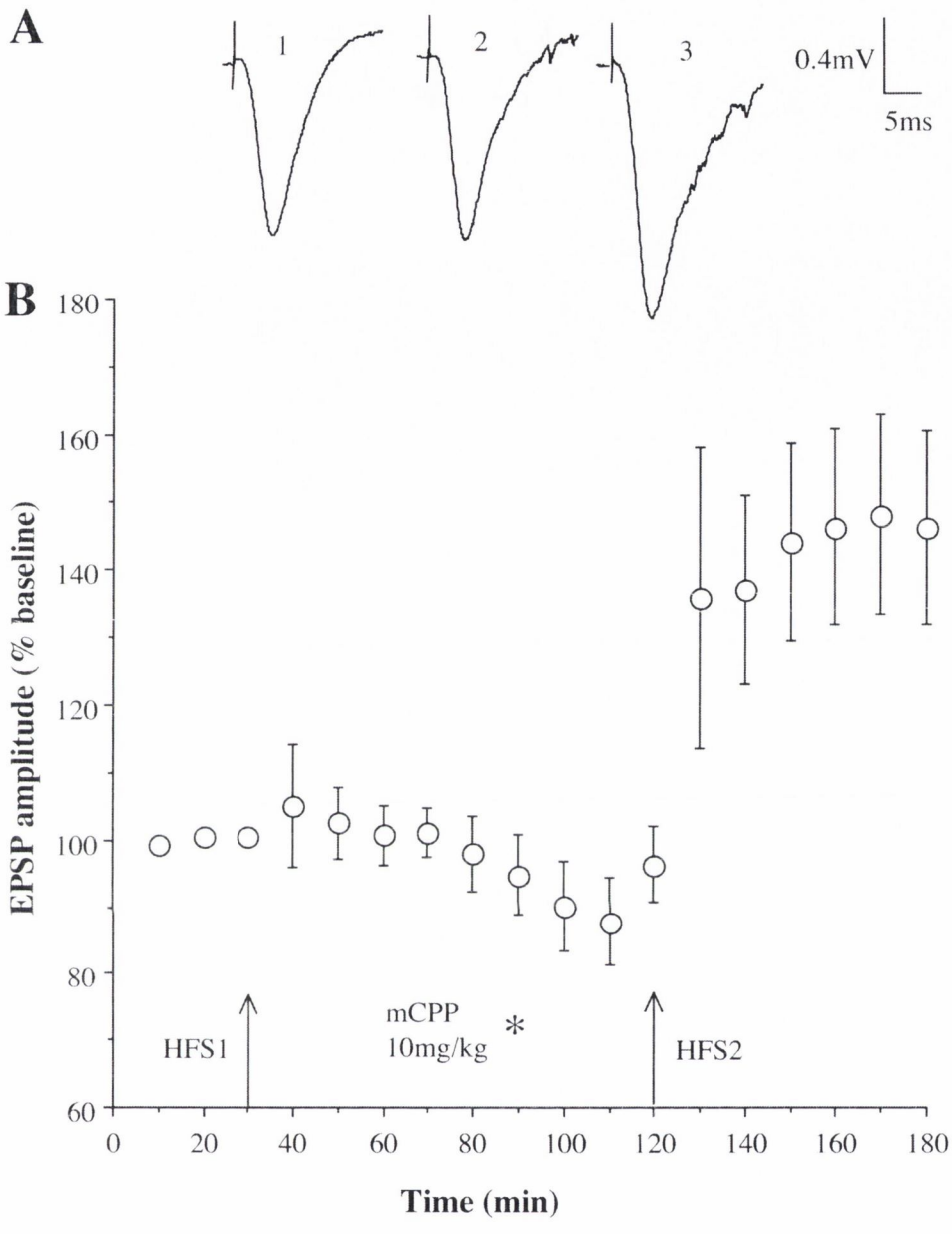

Figure 3.8 Effect of mCPP on LTP induction in stressed rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS1 and 60 min after (3) HFS2. (B) Mild stress prevented the induction of LTP by HFS1 (95±6% at 60 min post HFS1, n=6, $p>0.1$ compared with baseline). Administration of mCPP (a 5-$HT_{2B/2C}$ receptor agonist, 10mg/kg, i.p.) recovers LTP (146±14% at 60 min post HFS2, n=6, $p<0.05$ compared with baseline). Data expressed as mean±s.e.m.

3.9 mCPP does not cause a change in baseline synaptic transmission in stressed animals.

This experiment was designed to determine if mCPP had an effect on synaptic transmission in stressed animals independent of HFS.

Animals were exposed to acute mild elevated platform stress. A stable baseline for 30 minutes was obtained (98.9±1.5% for the 10 minutes prior to HFS). Application of HFS did not result in LTP (109.5±4.5% at 60 minutes post HFS, n=5, $p>0.1$, compared with baseline, paired student's t-test). Administration of mCPP (10mg/kg, i.p.) did not statistically significantly alter synaptic transmission (114.4±2.8%, at 90 min post HFS, n=5, $p>0.1$ compared with 10 minute epoch pre injection, paired student's t-test). There was no application of a second HFS. This data set clearly shows that the LTP shown in Figure 3.8 was due to high frequency stimulation and not simply attributable to a mCPP mediated increase in baseline synaptic transmission.

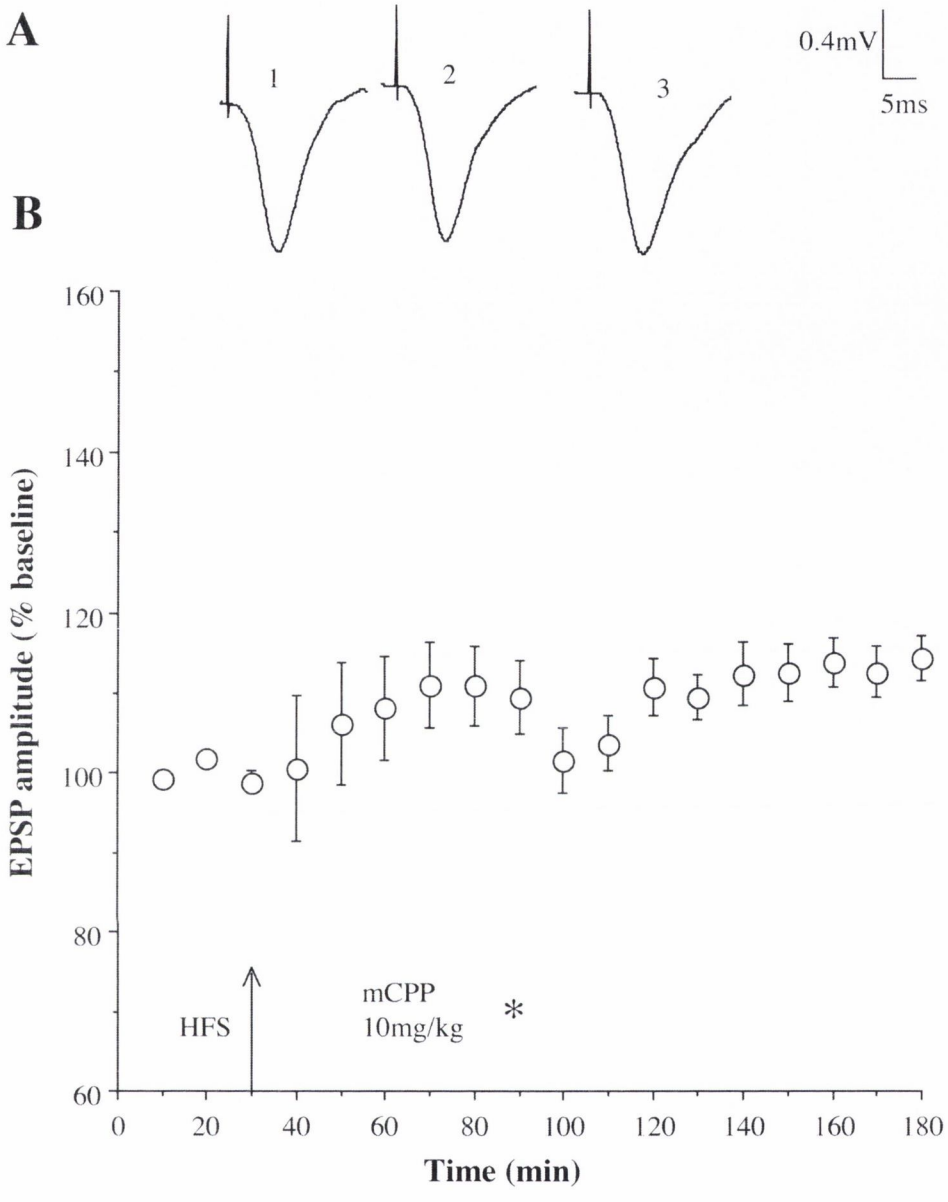

Figure 3.9 Effect of mCPP on baseline synaptic transmission in stressed rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS and 90 min post mCPP (10mg/kg, i.p.) administration (3). (B) Mild stress prevented the induction of LTP (109±4% at 60 min post HFS, n=5, $p>0.1$ compared with baseline). Administration of mCPP did not significantly alter synaptic transmission (114±3% at 90 min post i.p. injection, n=5, $p>0.1$ compared with 10 min epoch pre-injection). Data expressed as mean±s.em.

3.10 BW723c86, a 5-HT_{2B} receptor agonist overcomes stress-induced inhibition of LTP.

As mCPP is both a 5-$HT_{2B/2C}$ receptor agonist it was decided to investigate agonists which had a more selective 5-HT profile. BW723c86 is a 5-HT_{2B} receptor selective agonist (Baxter et al. 1995; Kennett et al. 1997a). Animals were exposed to acute elevated platform stress. A stable baseline was recorded for 30 minutes (99.7±0.8%, n=5 for the 10 minutes prior to HFS1). Application of HFS1 failed to induce LTP (103.8±2.3%, n=5 at 60 minutes post HFS1, $p>0.1$ compared with baseline, paired student's t-test). At 60 minutes post HFS1 BW723c86 (30mg/kg) was administered i.p. There was no evidence to suggest that BW723c86 affected baseline synaptic transmission. Thirty minutes after BW723c86 administration HFS2 was applied. HFS2 induced stable LTP (121.3±6.3% at 60 minutes post HFS2, n=5, $p<0.05$ compared with baseline, paired student's t-test). Although this LTP was numerically not very large it was statistically significantly different from baseline transmission values. Furthermore, the level of LTP was not statistically significantly less than the level of LTP in non-stressed controls (121.3±6.3% vs 132.3±4.6%, $p>0.1$, unpaired student's t-test). This result implicates 5-HT_{2B} receptor activation in the process by which LTP is recovered in stressed animals due to manipulation of the serotonergic system.

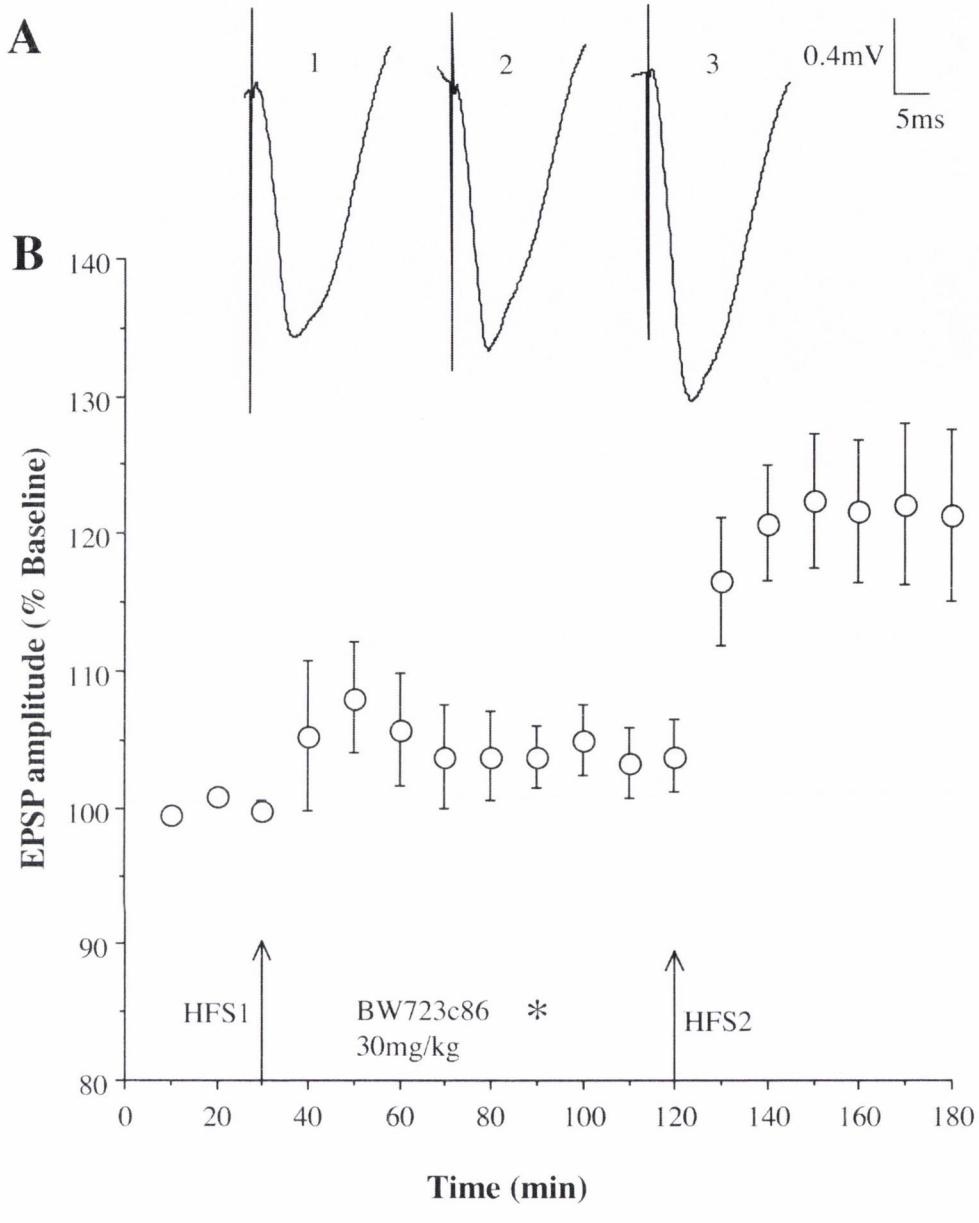

Figure 3.10 Effect of the 5-HT_{2B} receptor agonist BW723c86 on LTP in stressed rats.

(A) Insets show typical fEPSPs recorded ~ 5min before (1) and 60 min after (2) HFS1, and 60 min after HFS2 (3). (B) Mild stress prevented the induction of LTP by HFS1 (104±2% at 60 min post HFS1, n=5, $p>0.05$ compared with baseline). Administration of BW723c86 (30mg/kg, i.p.) a 5-HT_{2B} receptor agonist overcomes stress-induced inhibition of LTP following HFS2 (121±6%, n=5, $p<0.05$ compared with baseline). Data expressed as mean±s.e.m.

3.11 MK-212, a 5-HT_{2C} receptor agonist overcomes stress-induced inhibition of LTP.

Animals were exposed to acute mild elevated platform stress. A stable baseline was recorded for 30 minutes (100.4±0.7%, n=6 for the 10 minutes prior to HFS1). Application of HFS1 failed to induce LTP (106.5±3.2% at 60 minutes post HFS1, n=6, $p>0.05$, compared with baseline, paired student's t-test). At 60 minutes post HFS1, MK-212 (3mg/kg) a 5-HT_{2C} receptor selective agonist (Conn & Sanders-Bush 1987; Lee et al. 1992; Hemrick-Luecke & Fuller 1996) was administered i.p. There was no evidence to suggest that MK-212 (3mg/kg) had any effect on baseline synaptic transmission in the intervening period between its administration and HFS2. HFS2 resulted in a stable and robust LTP (130.8±5.3% at 60 minutes post HFS2, n=5, $p<0.01$, compared with baseline, paired student's t-test). There was no statistically significant difference between the level of recovered LTP (shown here) and that found in control non-stressed animals (130.8±5.3% vs 132.2±4.6%, $p>0.5$, unpaired student's t-test). This suggests that activation of 5-HT_{2C} receptors has the ability to overcome stress-induced inhibition of LTP.

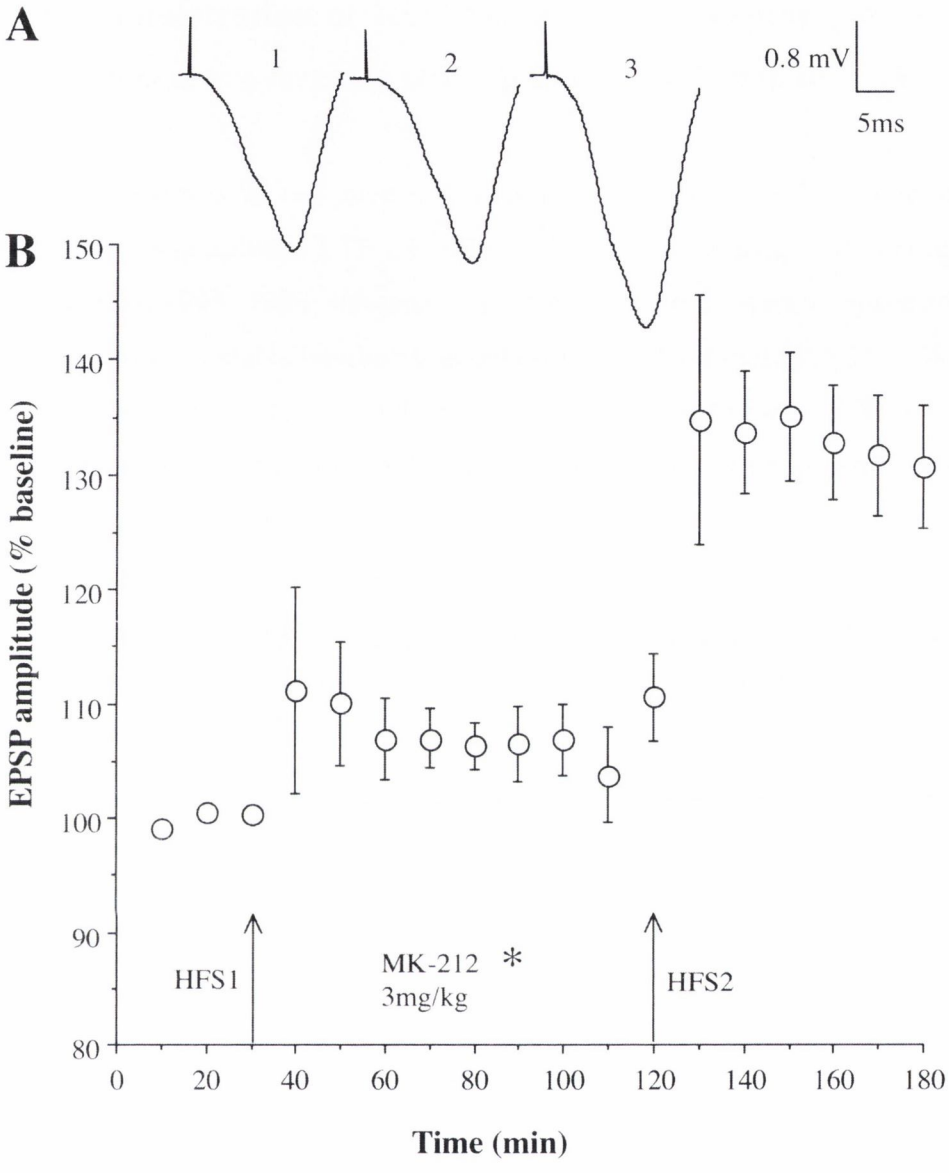

Figure 3.11 Effect of the 5-HT_{2C} receptor agonist MK-212 on LTP in stressed rats. (A) Insets show typical fEPSPs ~ 5 min before (1) and 60 min after (2) HFS1, and 60 min after HFS2 (3). (B) Mild stress prevented the induction of LTP by HFS1 (107±3% at 60 min post HFS1 n=6, $p>0.05$ compared with baseline). Administration of MK-212 (3mg/kg, i.p.) a 5-HT_{2C} receptor agonist overcame LTP inhibition (131±5% at 60 min post HFS2, n=6, $p<0.05$ compared with baseline). Data expressed as mean±s.e.m.

3.12 Administration of RS67333, a 5-HT_4 receptor partial agonist is not sufficient to overcome stress-induced inhibition of LTP.

This experiment was designed to assess the ability of a 5-HT_4 receptor agonist to overcome stress-induced LTP inhibition. RS67333 is a partial 5-HT_4 receptor agonist (Eglen et al. 1995, 1995; Fontana et al. 1997). Animals were exposed to acute mild elevated stress. A stable baseline was obtained for 30 minutes (105.3±1.7%, n=6 for the 10 minutes prior to HFS1). Application of HFS1 failed to induce LTP (106.9±5.7% at 60 minutes post HFS1, n=6, $p>0.5$ compared with baseline, paired student's t-test). Administration of RS67333 (10mg/kg, i.p.), a 5-HT_4 receptor partial agonist at 60 min post HFS1 did not affect baseline synaptic transmission. Application of HFS2 at 90 min post HFS1 failed to induce LTP (115.0±11.9% at 60 minutes post HFS2, n=6, $p>0.1$ compared with baseline, paired student's t-test). However, the level of LTP was not statistically significantly less than the level of LTP in non-stressed control animals (115.0±11.9% vs 132.3±4.6%, $p>0.1$, unpaired student's t-test).

3.13 Effect of RS67333, a 5-HT_4 receptor partial agonist in non-stressed rats.

This set of experiments involved investigating the effects of 5-HT_4 receptor activation in non-stressed rats. A stable baseline was obtained for 30 minutes (99.82±0.2%, n=6 for the 10 minutes prior to RS67333 administration). Following baseline recording, RS67333 (10mg/kg), a 5-HT_4 receptor partial agonist was administered i.p. RS67333 did not affect baseline transmission. 30 minutes after RS67333 administration HFS was applied. The variability in the post HFS data was such that although numerically there exists a clear LTP (129.0±13.0%) it is not statistically significant ($p=0.074$ compared with baseline, n=6, paired student's t-test). However, the difference between the 10 minute epoch pre-HFS and 60 minutes post HFS was statistically significant ($p=0.05$, n=6, paired student's t-test). In addition, there was no significant difference between

the LTP induced in this experiment and that induced in the control study (129.0±13.0% vs 132.3±4.6% at 60 min post HFS, $p>0.5$, $n=6$, unpaired student's t-test).

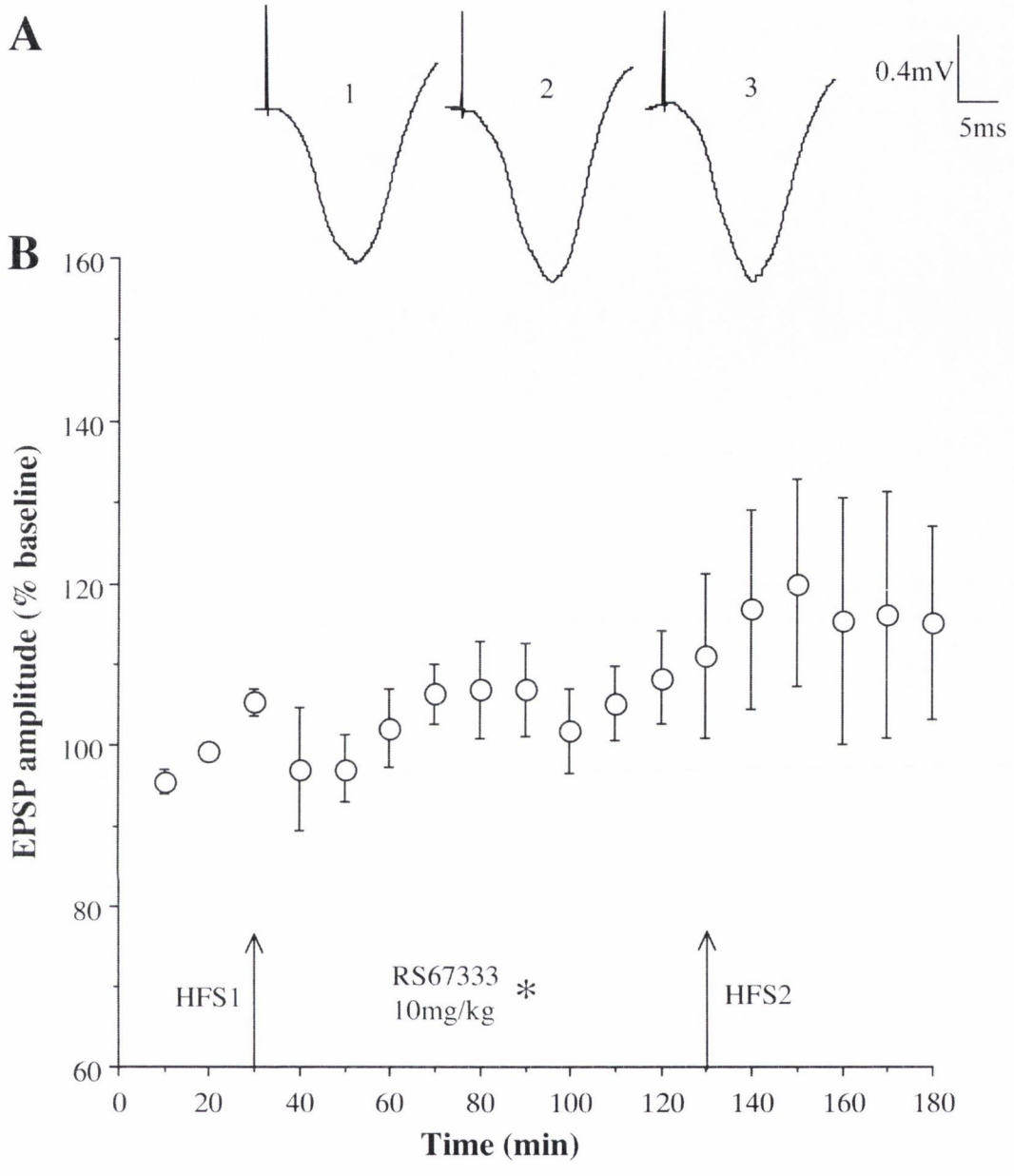

Figure 3.12 Effect of a 5-HT_4 receptor agonist RS67333 on stress induced inhibition of LTP.

(A) Insets show typical fEPSPs recorded ~ 5min before (1) and 60 min after (2) HFS1, and 60 min after HFS2 (3). (B) Mild stress prevented the induction of LTP by HFS1 (107±6%, at 60min post HFS1, n=6, p>0.5 compared with baseline). Administration of RS67333 (10mg/kg, i.p.), a 5-HT_4 partial agonist failed to overcome inhibition of LTP following a second HFS (HFS2) (115±12% at 60 min post HFS2, n=6, p>0.1 compared with baseline). Data expressed as mean±s.e.m.

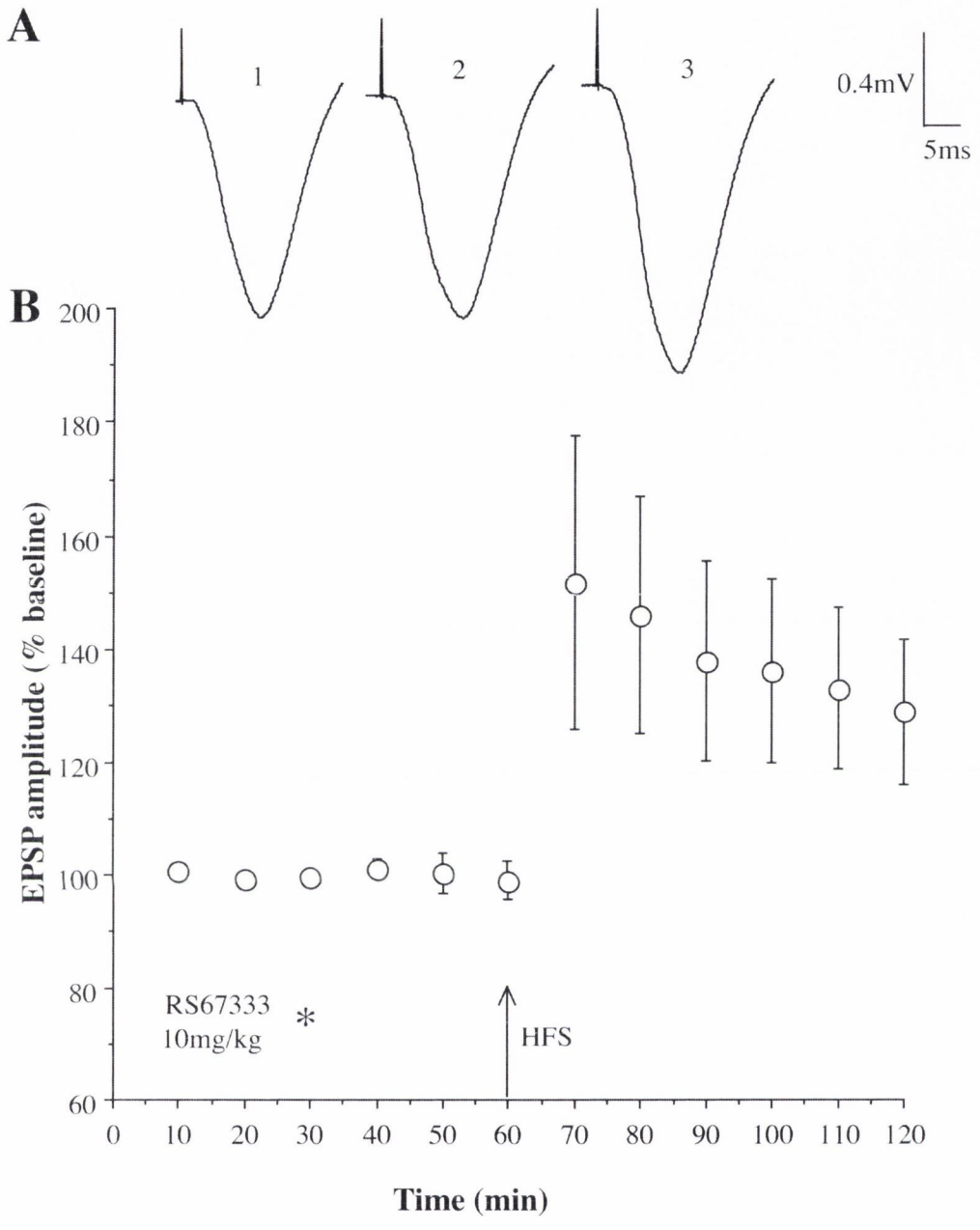

Figure 3.13 Effect of RS67333 on LTP in non-stressed rats.

(A) Insets show typical fEPSPs ~ 5min before (1) and 30 min after (2) RS67333 administration and 60 min after (3) HFS. (B) Administration of RS67333, a 5-HT_4 partial agonist (10mg/kg, i.p.) did not affect the induction of LTP by HFS (129±13% at 60 min post HFS, n=6, p=0.05 compared with 10 min epoch pre-HFS). Data expressed as mean±s.e.m.

3.14 Effect of D-AP5 (100 nmol) on LTP induction in CA1 *in vivo*.

It was decided to determine the specificity of the ability of the 5-HT releaser to overcome the inhibition of LTP by stress by examining its effects on LTP inhibition by D-AP5, a competitive NMDA receptor antagonist. First we examined the ability of a relatively low dose of D-AP5 to prevent the induction of LTP in CA1 in non-stressed animals. After recording 30 minutes of stable synaptic transmission, D-AP5 (100 nmol) was injected i.c.v. concomitant to i.p. vehicle administration. Application of HFS resulted in a numerically very small although statistically significant increase in synaptic transmission (109.6±2.4%, n=8, $p<0.01$ compared with baseline, paired student's t-test). A control set of experiments was also undertaken in which distilled water (vehicle) (5µl) was injected i.c.v. in place of D-AP5. There was no significant i.c.v. injection effect on synaptic transmission. Application of HFS resulted in a robust, stable and statistically significant LTP (129.1±8.5%, at 60 minutes post HFS, n=6, $p<0.05$ compared with baseline, paired student's t-test). The treatment with D-AP5 resulted in significantly less potentiation of synaptic transmission when compared to vehicle i.c.v. injected animals (109.6±2.4% vs 129.1±8.5%, $p<0.05$, unpaired student's t-test). Therefore, the inhibition of LTP by D-AP5 although not entirely complete was both numerically very considerable and statistically significant.

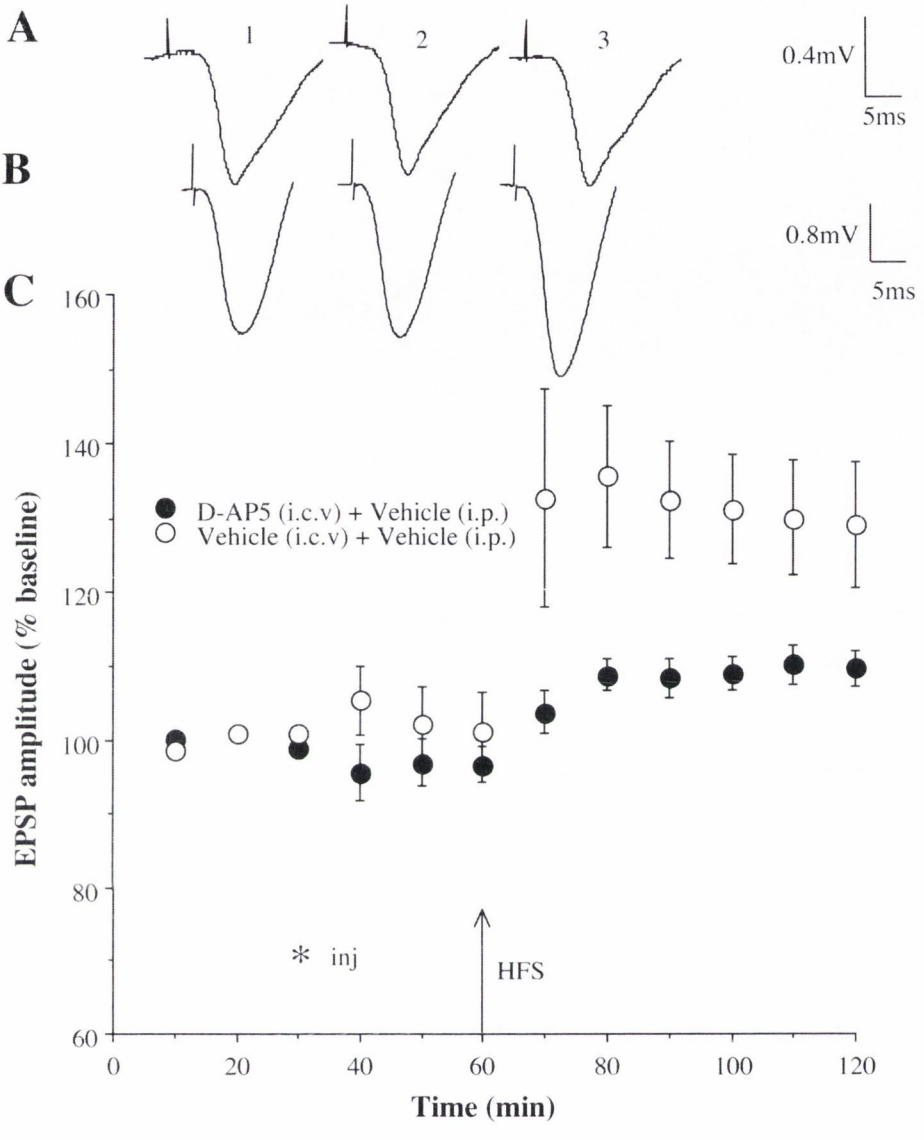

Figure 3.14 Effect of D-AP5 on LTP in non-stressed rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 30 min after (2) D-AP5 (i.c.v) and Vehicle (i.p.) coadministration and 60 min after (3) HFS. (B) Insets show typical fEPSPs recorded ~ 5min before (1) and 30 min after (2) vehicle (i.c.v. and i.p.) coadministration and 60 min after (3) HFS. (C) Administration of D-AP5 (100 nmol) causes a statistically significant reduction in LTP (●, D-AP5 + Vehicle 110±2%, n=8; O, Vehicle + Vehicle 129±9%, n=6 at 60 min post HFS, $p<0.05$,unpaired student's t-test). Data expressed as mean±s.e.m.

3.15 Effect of co-administration of (±)fenfluramine on the inhibition of LTP due to D-AP5.

This experiment was designed to investigate whether a compound which modulates extracellular serotonin levels and has been shown to have the ability to overcome a stress induced LTP block may also overcome a block in LTP due to another mechanism i.e. NMDA receptor antagonism. Non-stressed animals were anaesthetised and a 30 minute baseline was recorded (102.0±0.3%, n=5 for the 10 minutes prior to drug co-administration). D-AP5 (100nmol, i.c.v.) was administered concomitantly with (±)fenfluramine (5mg/kg, i.p.). After 30 minutes of further baseline recordings, HFS was applied. HFS failed to induce a statistically significant STP (105.9±6.9%, n=5, for the first 10 minute epoch post HFS, $p>0.5$ compared to baseline, paired student's t-test). However, at 60 minutes post HFS there was a significant increase in synaptic transmission (LTP) (126.8±7.3% at 60 minutes post HFS, n=5, $p<0.05$ compared with baseline, paired student's t-test). The level of LTP was greater than the magnitude of potentiation seen in D-AP5 (i.c.v) and vehicle (i.p.) treated animals (126.8±7.3% vs 109.6±2.4%, $p<0.05$, unpaired student's t-test). Also the level of LTP was not significantly less than that of the vehicle only (i.c.v and i.p.) treated animals (126.8±7.3% vs 129.1±8.5%, $p>0.5$, unpaired student's t-test). This suggests that the ability of the serotonergic system to overcome LTP blockade is not confined solely to LTP which has been blocked by stress.

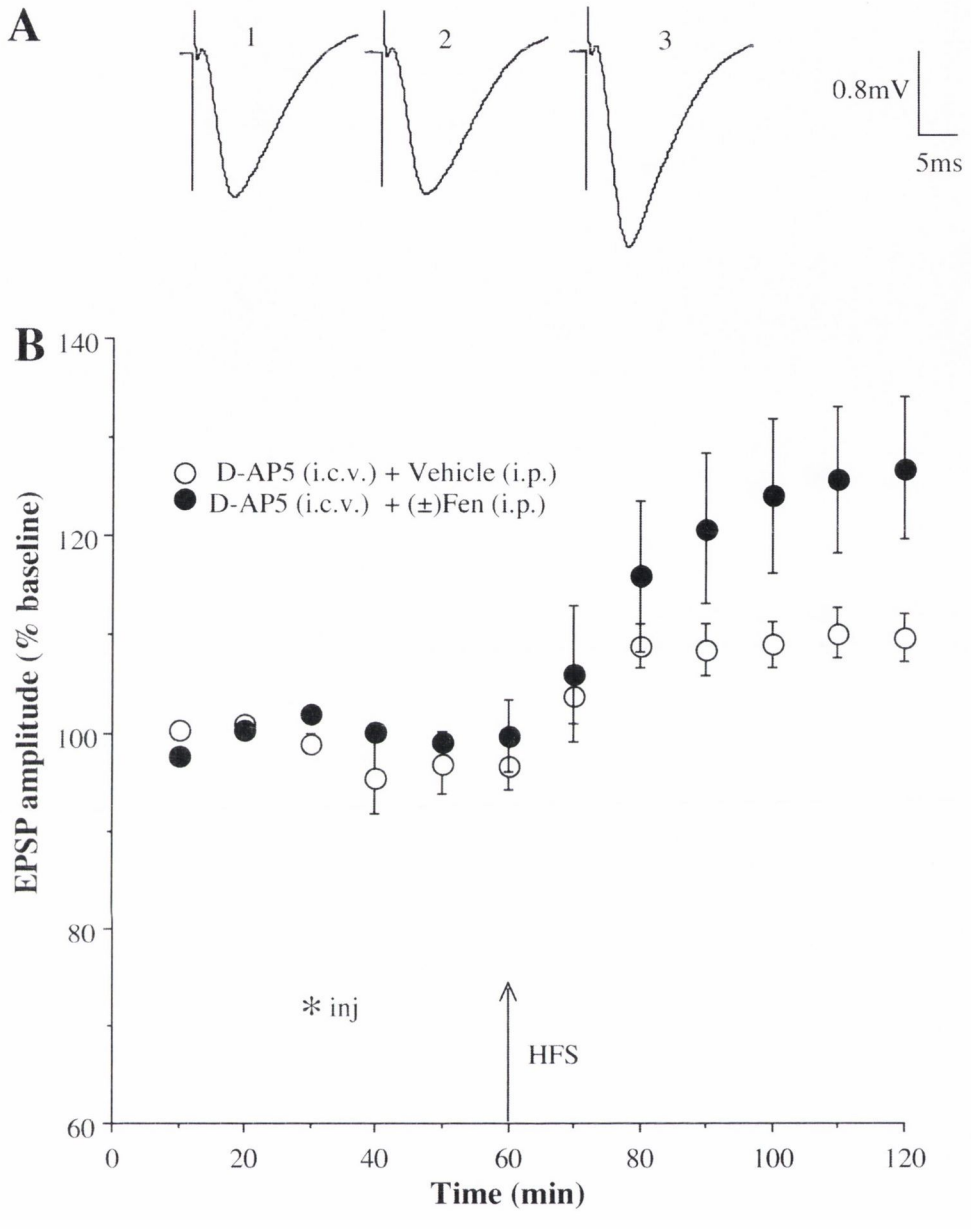

Figure 3.15 Effect of (±)fenfluramine on the D-AP5 mediated block of LTP.

(A) Insets show typical fEPSPs recorded ~ 5min before (1) and 30 min after (2) D-AP5 (i.c.v.) and (±)Fen (i.p.) co-administration and 60 min post HFS (3). (B) The induction of LTP is significantly greater following co-administration of D-AP5 (100 nmol) and (±)Fen (5mg/kg) when compared to co-administration of D-AP5 (100 nmol) and Vehicle (●, D-AP5 + (±)Fen 127±7%, n=5; O, D-AP5+Vehicle 110±2% n=8 at 60 min post HFS $p<0.05$ unpaired student's t-test). Data expressed as mean±s.e.m.

The Flinders Model of Depression.

This next set of experiments were designed to investigate if synaptic plasticity in the CA1 area was impaired in the Flinders model of depression. As well as investigating the intact model itself, manipulations such as maternal separation and chronic anti-depressant drug treatment were also investigated.

3.16 Effect of HFS in Flinders Resistant Line (FRL) rats.

The Flinders resistant line rats are the control 'non-depressed' rats in the Flinders depression model. A stable 30 minute baseline was recorded (101.1±1.6%, n=7, for the 10 minutes prior to HFS). Application of HFS resulted in robust LTP. There was an immediate increase in synaptic transmission (121.3±4.5% at 10 minutes post HFS, $p<0.01$ when compared with baseline, paired student's t-test) which then stabilized (127.1±8.0% at 60 minutes post HFS, $p<0.01$ compared with baseline, paired student's t-test).

3.17 Effect of HFS in chronic escitalopram treated FRL rats.

This experiment was designed to assess the effect of chronic escitalopram (anti-depressant) treatment on the ability of HFS to induce LTP in FRL rats. Escitalopram was administered as a constituent of the food pellet. This pellet was available ad libitum for 2 weeks prior to experimentation. A stable baseline was obtained for 30 minutes (100.4±0.9%, n=6, for the 10 minutes prior to HFS). Application of HFS failed to induce LTP (102.8±7.2% at 60 minutes post HFS, n=6, $p>0.5$ when compared to baseline, paired student's t-test). Also, there was a statistically significant difference in synaptic transmission post HFS between FRL control animals and FRL escitalopram treated animals (127.1±8.0% vs 102.8±7.2% at 60 minutes post HFS, $p<0.05$, unpaired student's t-test).

3.18 Effect of HFS in maternally separated FRL rats.

This experiment was designed to assess the effect of maternal separation on LTP induction in FRL rats. For maternal separation protocol see Section 2.12. A stable baseline was obtained for 30 minutes (99.2±1.0%, n=7, for the 10 minutes prior to HFS). Application of HFS induced a small although statistically significant increase in synaptic transmission (115.0±4.9% at 60 minutes post HFS, n=7, $p<0.05$, compared with baseline, paired student's t-test). Although the level of LTP was numerically on average considerably less than that of FRL control animals (127.1±8.0% vs 115.0±4.9%), this reduction was not statistically significant ($p>0.1$, unpaired student's t-test).

3.19 Effect of HFS in chronic escitalopram treated maternally separated FRL rats.

This experiment was designed to assess the effect of chronic escitalopram treatment on maternally separated FRL rats. Although chronic escitalopram treatment inhibited LTP induction in control FRL rats (Figure 3.18) it was hypothesised that escitalopram may enhance LTP in animals that had previously undergone a chronic stressor such as maternal separation.

A stable baseline was obtained for 30 minutes (98.9±1.5%, n=6, for the 10 minutes prior to HFS). Application of HFS failed to induce a statistically significant LTP (111.6±7.1% at 60 minutes post HFS, n=6, $p>0.1$ compared with baseline, paired student's t-test). The level of potentiation was on average less than the level of potentiation seen in maternally separated rats (115.0±4.9% vs 111.6±7.1%). However there was no significant statistical difference between these groups ($p>0.5$, unpaired student's t-test).

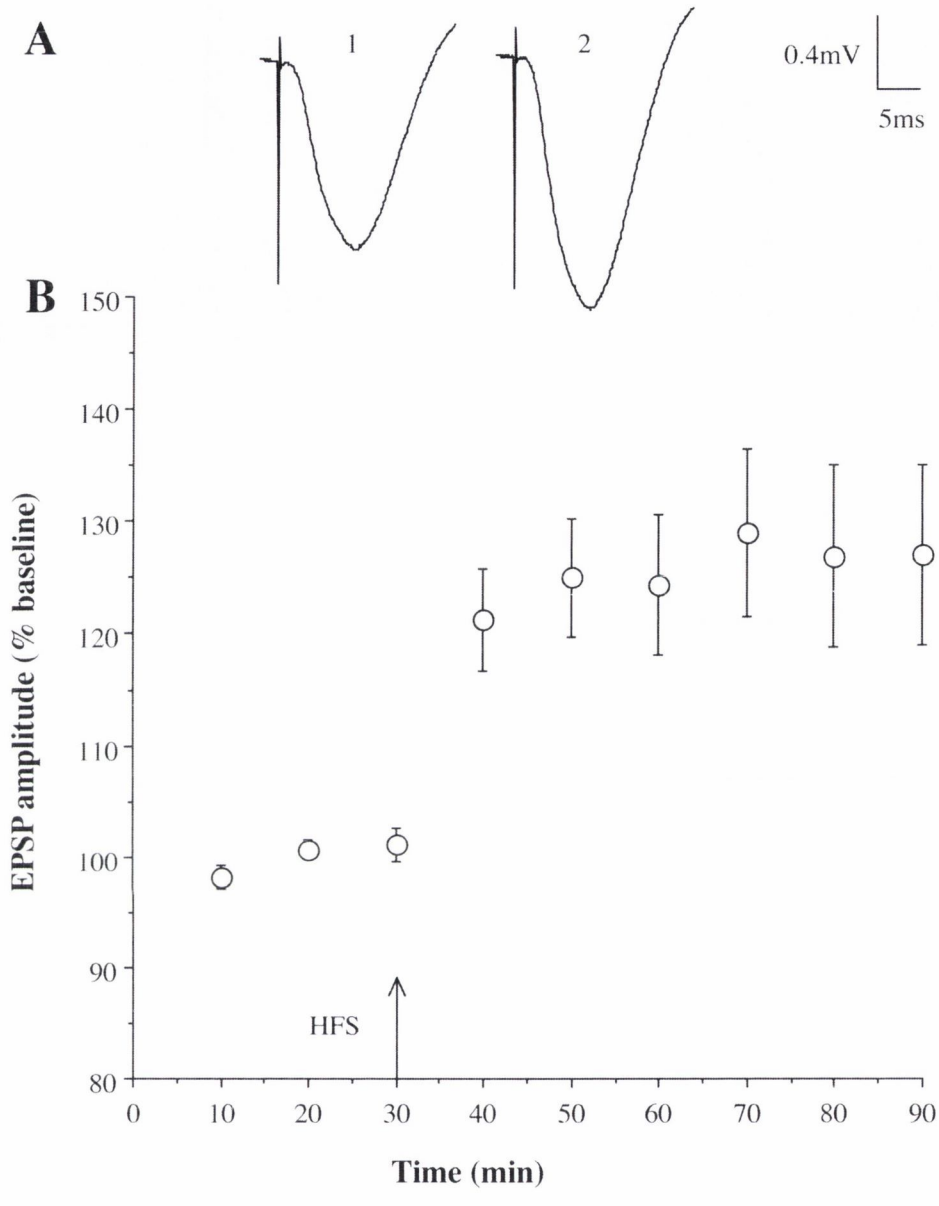

Figure 3.16 Effect of HFS on synaptic transmission in Flinders Resistant Line (FRL) rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS induced stable LTP (127±8%, at 60 min post HFS, n=7, $p<0.01$ compared with baseline). Data expressed as mean±s.e.m.

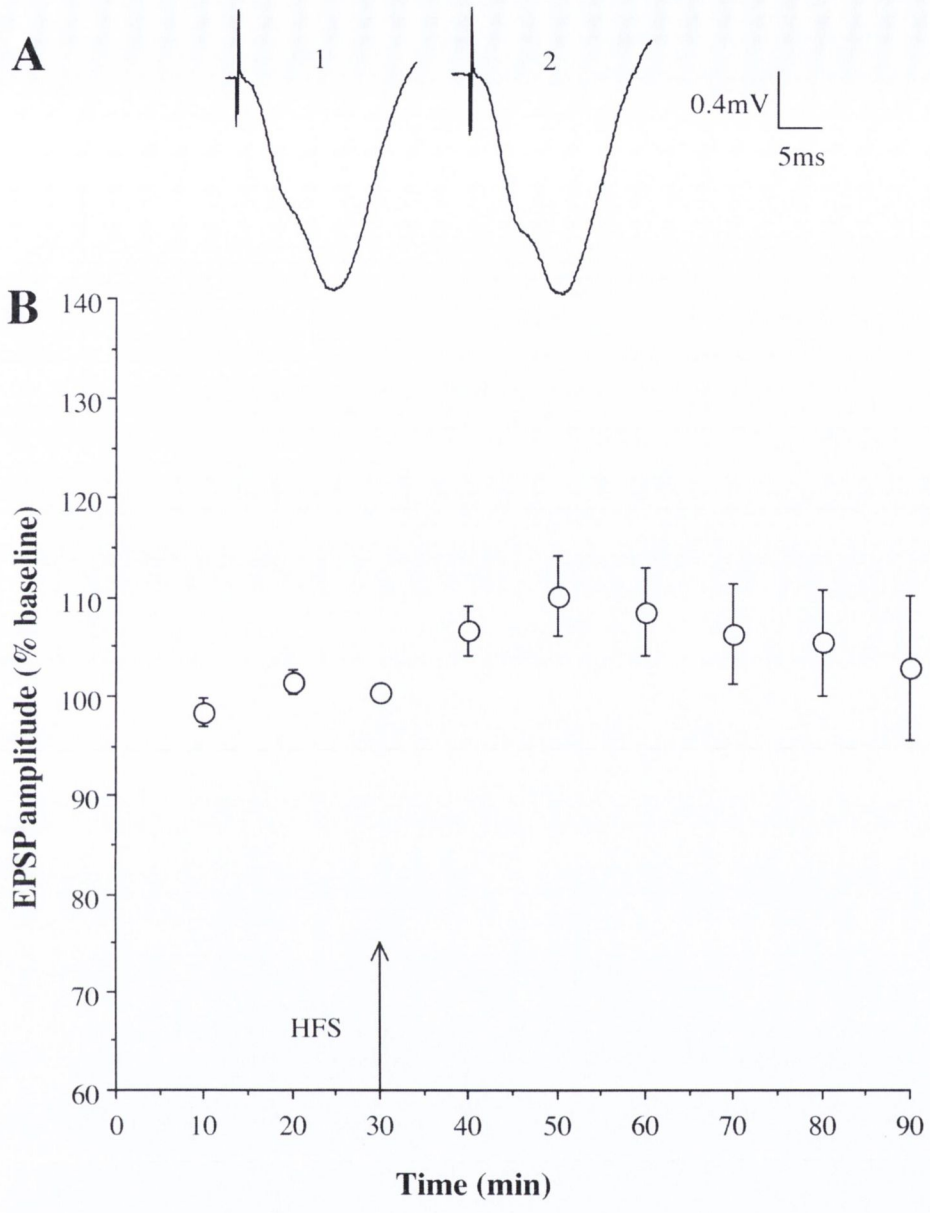

Figure 3.17 Effect of HFS on synaptic transmission in escitalopram treated FRL rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS failed to induce LTP (103±7% at 60 min post HFS, n=6, $p>0.5$ compared with baseline). Data expressed as mean±s.e.m.

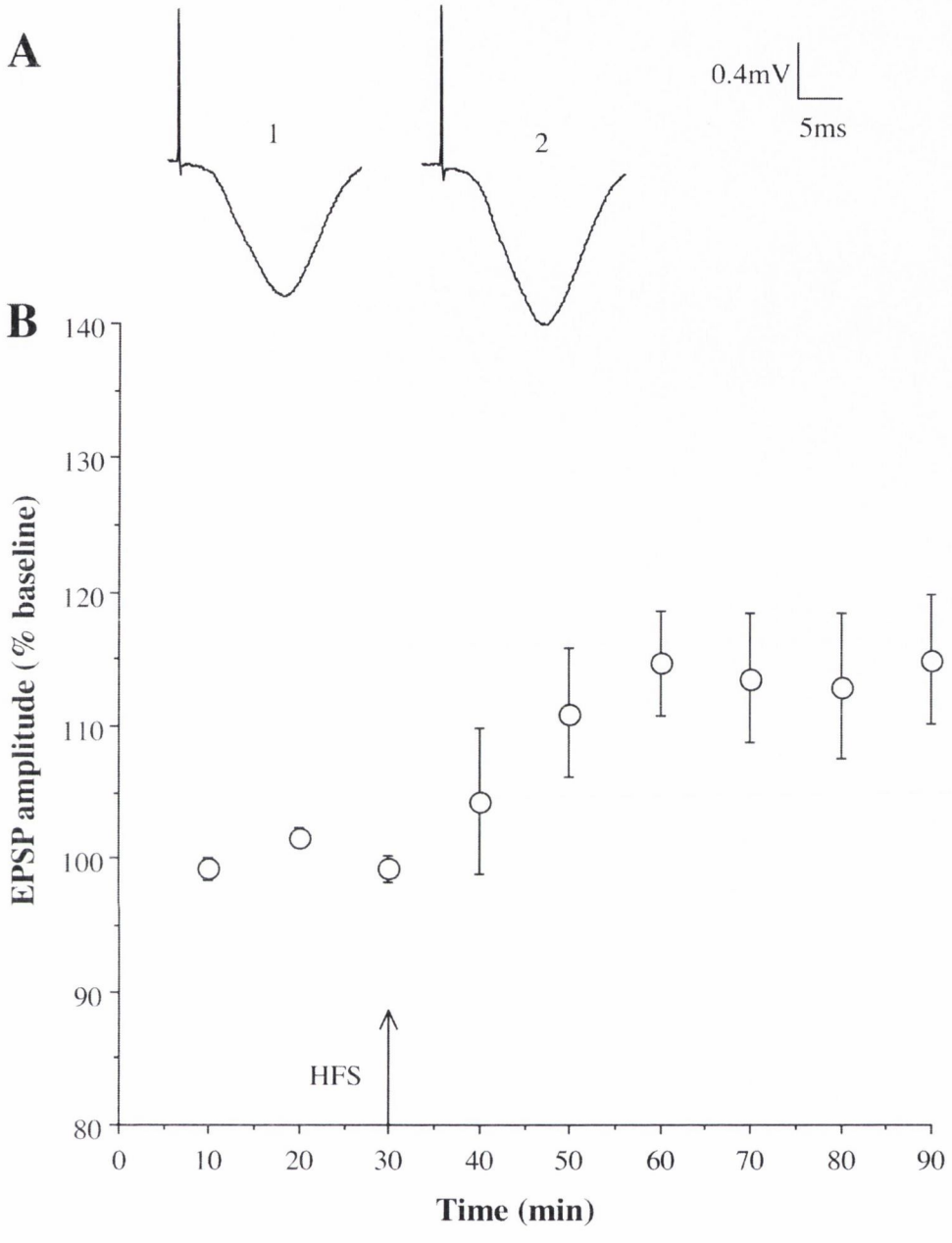

Figure 3.18 Effect of HFS on synaptic transmission in maternally separated FRL rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS induced stable LTP (115±5%, at 60 min post HFS, n=7, $p<0.01$ compared with baseline). Data expressed as mean±s.e.m.

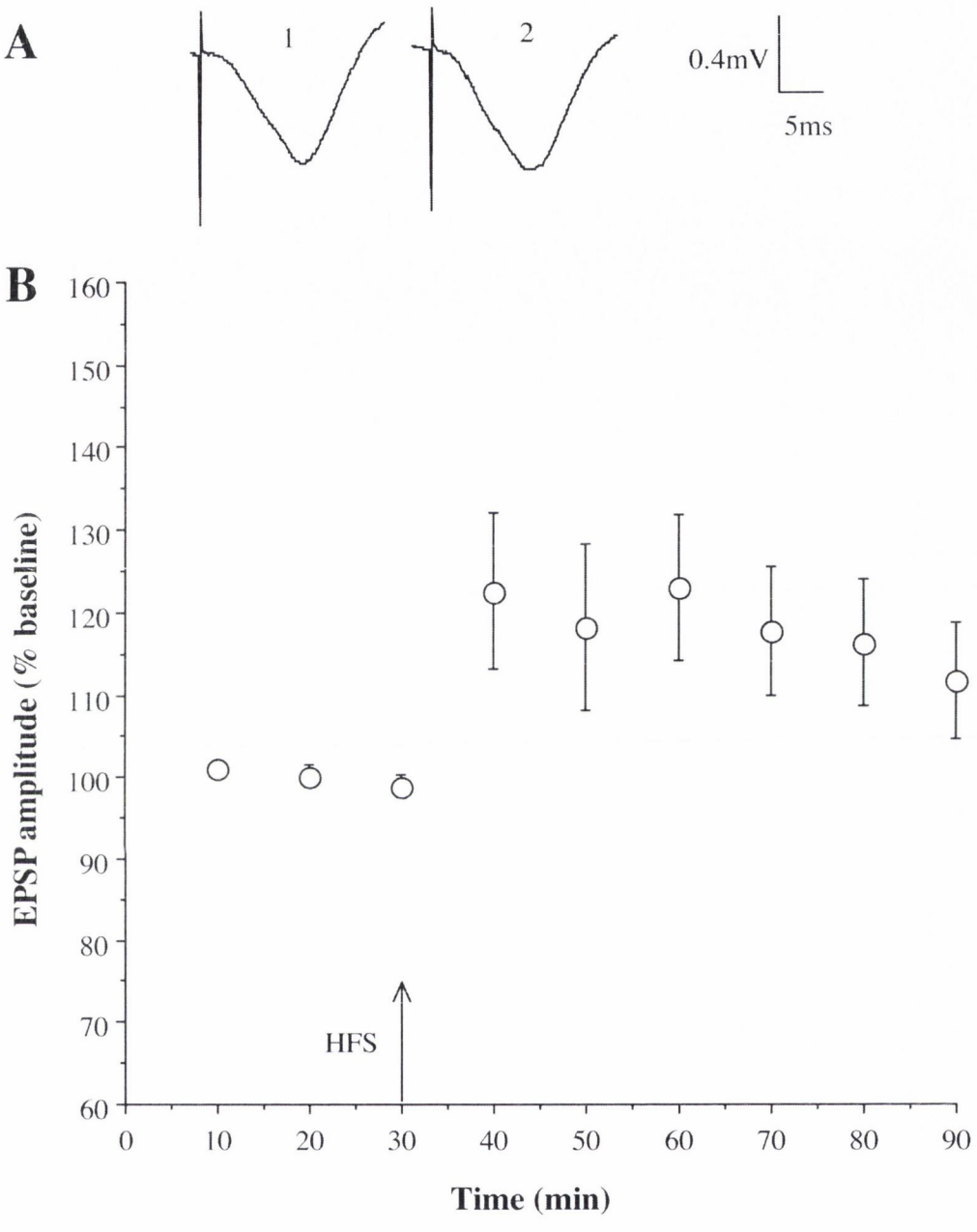

Figure 3.19 Effect of HFS on synaptic transmission in escitalopram treated maternally separated FRL rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS failed to induce LTP (112±6%, at 60 min post HFS, n=6, $p>0.1$ compared with baseline). Data expressed as mean± s.e.m.

3.20 Effect of HFS in Flinders Sensitive Line (FSL) rats.

The Flinders Sensitive Line rats are the depression/stress susceptible animals in the Flinders model of depression. This set of experiments are designed to assess the effects of HFS on synaptic transmission in male FSL rats. A stable baseline was obtained for 30 minutes (100.1±0.9%, n=7, for the 10 minutes prior to HFS). Application of HFS resulted in a numerically very small albeit statistically significant increase in synaptic transmission (108.1±2.8%, n=7, $p<0.05$ compared with baseline, paired student's t-test). This level of potentiation was however significantly less than the level found in FRL control animals (127.1±8.0% vs 108.1±2.8%, $p<0.05$, unpaired student's t-test).

3.21 Effect of HFS in chronic escitalopram treated FSL rats.

We also investigated whether treatment with escitalopram ad libitum would affect induction of LTP in FSL animals. As mentioned previously escitalopram was administered as a constituent of the food pellet for a period of 2 weeks.

A stable baseline was obtained for 30 minutes (99.3±1.1%, n=6, for the 10 minutes prior to HFS). Application of HFS failed to induce LTP (95.4±6.5%, at 60 minutes post HFS, n=6, $p>0.5$ compared with baseline, paired student's t-test). This paralleled the negative impact of escitalopram treatment on FRL animals. There was no statistically significant difference between FSL escitalopram treated animals and FRL escitalopram treated animals (95.4±6.5% vs 102.8±7.2%, $p>0.5$, at 60 minutes post HFS, unpaired student's t-test). However, there was a trend towards statistical significance between FSL control animals and FSL escitalopram treated animals (108.1±2.8% vs 95.4±6.5% at 60 minutes post HFS, $p<0.1$, unpaired student's t-test).

3.22 Effect of HFS in maternally separated FSL rats.

This experiment was designed to investigate the effect of maternal separation on LTP induction in FSL rats. A stable baseline was obtained for 30 minutes (101.2±1.5%, n=6, for the 10 minutes prior to HFS). Application of HFS induced an increase in synaptic transmission (118.2±6.3%, n=6, at 60 minutes post HFS). While this increase was numerically reasonably large it was not quite statistically significant ($p=0.065$ compared with baseline, paired student's t-test). Furthermore, there was no statistically significant difference between the level of HFS induced potentiation seen in FSL maternally separated rats and FRL maternally separated rats (118.2±6.3% vs 115.0±4.9%, $p>0.5$, unpaired student's t-test). In addition the level of potentiation seen here was on average numerically greater although not statistically different from that seen in FSL control animals (118.2±6.3% vs 108.1±2.8%, at 60 minutes post HFS, $p>0.1$, unpaired student's t-test).

3.23 Effect of HFS in chronic escitalopram treated maternally separated FSL rats.

A stable baseline was obtained for 30 minutes (100.9±0.4%, n=7, for the 10 minutes prior to HFS). Application of HFS failed to induce LTP (105.8±7.8%, n=7, at 60 minutes post HFS, $p>0.5$ compared with baseline, paired student't t-test). There was no significant difference at 60 minutes post HFS between escitalopram treated maternally separated FSL animals and FSL control animals (105.8±7.8% vs 108.1±2.8%, $p>0.5$, unpaired student's t-test). There was no significant difference in synaptic transmission post HFS between non-treated maternally separated FSL animals and escitalopram treated maternally separated FSL animals (118.2±6.3% vs 105.8±7.8%, $p>0.1$, unpaired student's t-test). Also there was no significant difference between FRL escitalopram treated maternally separated animals and FSL escitalopram treated maternally separated animals (111.6±7.1% vs 105.8±7.8%, $p>0.5$, unpaired student's t-test).

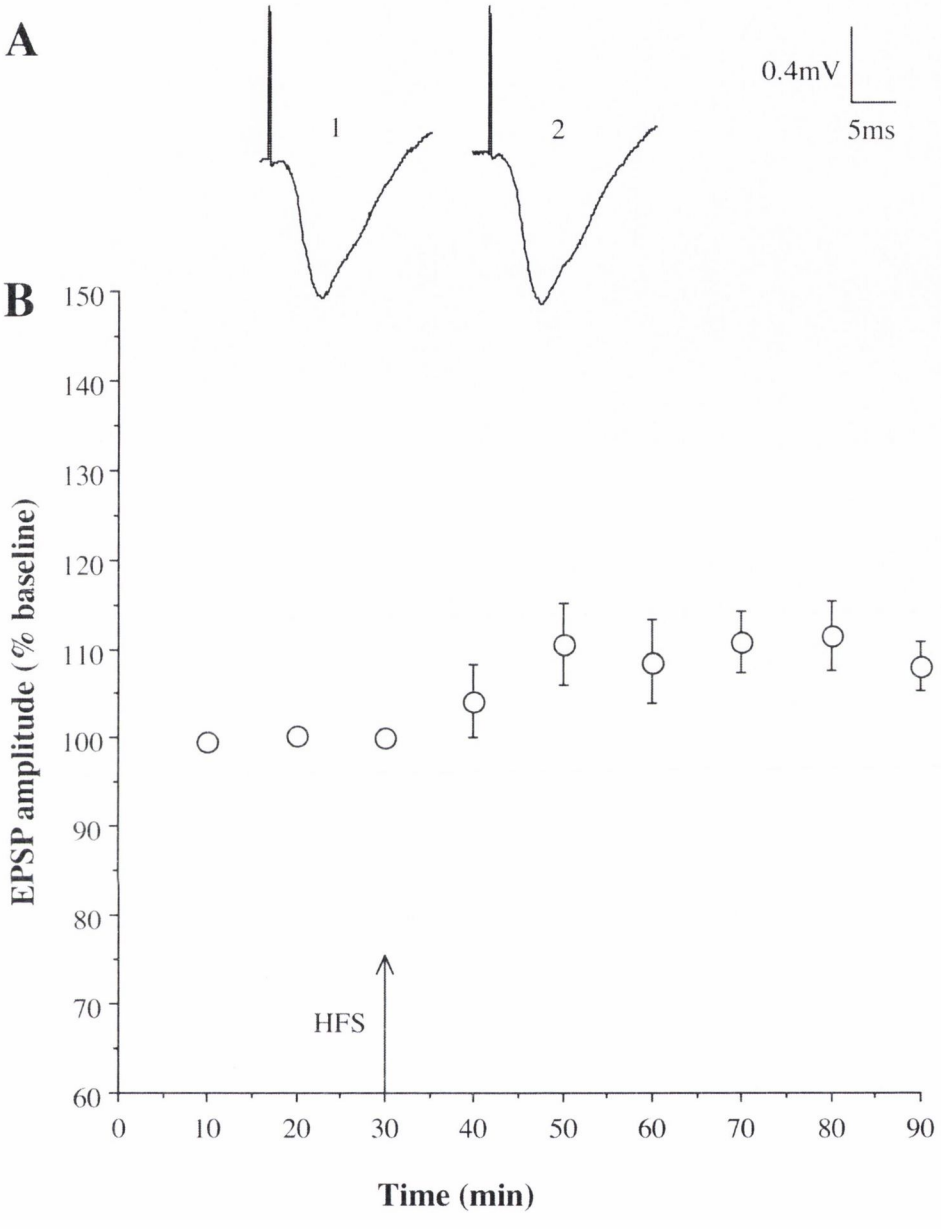

Figure 3.20 Effect of HFS on synaptic transmission in Flinders Sensitive Line (FSL) rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS induced a significant increase in EPSP amplitude (108±3%, at 60 min post HFS, n=7, $p<0.05$ compared with baseline). Data expressed as mean±s.e.m.

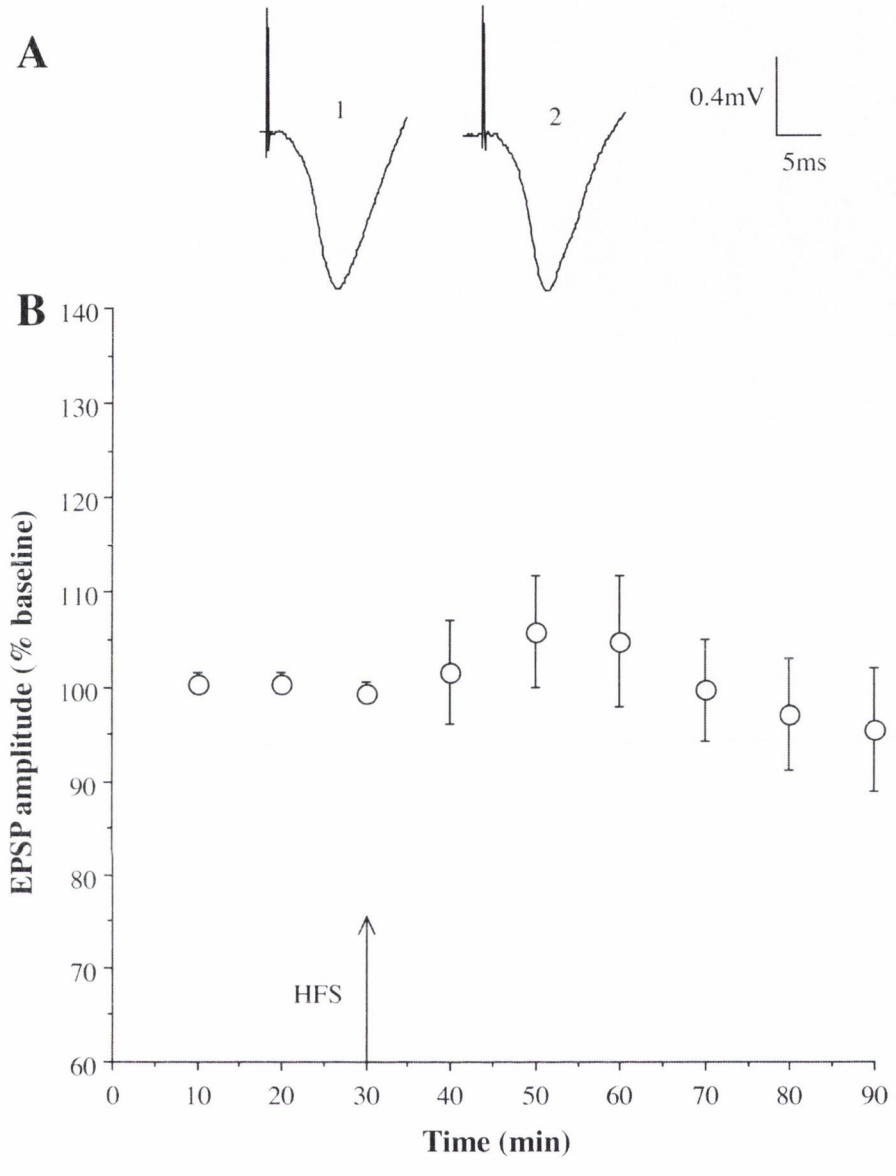

Figure 3.21 Effect of HFS on synaptic transmission in escitalopram treated FSL rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS failed to induce stable LTP (95±7% at 60 min post HFS, n=6, $p>0.5$ compared with baseline). Data expressed as mean±s.e.m.

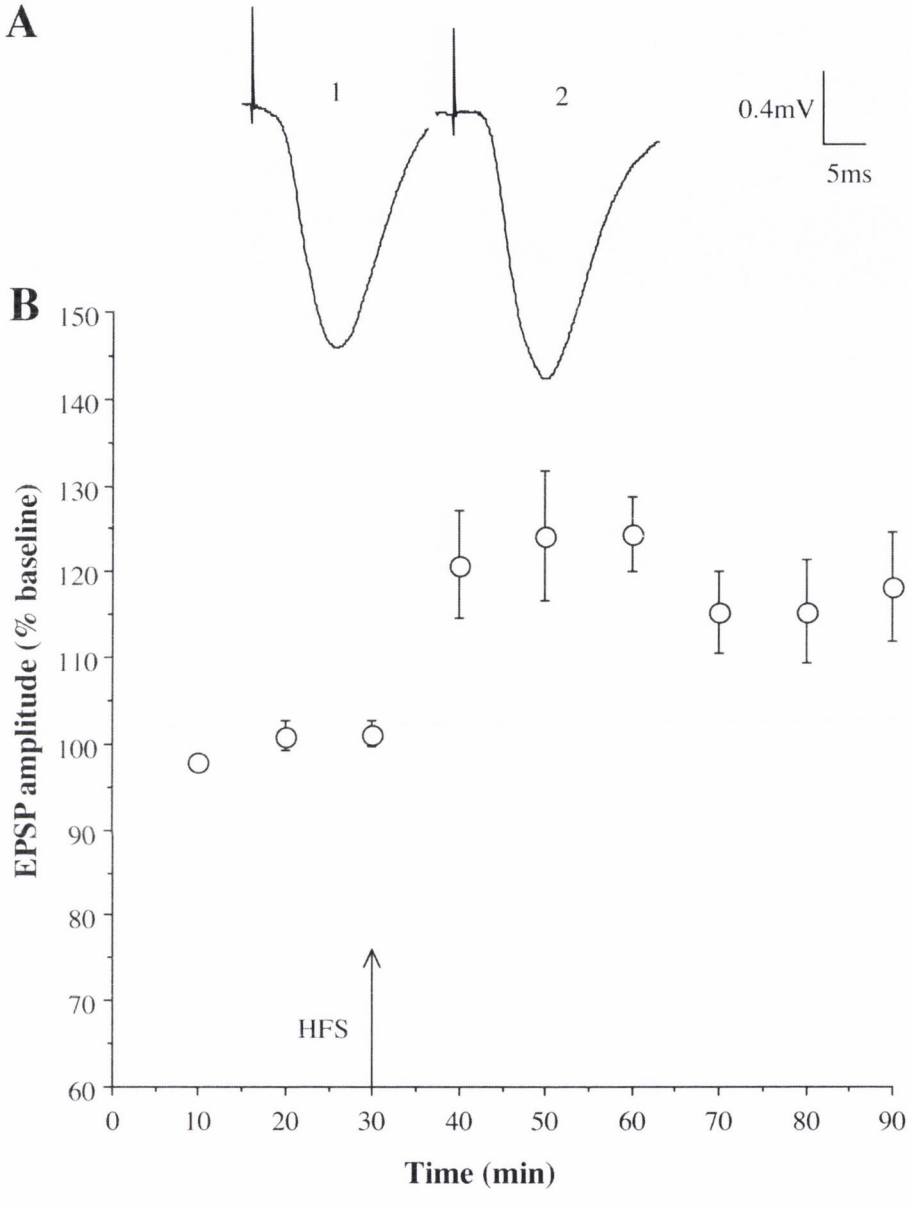

Figure 3.22 Effect of HFS on synaptic transmission in maternally separated FSL rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS failed to induce stable LTP (118±6%, at 60 min post HFS, n=6, $p>0.05$ compared with baseline). Data expressed as mean±s.e.m.

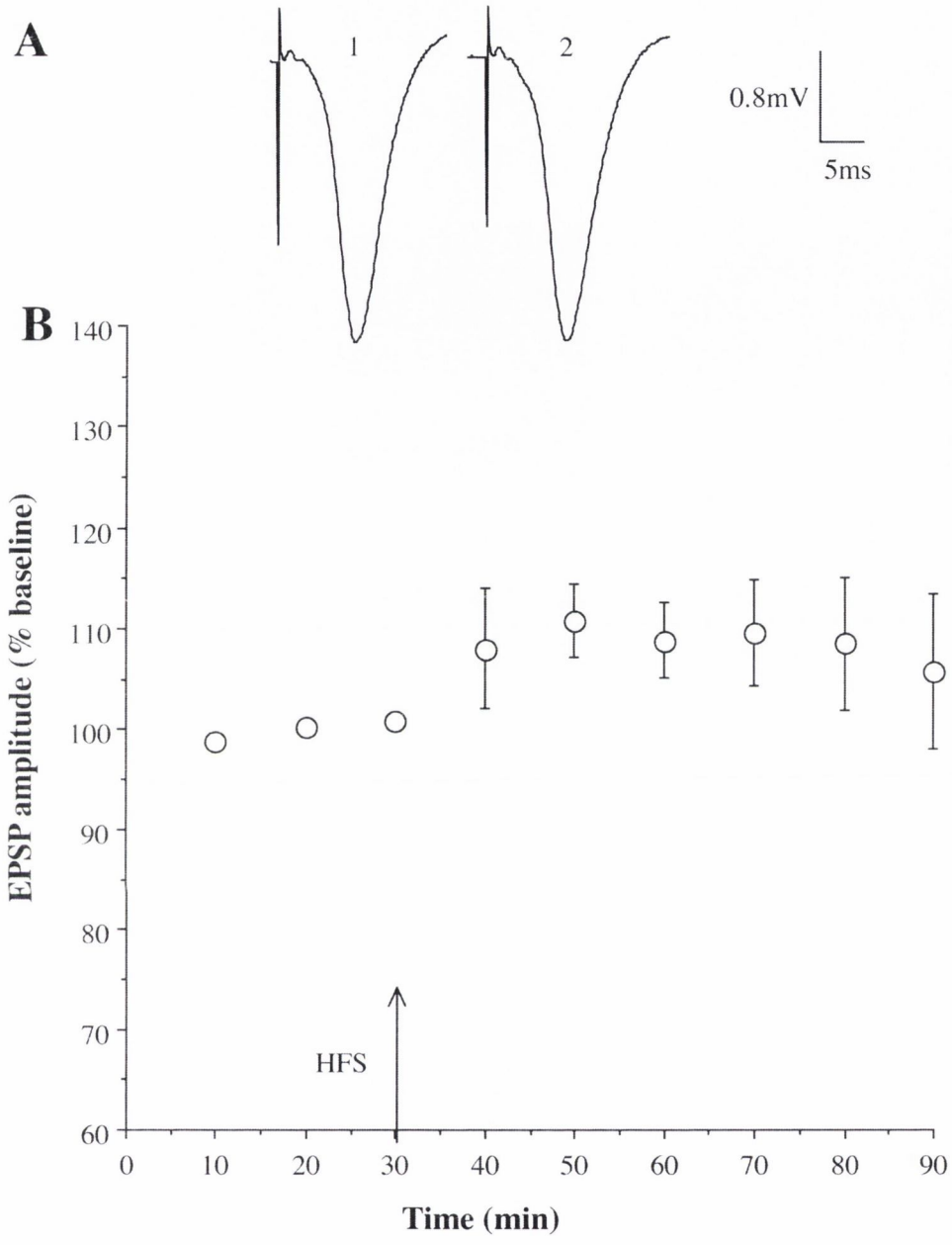

Figure 3.23 Effect of HFS on synaptic transmission in escitalopram treated maternally separated FSL rats.

(A) Insets show typical recordings of fEPSPs ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS failed to induce stable LTP (106±8%, at 60 min post HFS, n=7, $p>0.5$ compared with baseline). Data expressed as mean±s.e.m.

3.24 Paired Pulse Facilitation (PPF) in Flinders Depression Model.

Each group (except FSL control animals) showed decreasing levels of paired pulse facilitation with increasing time interval between initial stimulus and second stimulus. There was a significant reduction in PPF at 40ms between FRL control animals and FSL control animals (34.5±4.9% vs 17.0±3.6%, $p<0.05$, unpaired student's t-test). The difference between all other FSL groups and their respective counterpart FRL groups was not statistically significant. However the difference in PPF between FSL control animals and FRL control animals at 120ms did border on statistical significance (25.0±3.0% vs 14.7±4.2%, $p=0.069$, unpaired student's t-test). Correlation studies were undertaken to see if the level of PPF inducible pre-HFS was correlated with subsequent potentiation at 60min post HFS. FRL control animals showed a strong positive correlation between PPF at 40ms and potentiation at 60min post HFS (Correlation coefficient:0.759, $p<0.05$, $n=7$). In addition FRL control animals also showed strong positive correlation between PPF at 80ms and potentiation at 60 min post HFS (Correlation coefficient:0.898, $p<0.01$, $n=7$). FSL control animals showed a strong positive correlation between PPF at 40ms and potentiation at 60min post HFS (Correlation coefficient: 0.899, $p<0.05$, $n=7$).

Table 3.24 Paired Pulse Facilitation in the Flinders Depression Model.

GROUP	40ms	80ms	120ms
FRL control (n=7)	34.5+4.9%	27.8+5.1%	25.0+3.0%
FRL maternally separated (n=7)	44.0+9.3%	38.9+7.5%	25.6+3.7%
FRL escitalopram treated (n=6)	27.1±8.8%	25.2+4.6%	21.9+4.1%
FRL maternally separated & escitalopram treated (n=6)	41.6+11.3%	36.0+11.7%	24.3±7.6%
FSL control (n=7)	17.0+3.6%	26.9+5.3%	14.7±4.2%
FSL maternally separated (n=6)	49.1+11.5%	42.0+10.0%	30.8+8.1%
FSL escitalopram treated (n=6)	37.2±15.6%	25.2±8.0%	14.4±5.1%
FSL maternally separated & escitalopram treated (n=7)	44.4+7.4%	34.2+6.8%	24.6+4.9%

The above table shows the paired pulse facilitation data for the Flinders Depression Model at 40ms, 80ms and 120ms intervals respectively. PPF is expressed as [((EPSP2-EPSP1)/EPSP1)X100)%]. Data expressed as mean±s.e.m.

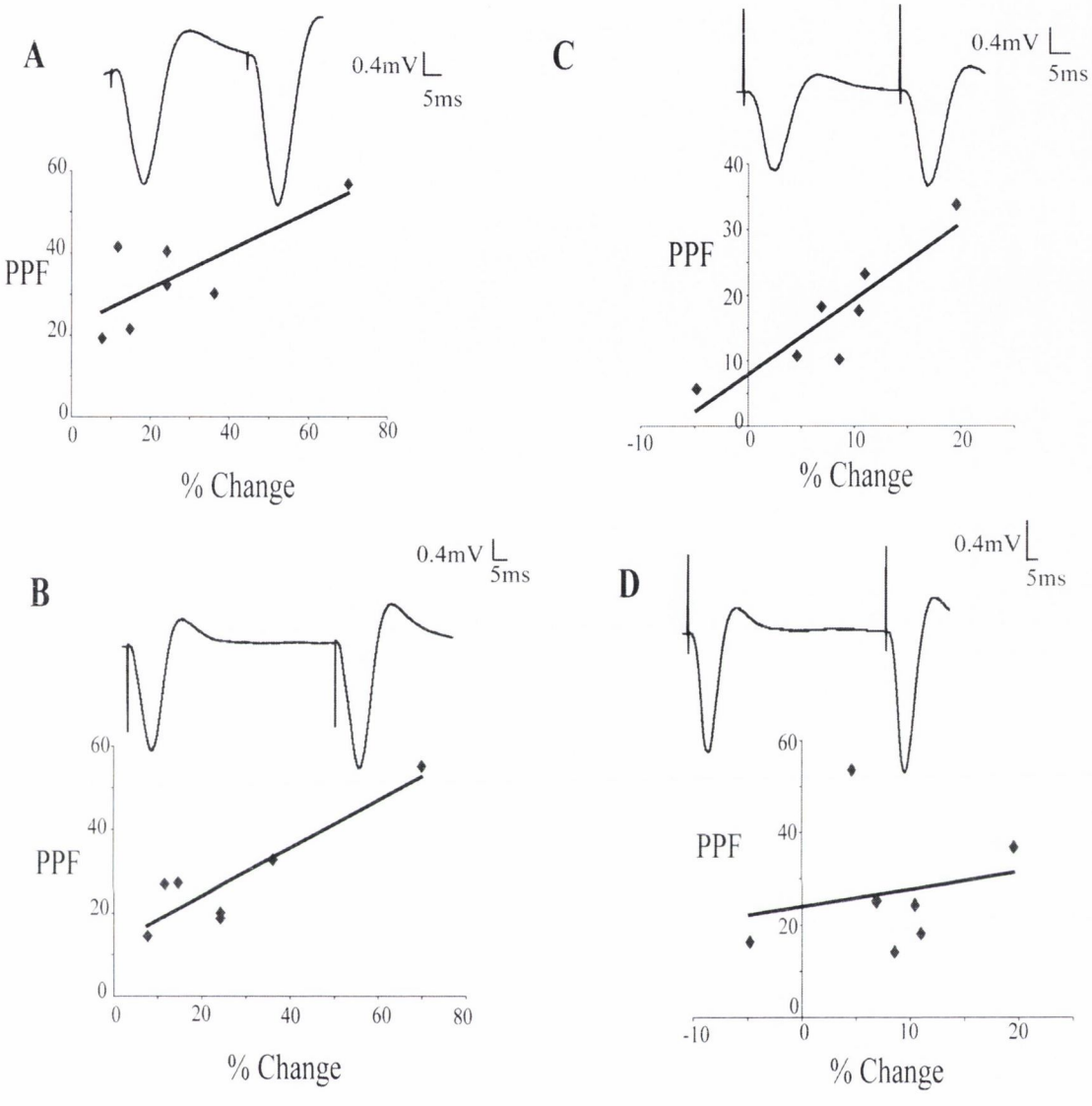

Figure 3.24 Initial PPF correlates with HFS-induced changes in certain Flinders subgroups.

Insets show examples of PPF traces from individual rats.(A) FRL at 40ms r=0.759, $p<0.05$, n=7 (B) FRL at 80ms r=0.898, $p<0.01$, n=7 (C) FSL at 40ms r=0.899, $p<0.05$, n=7 (D) FSL at 80ms r=0.194, $p>0.5$, n=7.

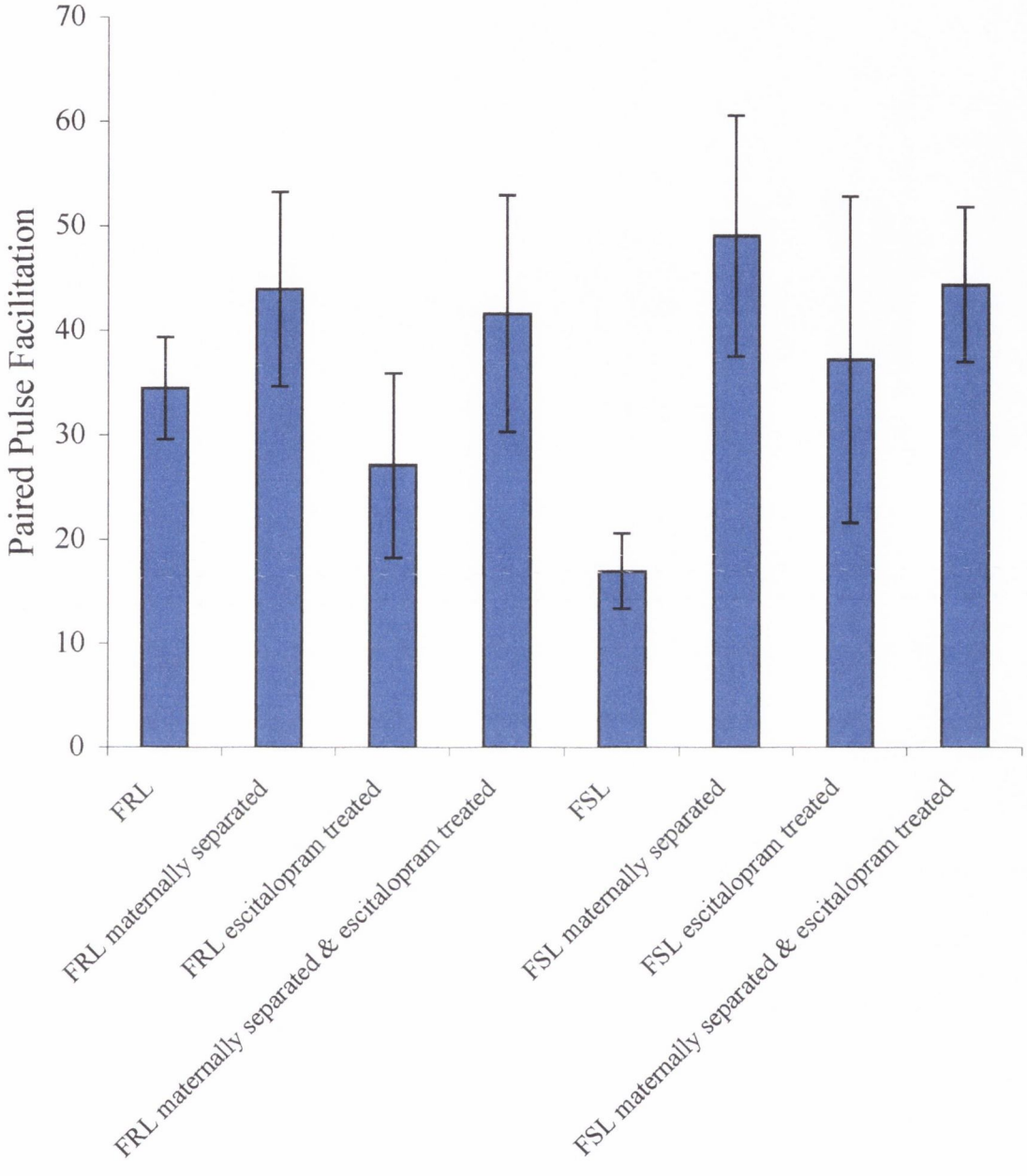

Figure 3.24.1 Paired Pulse Facilitation at 40ms for the Flinders Depression Model

The above figure shows the paired pulse facilitation at 40ms associated with each of the subgroups of the Flinders Depression model.

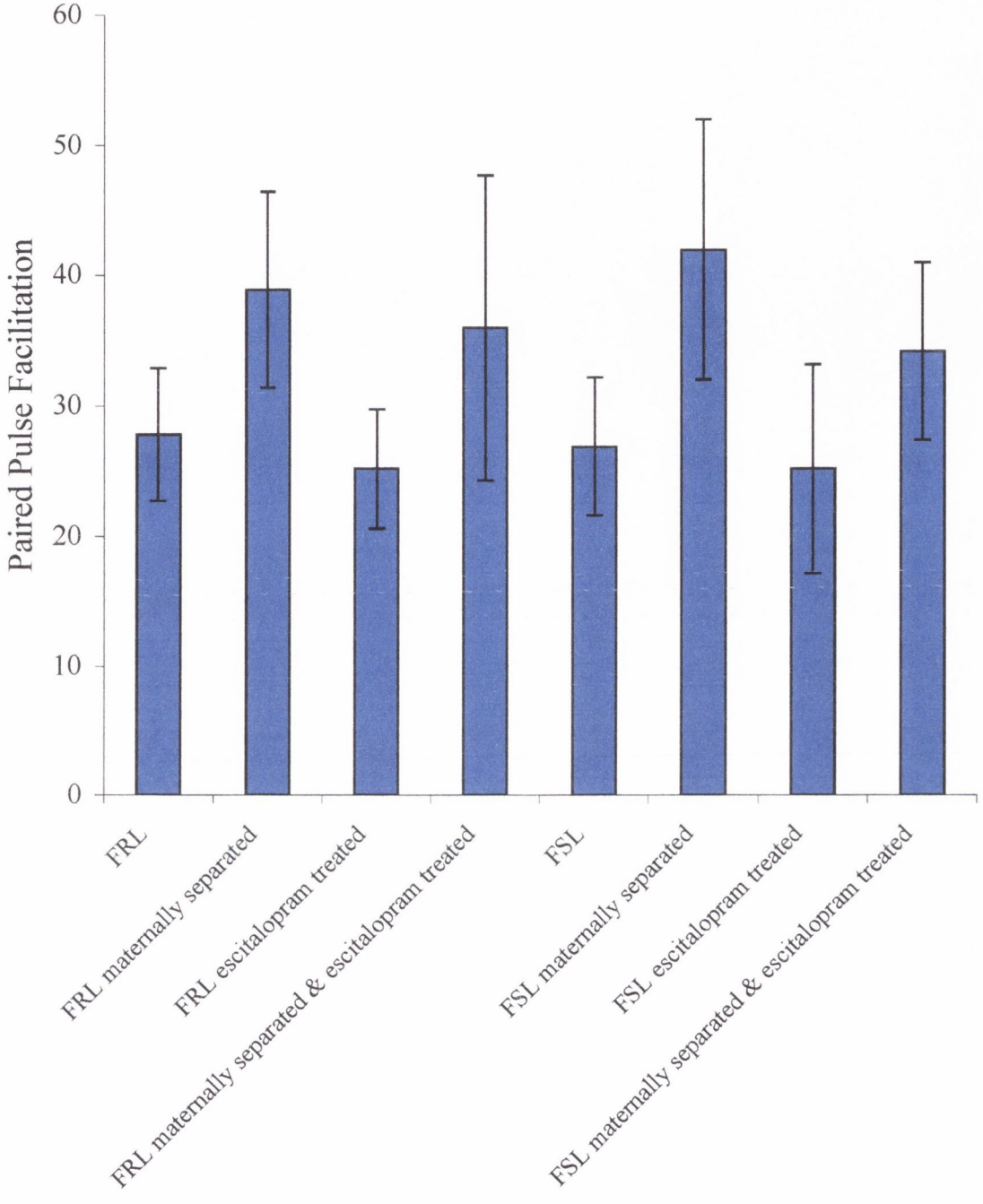

Figure 3.24.2 Paired Pulse Facilitation at 80ms for the Flinders Depression Model.

The above figure shows the paired pulse facilitation at 80ms associated with each of the sungroups of the Flinders Depression model.

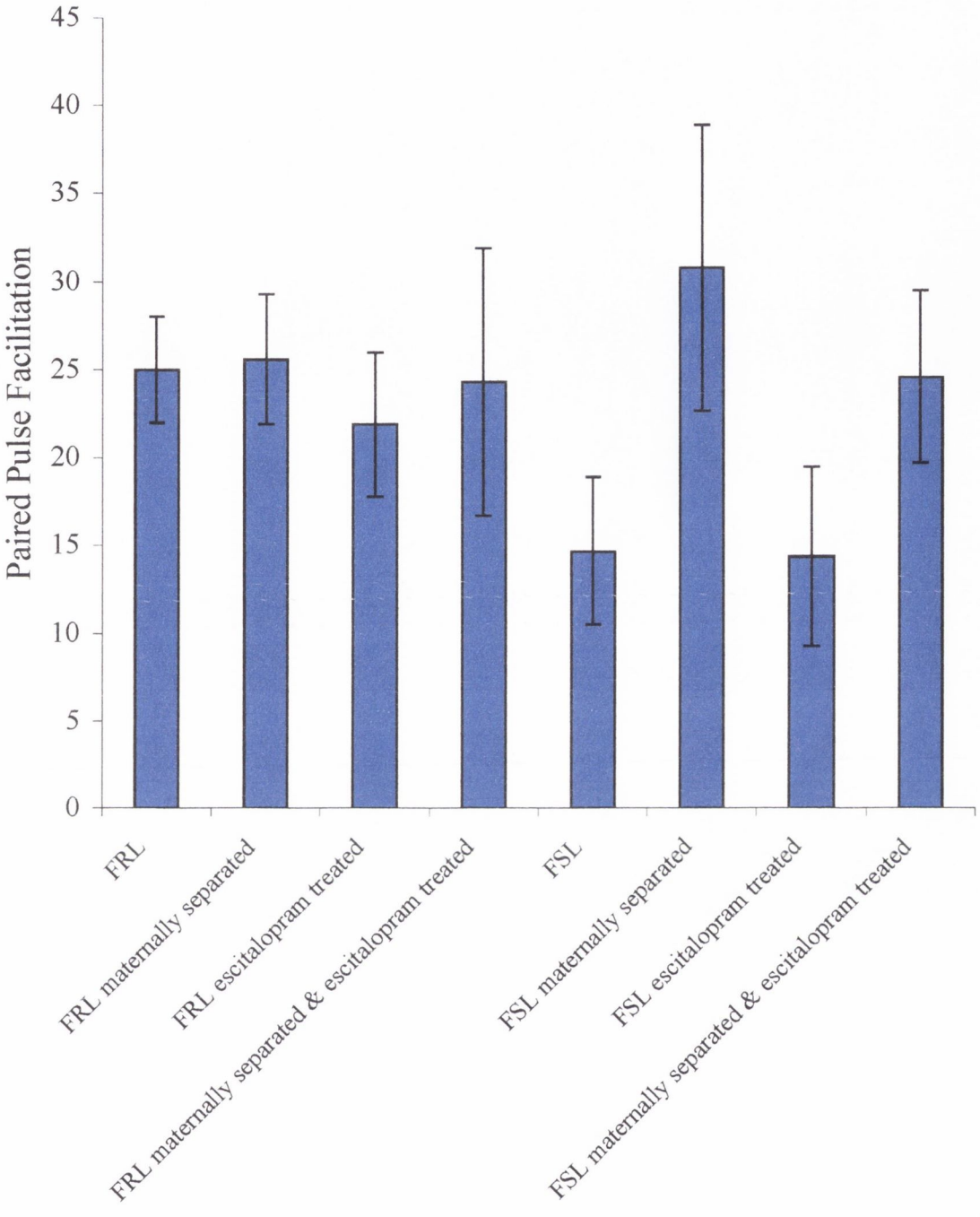

Figure 3.24.3 Paired Pulse Facilitation at 120ms for the Flinders Depression Model.

The above figure shows the paired pulse facilitation at 120ms associated with each of the subgroups of the Flinders Depression model.

3.25 Input/Output curves for the Flinders Depression Model.

The input/output curves of the various groups of treated and untreated Flinders animals were analysed. The current required to elicit 50% maximal EPSP response was used as the comparable measure. FRL control animals required statistically significantly less current to elicit a 50% maximum EPSP response when compared to all other groups studied. In particular FRL control animals were statistically different in terms of current required to elicit a 50% maximum EPSP response when compared to FSL control animals (5.25±0.18mA vs 6.1±0.26mA, $p<0.05$, unpaired student's t-test). There was no statistical difference between any other non-control FRL group and their respective FSL counterpart . There was no statistical difference in test EPSP amplitude between FRL and FSL animals. However the reduction in EPSP amplitude in the escitalopram treated FSL animals did border on statistical significance when compared to FRL escitalopram animals ($p=0.068$, unpaired student's t-test).

Table 3.25 Current required to induce a 50% Maximal EPSP response.

Group	Current (mA)	50% 0f Maximum EPSP amplitude (mV)
FRL control (n=7)	5.36±0.18	1.4±0.3
FRL maternally separated (n=7)	7.05±0.41	1.1±0.3
FRL escitalopram treated (n=6)	6.76±0.34	1.2±0.2
FRL maternally separated & escitalopram treated (n=6)	6.70±0.40	0.9±0.3
FSL control (n=7)	6.09±0.25	1.3±0.4
FSL maternally separated (n=6)	6.44±0.33	1.1±0.3
FSL escitalopram treated (n=6)	6.90±0.50	0.7±0.1
FSL maternally separated & escitalopram treated (n=7)	6.53±0.34	1.8±0.5

The above table shows the current required to elicit a 50% maximal EPSP response for the different subgroups of the Flinders study. Also shown is the average EPSP size for each of the groups. Data expressed as mean±s.e.m.

The Learned Helplessness Model of Depression.

This next set of experiments were designed to investigate if synaptic plasticity in the CA1 area was impaired in the congenital Learned Helplessness model of depression/stress. This study focused on rats which had been derived from a breeding program used to generate higher yields of each particular 'learned helpless' phenotype. Barbara Vollmayr, Mannheim, Germany supplied 3 distinct subgroups groups of the congenital learned helplessness model. The first subgroup were rats which were congenitally non-learned helpless and which expressed a non-learned helpless phenotype (cNLH-nlh). These rats are resistant to learned helplessness. The second group were congenitally learned helpless (exhibit a helpless phenotype without exposure to uncontrollable shock) and expressed the learned helpless phenotype (cLH-lh). The third group were congenitally learned helpless but expressed a non-learned helpless phenotype (cLH-nlh). All experiments in this section were carried out "blind".

3.26 Effect of HFS on synaptic transmission in rats with congenital non-Learned Helplessness (non-learned helpless phenotype).

This study was designed to assess the effect of HFS on congenital non-learned helpless (non-learned helpless phenotype) rats. These rats are the 'non-depressed' animals in the 'Learned Helplessness' depression model. The animals were classified with regards to their 'learned helplessness' phenotype as outlined in Section 2.11. As these are 'non-depressed' animals it was hypothesised that these animals should show the greatest level of LTP.

A stable baseline was obtained for 30 minutes (100.1±0.9%, n=11, for the 10 minutes prior to HFS) Application of HFS induced a statistically significant, albeit numerically small LTP (113.6±4.3% at 60 min post HFS, n=11, $p<0.01$ compared with baseline, paired student's t-test).

3.27 Effect of HFS on synaptic transmission in rats with congenital Learned Helplessness (learned helpless phenotype).

These animals are the offspring of parents both of whom displayed a learned helpless phenotype. As these are the supposed 'depressed' rats it is hypothesised that these animals should give less than normal LTP upon HFS. The animals were classified with respect to their learned helplessness phenotype as outlined in Section 2.11. A stable baseline for 30 minutes was obtained (100.6±1.4%, n=8 for the 30 minutes prior to HFS). Application of HFS induced statistically significant LTP (129.1±6.6%, n=8, at 60 min post HFS, $p<0.01$ compared with baseline, paired student's t-test). Although this level of LTP was numerically greater, it was not statistically significantly different from cNLH-nlh animals (113.6±4.3%, p=0.056, unpaired student's t-test).

3.28 Effect of HFS on synaptic transmission in rats with congenital Learned Helplessness (non-learned helpless phenotype).

As not all progeny from rats with congenital learned helplessness had a learned helpless phenotype it was decided to investigate the synaptic properties of congenital learned helpless rats which had a 'non-learned helpless' phenotype. As such, these rats can be described as having a genetic predisposition for depression although they are asymptomatic for the condition.

The animals were classified with respect to their learned helpless phenotype as outlined in Section 2.11. A stable baseline was obtained for 30 minutes (100.6±0.6%, n=10 for the 10 minutes prior to HFS). Application of HFS resulted in the induction of LTP (132.6±10.4% at 60 min post HFS, n=10, $p<0.05$ compared with baseline, paired student's t-test). Although this level of LTP was numerically greater, it was not statistically significantly different from cNLH-nlh animals (113.6±4.3%, p=0.097, unpaired student's t-test). Also, there was no significant difference in the level of LTP induced between cLH-lh and cLH-nlh animals (129.1±6.6% vs 132.6±10.4%, $p>0.5$, unpaired student's t-test).

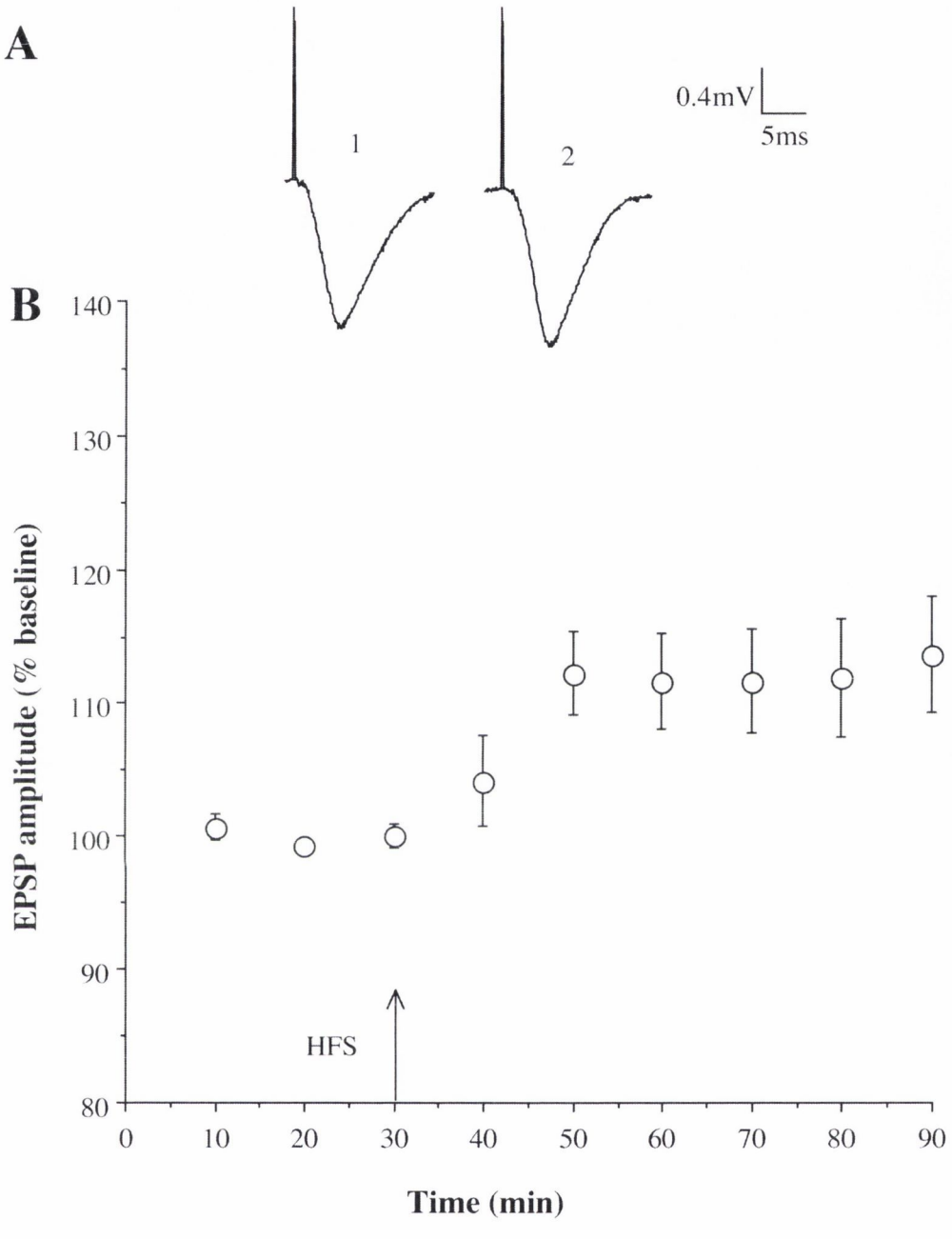

Figure 3.26 Effect of HFS on synaptic transmission in cNLH (non-learned helpless phenotype) rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS resulted in the induction of a statistically significant LTP (114±4% at 60 min post HFS, n=11, $p<0.01$ compared with baseline). Data expressed as mean±s.e.m.

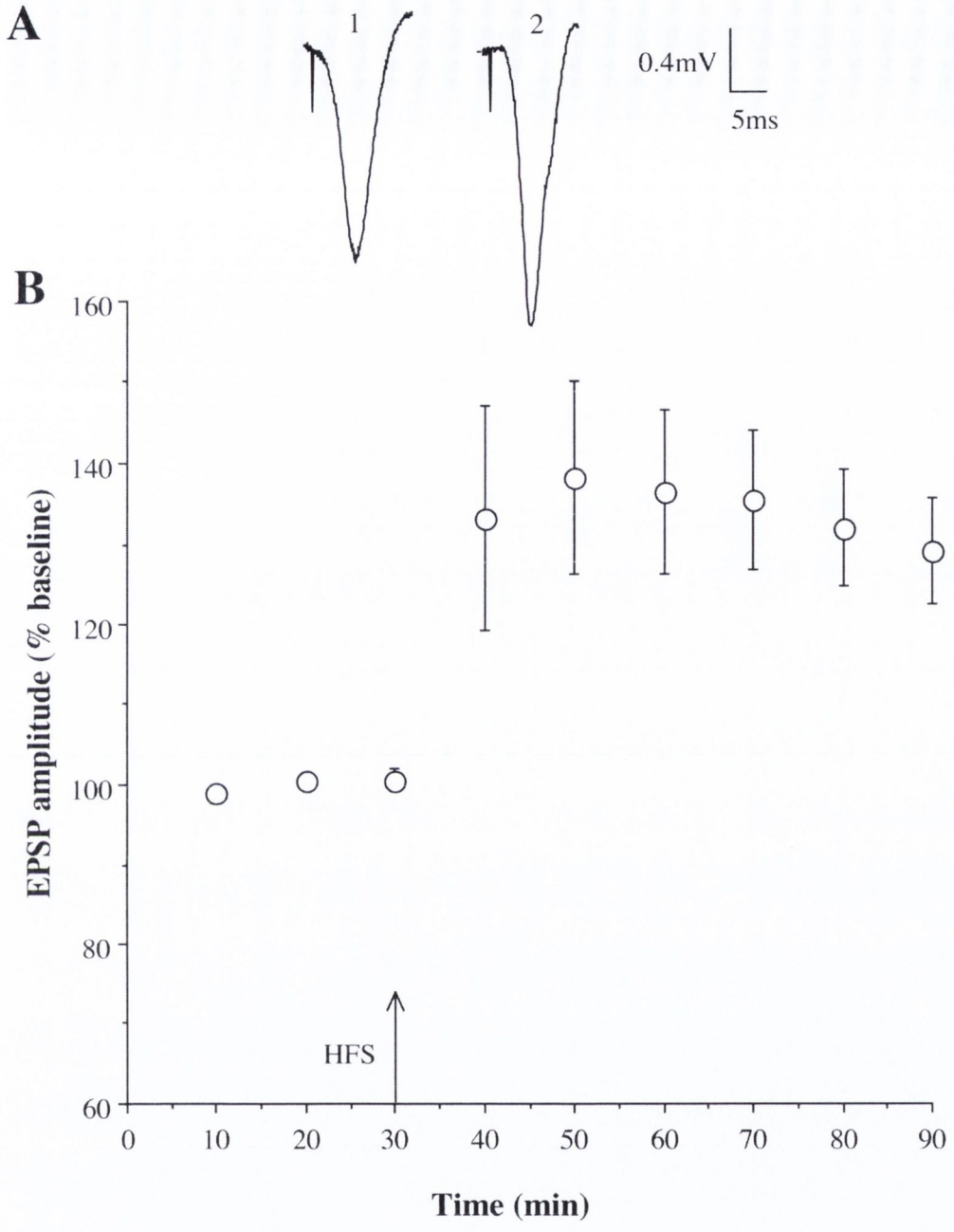

Figure 3.27 Effect of HFS on synaptic transmission in cLH (learned helpless phenotype) rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS induced a statistically significant LTP (129±7% at 60 min post HFS, n=8, $p<0.01$ compared with baseline). Data expressed as mean±s.e.m.

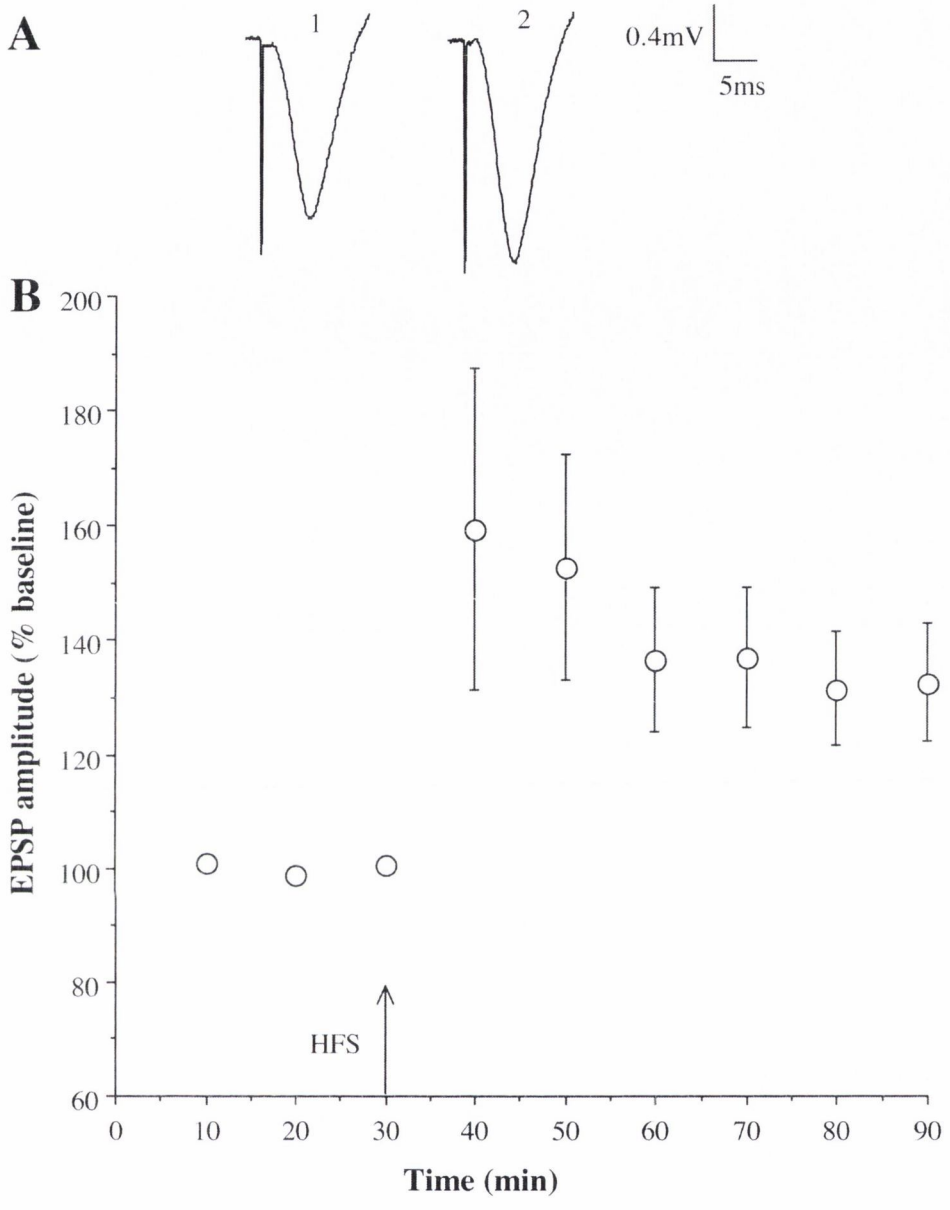

Figure 3.28 Effect of HFS on synaptic transmission in cLH (non-learned helpless phenotype) rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS resulted in the induction of statistically significant LTP (134±10% at 60 min post HFS, n=10, $p<0.05$ compared with baseline). Data expressed as mean±s.e.m.

3.29 Effect of HFS in male Sprague Dawley control animals.

As the results for the congenital Learned Helplessness study was somewhat unexpected it was decided to investigate 'Learned Helplessness' in outbred Sprague Dawley animals.

This first set of experiments was designed therefore to ascertain the level of LTP inducible in untrained, non-tested Sprague Dawley rats obtained from B. Vollmayr, Manheim, Germany. These animals were from the same source from which the animals of the congenital learned helplessness depression model were derived. A stable 30 minute baseline was recorded (102.4±1.0%, n=9 for the 10 minutes prior to HFS) Application of HFS resulted in the induction of LTP (132.0±6.7% at 60 min post HFS, n=9, $p<0.01$ compared with baseline, paired student's t-test).

3.30 Effect of HFS on synaptic transmission in Sprague Dawley (learned helpless phenotype) rats.

Sprague Dawley rats were classified with respect to their learned helpless phenotype as outlined in Section 2.11.

A stable baseline was obtained for 30 minutes (99.2±1.3%, n=5, for the 10 minutes prior to HFS). Application of HFS induced an increase in synaptic transmission (121.4±14.8% at 60 min post HFS, n=5). However, this increase was not statistically significant ($p>0.1$, compared with baseline, paired student's t-test). There was no significant difference between the level of LTP induced in non 'trained and tested' Sprague Dawley animals and Sprague Dawley (learned helpless phenotype) animals (132.0±6.7% vs 121.4±14.8%, $p>0.1$, unpaired student's t-test).

3.31 Effect of HFS on synaptic transmission in Sprague Dawley (intermediate phenotype) rats.

Sprague Dawley rats were classified with respect to their learned helpless phenotype as outlined in Section 2.11.

A stable baseline was obtained for 30 minutes (100.6±0.9%, n=5 for the 10 minutes prior to HFS). Application of HFS induced an increase in synaptic transmission (119.0±11.8% at 60 min post HFS, n=5). However similarly to the learned helpless phenotype rats this increase was not statistically significantly different from baseline values ($p>0.1$ compared with baseline, paired student's t-test). However, there was no significant difference between the level of LTP induced in non 'trained and tested' Sprague Dawley animals and Sprague Dawley (intermediate phenotype) animals (132.0±6.7% vs 119.0±11.8%, $p>0.1$, unpaired student's t-test).

3.32 Effect of HFS on synaptic transmission in Sprague Dawley (non-learned helpless phenotype) rats.

Sprague Dawley rats were classified with respect to their learned helplessness phenotype as outlined in Section 2.11. A stable baseline was obtained for 30 minutes (99.5%±1.1%, n=8, for the 10 minutes prior to HFS). Application of HFS failed to induce LTP (106.4±2.9% at 60 min post HFS, n=8, $p>0.05$ compared with baseline, paired student's t-test). There was a statistically significant reduction in the level of potentiation inducible between non 'trained and tested' Sprague Dawley animals and Sprague Dawley (non-learned helpless phenotype) animals (132.0±6.7% vs 106.4±2.9%, unpaired student's t-test, $p<0.01$).

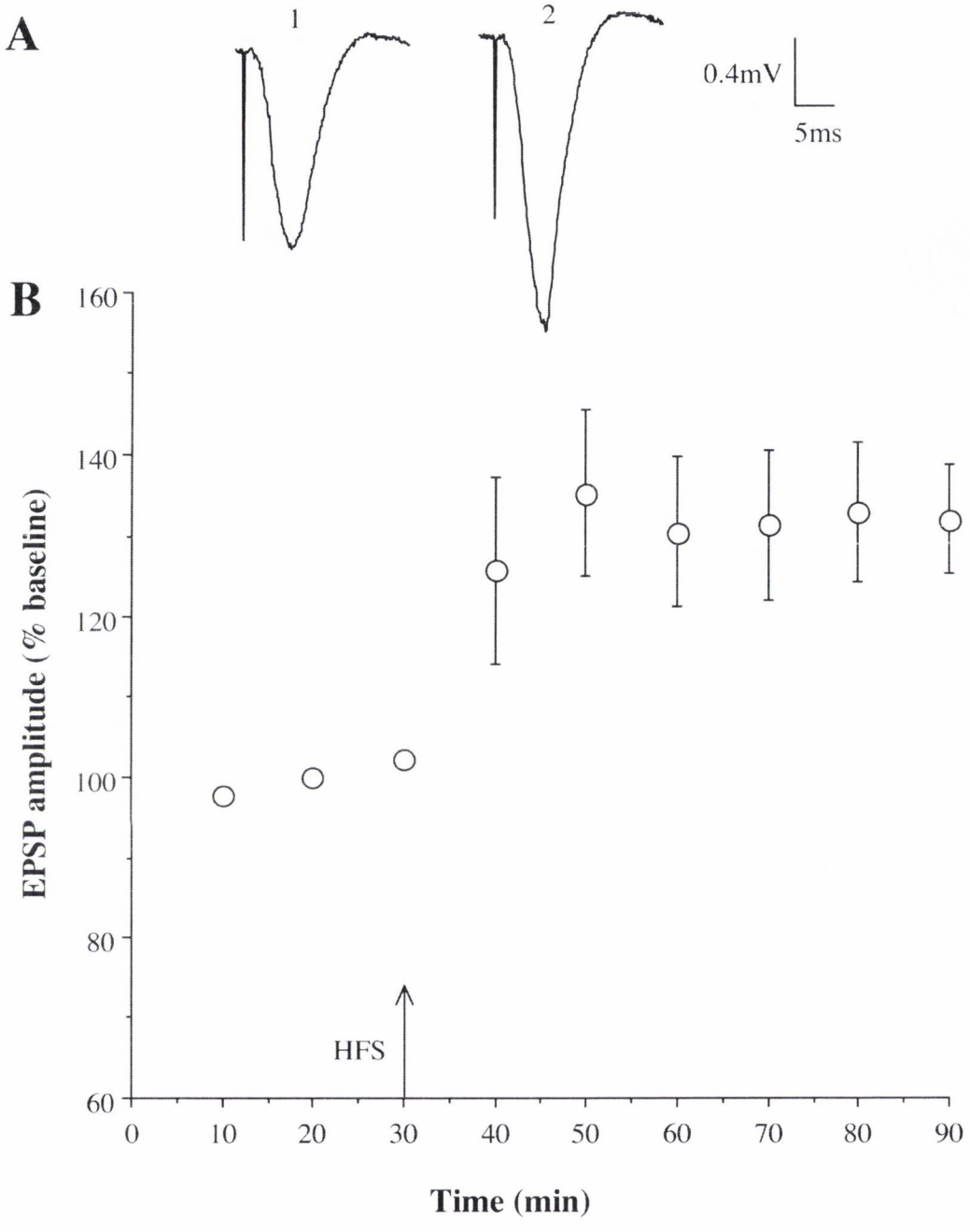

Figure 3.29 Effect of HFS on synaptic transmission in control, untrained, non-tested Sprague Dawley rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS induced a robust and stable LTP (132±7% at 60 min post HFS, n=9, $p<0.01$ compared with baseline). Data expressed as mean±s.e.m.

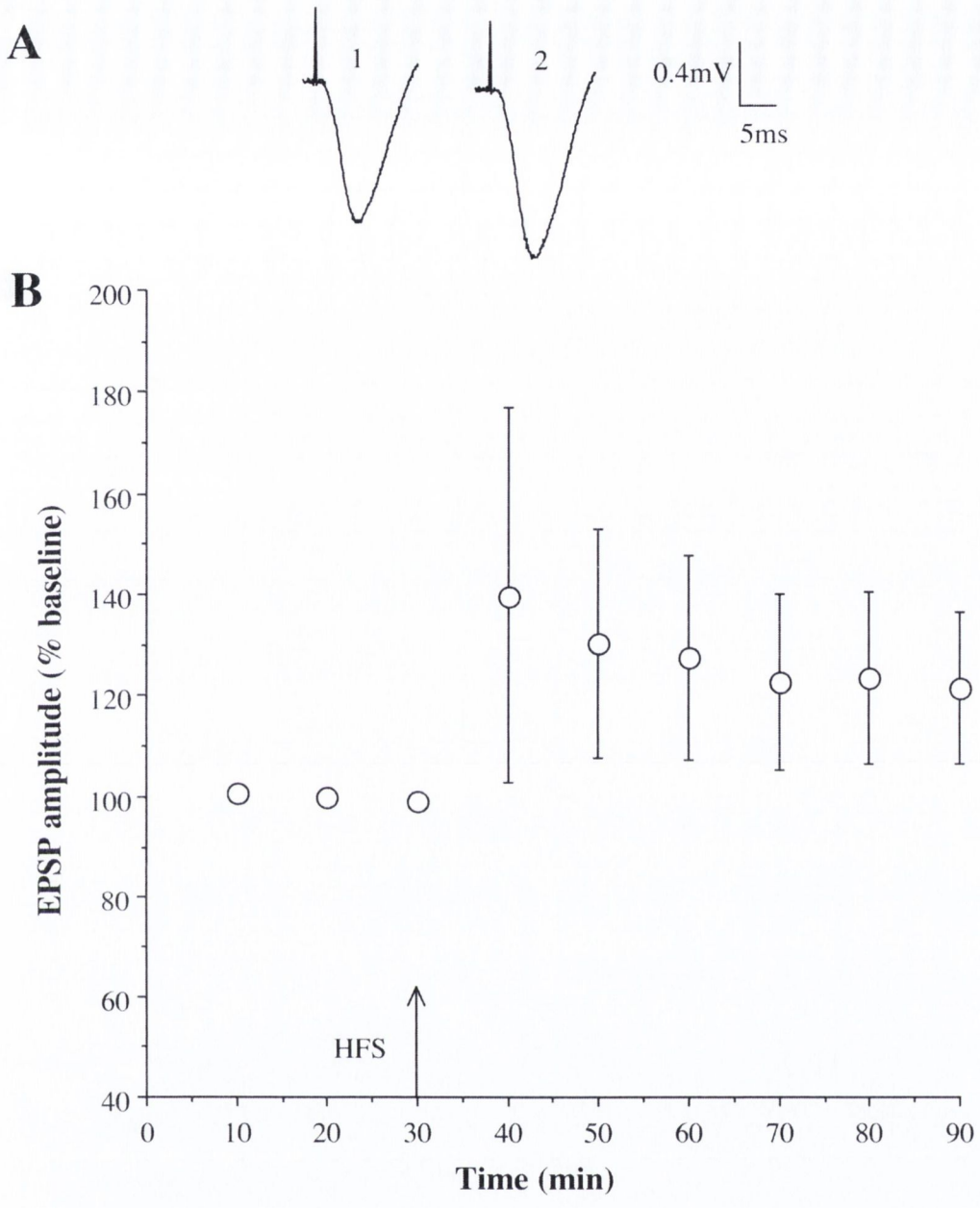

Figure 3.30 Effect of HFS on synaptic transmission in Sprague Dawley (learned helpless phenotype) rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS induced a non statistically significant increase in synaptic transmission (121±15% at 60 min post HFS, n=5, $p>0.1$ compared with baseline). Data expressed as mean±s.e.m.

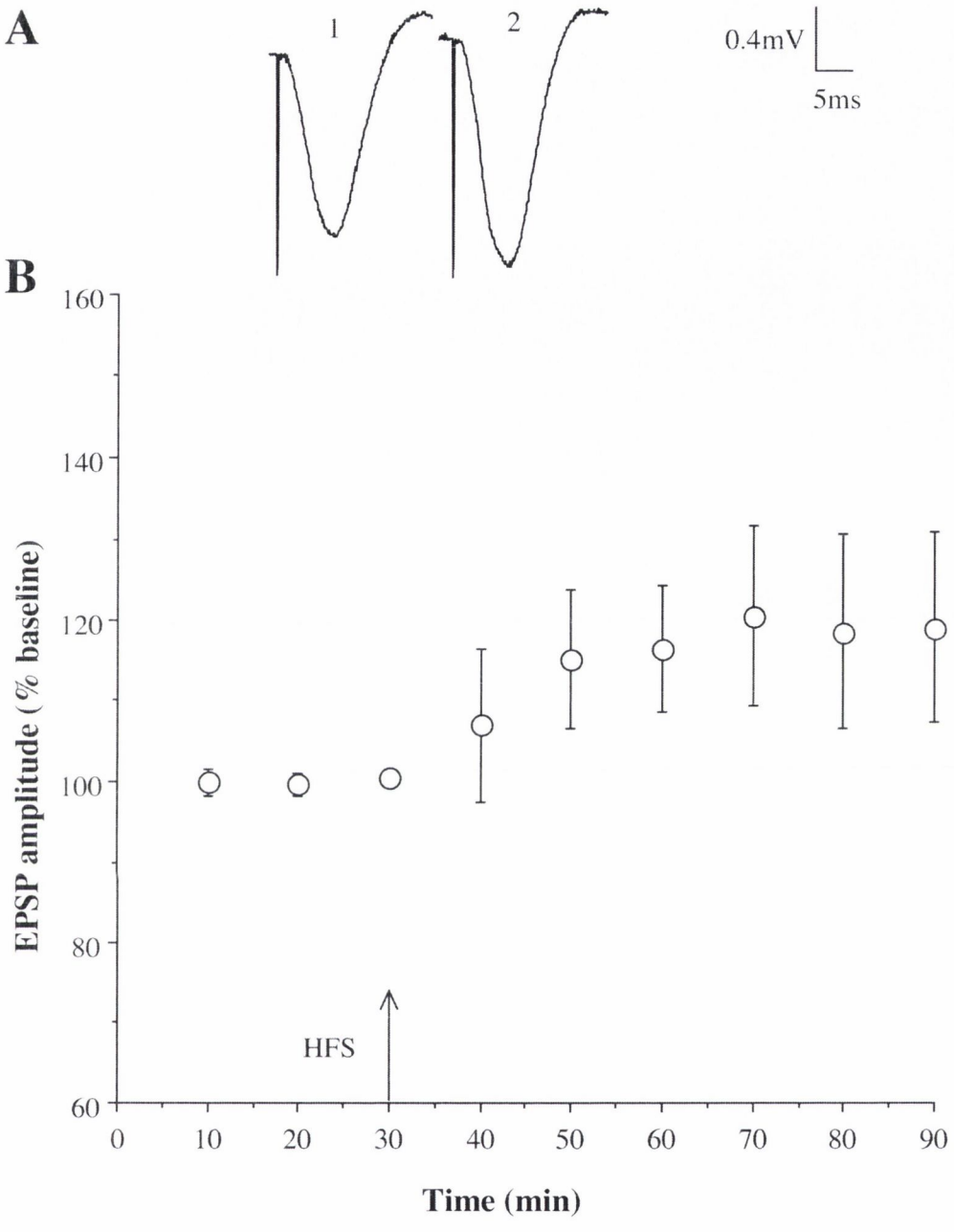

Figure 3.31 Effect of HFS on synaptic transmission in Sprague Dawley (intermediate phenotype) rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS resulted in a non-significant increase in synaptic transmission (119±11.8% at 60 min post HFS, n=5, $p>0.1$ compared with baseline). Data expressed as mean±s.e.m.

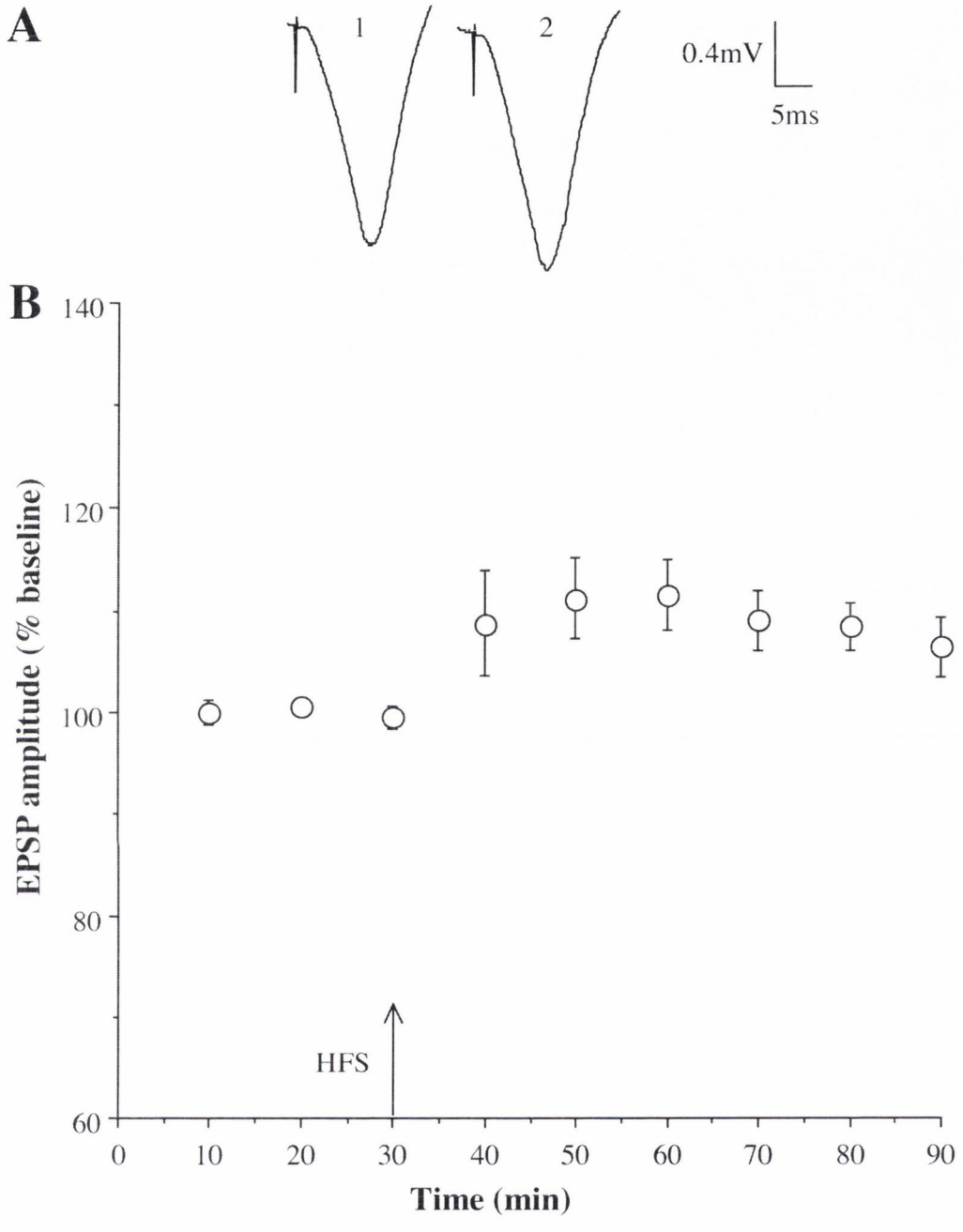

Figure 3.32 Effect of HFS on synaptic transmission in Sprague Dawley (non-learned helpless phenotype) rats.

(A) Insets show typical fEPSPs recorded ~ 5 min before (1) and 60 min after (2) HFS. (B) Application of HFS failed to induce LTP (106±3% at 60 min post HFS, n=8, $p>0.05$ compared with baseline). Data expressed as mean±s.e.m.

A comparison between Sprague Dawley control animals and animals which have been 'shocked' showed that there was significantly less LTP in animals that had been shocked (114.1±5.3%, n=18 vs 132.0±6.6%, n=9, p=0.05). This would suggest that an early stressor (such as uncontrollable shock exposure) may have a profound and long-lasting effect on the ability to induce LTP in CA1.

This result coincides with the unexpected finding that cLH-lh and cLH-nlh animals show on average a greater degree of potentiation following HFS than cNLH-nlh animals. As only cNLH animals received 'uncontrollable shock' on the 'training' day as well as 'controllable shock' on the 'testing' day (see Section 2.11), these animals received considerably more shock than their cLH counterparts. Also the context of the shock with respect to its controllability is different. Therefore the tendency for reduced LTP in cNLH-nlh animals when compared to cLH-lh/nlh animals (p=0.057) may be, in part at least, due to the increased exposure to shock or to the effect of inescapable shock exposure as part of the classification process rather than any inherent depression/stress related genetic/physiological difference between groups.

3.33 Paired Pulse Facilitation (PPF) in the Congenital Learned Helplessness Depression Model.

Each group showed decreasing levels of paired pulse facilitation with increasing time interval between initial stimulus and second stimulus. There was no statistically significant difference between groups at any specific time interval. Correlation studies were undertaken to see if the level of PPF inducible pre-HFS was correlated with potentiation at 60 min post HFS. Only cLH-nLH showed significant positive correlation between PPF at 40ms and LTP at 60 min post HFS (Correlation coefficient:0.665, $p<0.05$, n=10).

Table 3.33 Paired Pulse Facilitation in the Congenital Learned Helplessness model of depression.

Group	40ms	80ms	120ms
cLH-lh (n=8)	60.1±7.3%	37.8±8.3%	26.2±6.6%
cLH-nlh (n=10)	60.3±8.0%	44.2±5.9%	30.1±5.7%
cnLH-nlh (n=11)	52.4±5.6%	38.2±5.0%	28.7±6.3%

The above table show the paired pulse facilitation data for the congenital learned helplessness model of depression/stress at 40ms, 80ms and 120ms intervals respectively. PPF is expressed as [((EPSP2-EPSP1)/EPSP1)X100)%]. Data expressed as mean±s.e.m.

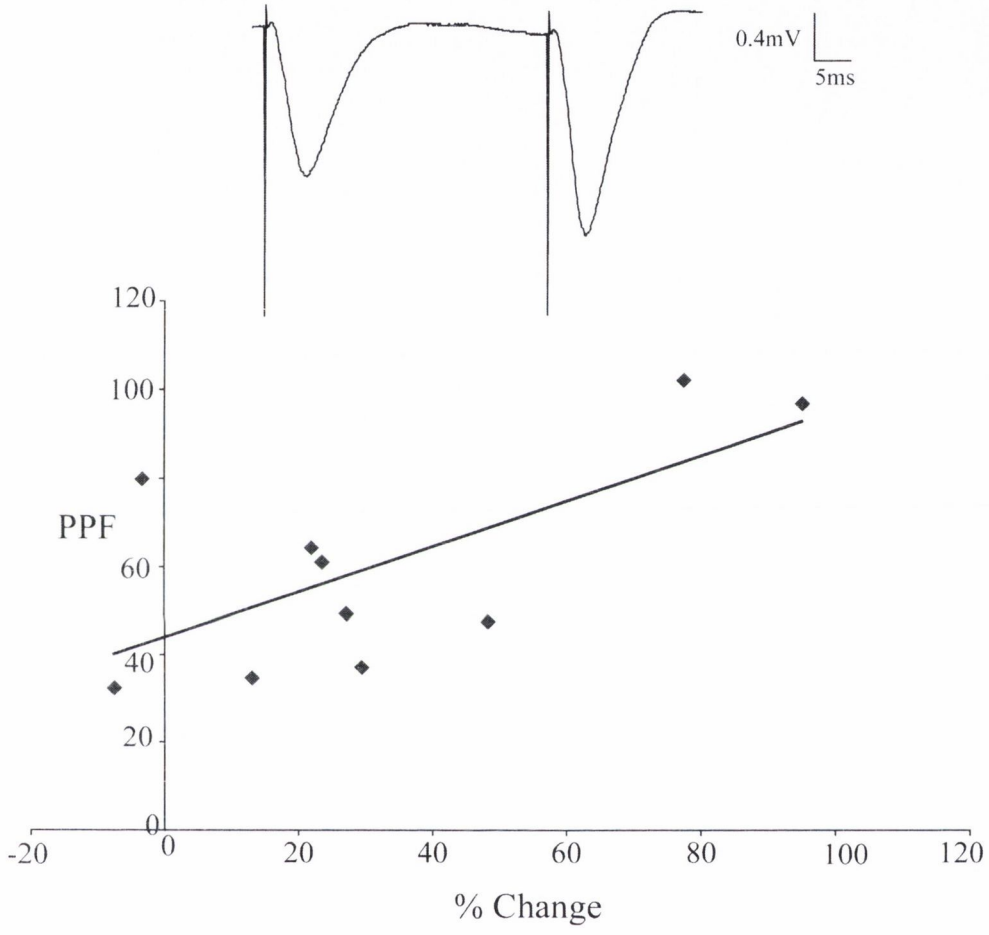

Figure 3.33 Initial PPF at 40ms correlates with HFS-induced changes in cLH-nlh rats.

The level of PPF inducible pre HFS is correlated with the level of potentiation at 60 min post HFS ($r=0.665$, $p<0.05$, $n=10$).

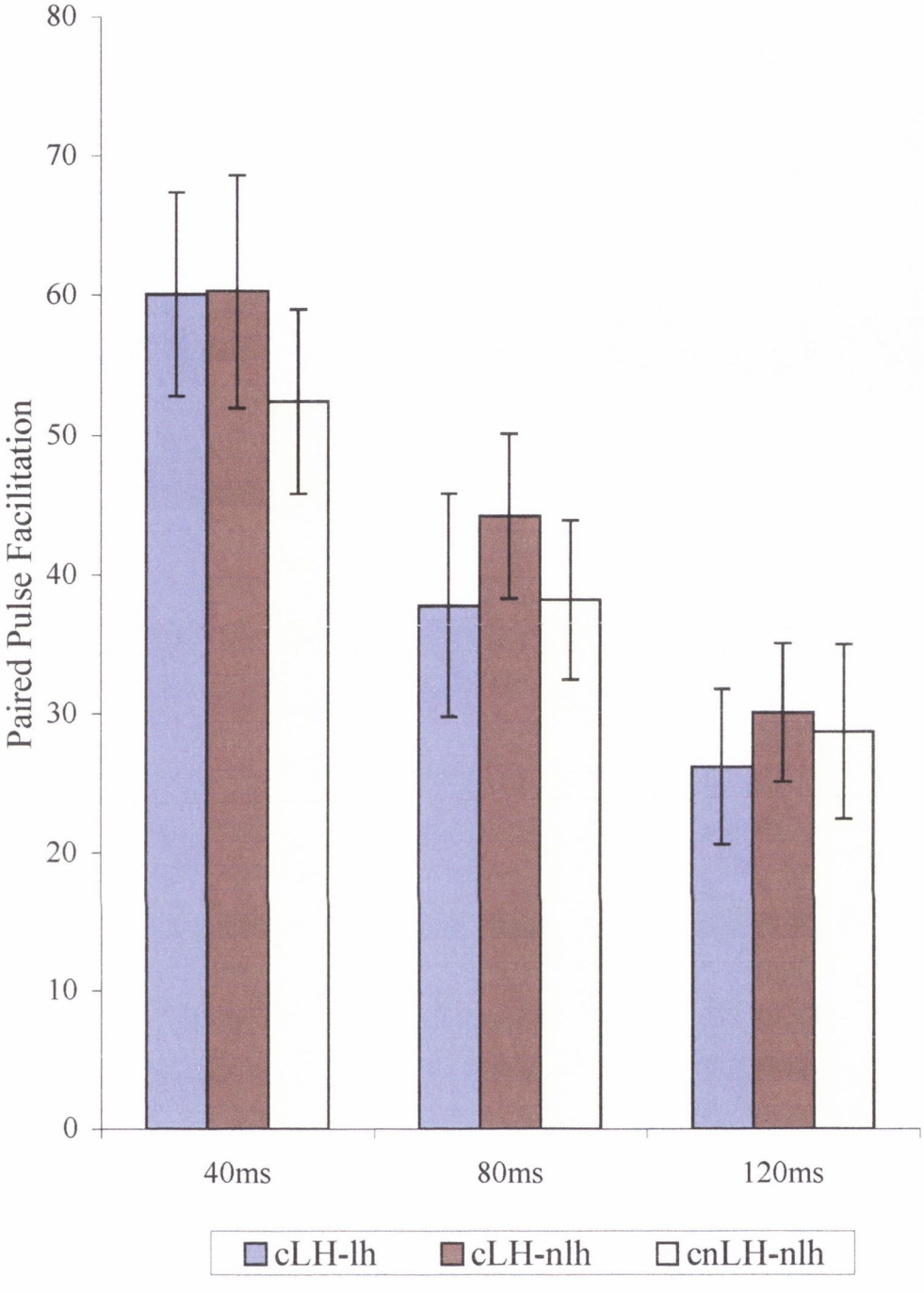

Figure 3.33.1 Paired Pulse Facilitation in the Congenital Learned Helplessness model of depression/stress.

The above figure shows the level of PPF at 40ms, 80ms, and 120ms for each of the congenital learned helpless subgroups.

3.34 Input/Output curves for the congenital Learned Helplessness model.

The input/output curves of the various members of the learned helplessness depression model were analysed. The current required to elicit 50% maximal EPSP response was use as the comparable measure. There was no statistically significant difference between any of the groups with respect to current. In addition there was no statistical difference between any of the groups with respect to test EPSP amplitude.

Table 3.34 Current required to induce a 50% maximal EPSP response.

Group	Current (mA)	50% of Maximum EPSP amplitude (mV)
Sprague Dawley control (n=9)	7.16±0.26	0.93±0.17
Sprague Dawley (lh Phenotype) (n=5)	6.80±0.66	0.96±0.28
Sprague Dawley (Intermediate Phenotype) (n=5)	8.42±0.96	0.61±0.12
Sprague Dawley (nlh Phenotype) (n=8)	7.04±0.45	0.89±0.17
Congenital non-Learned Helpless (nlh Phenotype) (n=11)	7.65±0.42	0.71±0.10
Congenital Learned Helpless (nlh Phenotype) (n=10)	7.37±0.69	0.72±0.14
Congenital Learned Helpless (lh Phenotype) (n=8)	6.76±0.93	0.77±0.16

The above table shows the current required to elicit a 50% maximal EPSP response. Also shown is the average EPSP size for each of the groups. Data expressed as mean±sem.

IV. Discussion

4.1 Effect of serotonergic modulation on the inhibition of LTP in CA1 following acute elevated platform exposure.

The present findings confirm that exposure to elevated platform stress, a relatively mild behavioural stressor, is sufficient to block LTP induction in CA1 (Shakesby et al. 2002; Xu et al. 1997). This block of LTP induction was robust and lasted for hours in the anaesthetised animal. This is consistent with the findings of others who investigated other acute stressors *ex vivo* in the hippocampal slice or in awake animals (Shors et al. 1989; Diamond et al. 1994). It appears that the anaesthesia locks the "brain" in the emotional state of its last waking moments ensuring that the physiological effects of stress are felt long after the stressors removal.

Many researchers have hypothesised how acute stress may block LTP induction. As stress activates a multitude of neurotransmitter (Kim & Diamond 2002) and neuroendocrine systems (Lopez et al. 1999) it is unlikely that *in vivo* a single system will act independently of others but rather each system will act in concert to block LTP induction. Many experiments have shown how removal of any one of the many constituents of the stress response is sufficient to prevent the inhibition of LTP by stress (Xu et al. 1998; Kim et al. 1996; Shors et al. 1990). Therefore the integrity of the stress pathway as a whole is of greater importance than any individual constituent thereof. It is clear that there are many points of the stress response at which suitable pharmacological intervention may inhibit the disruptive effects of stress on synaptic plasticity.

Interestingly, awake animals rapidly regain the ability to induce LTP in CA1 following elevated platform exposure if they habituate to or are removed from the stress (Xu et al. 1997). This clearly suggests that the effect of stress on LTP induction in CA1 is transitory i.e. reversible. Two distinct mechanisms may be suggested to account for the ability of the awake animal to rapidly recover from stress exposure and hence elicit LTP. Firstly, it may be that upon stressor removal, the levels of neurotransmitter/neuroendocrine molecules released by the stress response rapidly

return to normal. Although plausible this appears unlikely. More likely, LTP recovery may involve a physiological antagonist, that is, it involves the activation of a particular receptor whose down-stream processes allows it to by-pass the block of LTP induction. This process would have the ability to be rapid in onset.

Among the systems said to be activated following stress exposure is the serotonergic system (Graeff et al. 1996; Chaouloff et al. 1993; de Kloet 2000; Joels 2001). The serotonergic system is of particular interest because most successful agents for the treatment of anxiety and depression involve activation of the serotonergic system. Serotonin has a stimulatory effect on hypothalamic-pituitary-adrenal (HPA)-axis hormone secretion. Furthermore corticoadrenal steroid hormones modulate amine receptors in the hippocampus (Martire et al. 1989). Steroid hormones also target glutamate (NMDA, AMPA, kainate) receptors for modulation (Wu et al. 1991; Rupprecht 2003). This may be of particular importance with regards to LTP induction. Interestingly, the milieu of steroids released is stressor specific (Pacak & Palkovits 2001; Levine 1983). Different forms of stress affect the HPA-axis in different ways and this is mediated by different neurotransmitter systems (Van de Kar et al. 1991). Many studies have shown an increase in 5-HT release in the hippocampus following aversive stress exposure (Joseph & Kennett 1983; Vahabzadeh & Fillenz 1994; Wilkinson et al. 1996; Matsuo et al. 1996; Ge at al. 1997; Kirby et al. 1997). Interestingly, Robertson et al. (2005) reported that there was no significant effect of acute elevated platform exposure on 5-HT levels in the dorsal hippocampus. It has been shown that 5-HT can inhibit LTP in the CA1 area of the hippocampus (Corradetti et al. 1992; Passani et al. 1994; Staubli & Otaky 1994; Staubli & Xu 1995). Conversely, the treatment of anxiety and depression relies on agents which increase extracellular 5-HT. Therefore it can be hypothesised that the mode of action of serotonergic mediated antidepressants is complicated with respect to activation of distinct serotonergic receptors and their interaction with other systems.

The finding that (±)fenfluramine administration enables the induction of LTP in previously stressed animals suggests that raising extracellular 5-HT levels can

overcome stress-induced inhibition of LTP induction. As Shakesby et al. (2002) found similar results following tianeptine administration it may be suggested that the level of serotonin following acute stress exposure is of critical importance with respect to LTP induction and that further drug manipulation resulting in an increase or decrease in serotonin concentration may facilitate LTP induction. (±)Fenfluramine is a substituted amphetamine. It has the ability to cause a massive release of 5-HT centrally (Carboni & Di Chiara 1989; Trulson & Jacobs 1976; Zaczek et al. 1990). (+)Fenfluramine is the more active enantiomer in triggering the release of 5-HT. (±)Fenfluramine is rapidly metabolised to both (+)-norfenfluramine and (-)-norfenfluramine (Caccia et al. 1985; Campbell et al. 1988). All 4 species are biologically active. All are potent 5-HT releasing agents. In addition both (+)-norfenfluramine and (-)-norfenfluramine are potent 5-HT_{2C} receptor agonists (Kact, the concentration of agonist necessary to induce half of its maximal effect <20nM). (+)-Norfenfluramine is also a potent 5-HT_{2B} receptor agonist (Rothman et al. 2000a).

To confirm that a rise in extracellular 5-HT levels enabled the induction of LTP in previously stressed animals rather than any non-specific effect of (±)fenfluramine, tianeptine (Fattaccini et al.1990; Kamoun et al. 1989; Broqua et al. 1992; Kato & Weitsch 1988) , a 5-HT uptake enhancer was pre-administered. Pre-administration of tianeptine prevented (±)fenfluramine overcoming stress-induced inhibition of LTP. This would suggest that the recovery of LTP is mediated by an increase in extracellular 5-HT resulting in the activation of specific 5-HT receptors.

Shakesby et al. (2002) reported that (±)fenfluramine (5mg/kg) administration blocked LTP induction in non-stressed animals. We investigated whether this block in LTP was enantiomer specific. Neither treatment with (+)fenfluramine (2.5mg/kg) or (-) fenfluramine (2.5mg/kg) resulted in inhibition of LTP. This implies that both enantiomers may need to act in concert to block LTP in non-stressed animals. Surprisingly, on average, following (+) or (-)fenfluramine administration LTP was marginally though not statistically significantly facilitated. This is more consistent with the findings of Bliss et al. (1983) that a reduction in 5-HT levels may inhibit LTP

induction in the dentate gyrus thus suggesting that serotonin faciltates LTP induction. Indeed repeated treatment with the SSRI fluvoxamine facilitated LTP induction in the hippocampo-mPFC pathway *in vivo* (Ohashi et al. 2002). Opposing results were found in the CA1 area where fluvoxamine (an SSRI) was found to be inhibit LTP induction (Kojima et al. 2003).

As we had shown that an agent that raises extracellular 5-HT levels could overcome stress-induced inhibition of LTP induction our attention next turned to the 5-HT receptor superfamily and to the 5-HT_2 receptor in particular. The 5-HT_2 receptor was chosen as the hippocampus expresses postsynaptic 5-HT_{2A} receptors (Vaidya et al. 1997), 5-HT_{2B} receptors (Sanden et al. 2000) and 5-HT_{2C} receptors (Clemett et al. 2000; Abramowski et al. 1995). Secondly, the pharmacological effects of (±)fenfluramine administration were reported to be due, at least in part, to activation of 5-HT_2 receptors (Vickers et al. 1999; McCreary et al. 2003; Giambalvo & Price 2003). Interestingly, activation of 5-$HT_{2A/2C}$ receptors facilitates memory formation (Buhot et al. 2000).

Preadministration of cinanserin a non-subtype selective 5-HT_2 receptor antagonist prevented (±)fenfluramine enabling the induction of LTP in previously stressed animals. This result implicates 5-HT_2 receptor activation in the serotonergic mediated relief of LTP inhibition following acute stress exposure.

Additional evidence of a role for 5-HT_2 receptor activation in overcoming stress-induced LTP inhibition was provided by mCPP, a 5-$HT_{2B/2C}$ receptor agonist. Administration of mCPP enabled the induction of LTP following stress exposure. It should be noted however, that mCPP, in addition to its direct agonist properties, is a 5-HT releasing agent (Baumann et al. 1993). Interestingly, mCPP is the major metabolite of the antidepressant trazodone (Haria et al. 1994).

To further elucidate which 5-HT_2 receptor subtype enabled the induction of LTP post stress exposure, BW723c86, a 5-HT_{2B} subtype selective agonist was administered. Like

mCPP this compound too overcame the stress induced inhibition of LTP. MK-212, a 5-HT_{2C} receptor agonist also enabled the induction of LTP post stress exposure. It therefore appears that activation of either 5-HT_{2B} or 5-HT_{2C} receptors is sufficient to overcome stress-induced inhibition of LTP induction. This shared action of agonists at 5-HT_{2B} and 5-HT_{2C} receptors may be because both subtypes of the 5-HT_2 receptor family harness the same PLC molecular machinery. Similarly, although not tested, it seems likely that 5-HT_{2A} receptor activation would also enable LTP induction following stress exposure.

5-HT_2 receptors have been implicated in disorders of anxiety and stress. As yet no consensus has been reached regarding whether stress exposure results in subsequent activation or inhibition of 5-HT_2 systems. Suicide victims have been shown to have altered 5-HT_{2C} receptor editing (Gurevich et al. 2002). 5-HT_{2C} agonists have been reported to have antidepressant effects (Moreau et al. 1996; Martin et al. 1998). 5-HT_{2A} and 5-HT_{2C} receptor activation stimulates the release of HPA-axis hormones (Jorgensen et al.1992; Levy & Van de Kar 1992; Lee et al. 1992; Van de Kar 2001). Specifically, DOI, a 5-HT_2 receptor agonist has been shown to activate the hypothalamic-pituitary-adrenal axis (Raghavendra & Kulkarni 2000) and to increase plasma corticosterone levels (Welch & Saphier 1994). Interestingly, 5-HT_{2C} receptor knockout mice are hyper-responsive to repeated stress (Chou-Green et al. 2003). This may suggest a deficit in habituation to stress or possibly an impaired coping mechanism. Stress exposure is reported to result in increased numbers of 5-HT_2 receptor binding sites in the cortex (McKittrick et al. 1995; Torda et al. 1988) and increased sensitivity to 5-HT_2 receptor agonists (Gorzalka et al. 1998; Nankai et al. 1995).

5-HT_2 receptor agonists have also been shown to enhance learning in two learning paradigms (conditioned avoidance response in rats and the nictitating membrane response in the rabbit) (Harvey 1996). In addition, in the frontal cortex, 5-$HT_{2A/2C}$ receptor activation has a facilitatory effect on acetylcholine release (Hirano et al. 1995). Moreover, in the hippocampus, activation of 5-HT_2 receptors also resulted in enhanced release of acetylcholine (Nair & Gudelsky 2004; Zhelyazkova-Savova et al. 1999).

Furthermore, 5-HT_{2C} receptor knockout mice show deficits in the spatial version of the water maze (Tecott et al. 1998). Somewhat surprisingly these knockout animals have normal LTP in both CA1 and CA3. They do however exhibit reduced LTP in the dentate gyrus. It would be interesting to see if acute elevated platform stress could block LTP induction in CA1 of 5-HT_{2C} receptor knockout mice and if so whether (±)fenfluramine administration could relieve this block.

The behavioural effects of serotonin in stress and depression remain controversial (Zangrossi et al. 2001). Behaviourally, BW723c86 has been shown to have anxiolytic-like actions in the rat Vogel conflict test (Kennett et al. 1998). In addition, injection of BW723c86 into the medial amygdala was reported to be anxiolytic (Duxon et al. 1997b). Further evidence for the anxiolytic profile of BW723c86 was provided by Nic Dhonnchadha et al. (2003) who showed that in a mouse model BW723c86 provoked an anxiolytic-like response in the Four plates test and in the elevated plus maze. They also reported that mCPP had an anxiolytic profile in the elevated plus maze. However, this is contradictory to other work which has described an anxiogenic role for a similar dose of mCPP (Gibson et al. 1994; Meert et al. 1997). The discrepancy may relate to subtle differences in protocol/test used or inherent differences between rat and mouse models. Furthermore, mCPP was reported to have both an anxiolytic and anxiogenic effect in the rat elevated T-maze (Mora et al. 1997). This may be accounted for by the fact that the elevated T-maze is a model of both conditioned and unconditioned fear which would enable mCPP to elicit both an anxiogenic and anxiolytic profile. Interestingly, (±)fenfluramine administration was found to alleviate anxiety in the elevated T-maze (Graeff et al. 1998).

Of course, as this is an *in-vivo* model we cannot rule out extra-hippocampal effects of serotonin release or 5-HT_2 receptor activation as possible mediators of CA1-LTP recovery following stress exposure. For example, the amygdala is known to play a critical role in mood and emotion (Davis 1992; Ledoux 1995). Activation of 5-HT_2 receptors by DOI facilitates LTP in the BLA (Chen et al. 2003). These authors also suggested that activation of the 5-HT_{2C} receptor was particularly important in this

process. In addition they provided evidence showing that DOI acts via an NMDA receptor mediated mechanism to facilitate LTP. Activation of 5-$HT_{2A/2C}$ receptors has also been reported to excite GABAergic interneurons in the DRN-PAG suppressing 5-HT neuron firing (Liu et al. 2000). Indeed activation of 5-HT_2 receptors in visual cortex inhibits LTP (Edagawa et al. 2000).

Interestingly, Kim et al. (2001) described how amygdalar lesions prevent LTP impairment due to stress exposure in CA1. They also found similar results with regard to stress-induced memory impairment using the Morris water maze paradigm. As the amygdala is accepted as having a role in modulating memory function in various brain structures (Gallagher & Knapp 1978; Roozendaal et al. 1998; Packard & Chen 1999; McGaugh 2000) it may be that the ability to induce LTP in CA1 following stress exposure may be mediated via serotonin's action on the amygdala rather than any direct action on the hippocampus itself.

Administration of the 5-HT_4 receptor partial agonist RS67333 did not affect the induction of LTP in CA1 in non-stressed animals. A partial agonist was used because a selective full agonist that can cross the blood brain barrier was not available at the time of the experiments. Furthermore in stressed animals i.p. administration of RS67333 was not sufficient to enable the induction of LTP. This suggests that activation of the 5-HT_4 receptors is not involved in the serotonergic mediated relief of stress-induced LTP inhibition. Similarly, Cryan & Lucki (2000) have shown that the antidepressant-like behavioural effects of the SSRI fluoxetine in a forced swim test are not dependent on 5-HT_4 receptor activation. However activation of 5-HT_4 receptors has been reported to increase the release of 5-HT in the hippocampus (Ge & Barnes 1996). 5-HT_4 receptor activation has been associated with improved cognitive function possibly due to its ability to facilitate cholinergic transmission (Consolo et al. 1994; Matsumoto et al. 2001). Fontana et al. (1997) showed how RS67333 could overcome atropine-impaired performance in the Morris water maze. Furthermore RS67333 can enhance both place and object recognition memory in both young and old rats (Lamirault & Simon 2001). Marchetti et al. (2004) reported that neither administration of 5-HT_4 receptor agonists

nor antagonists had any effect on baseline synaptic transmission in the dentate gyrus. However they did show that RS67333 did increase LTP induction and maintenance while a 5-HT_4 antagonist facilitated depotentiation 24 hours later. Furthermore activation of 5-HT_4 receptors stimulates adenylyl cyclase activity in the hippocampus (Torres et al. 1995; Markstein et al. 1999) resulting in increased synthesis of cAMP, a molecule implicated in LTP processes (Otmakhova et al. 2000). Activation of 5-HT_4 receptors was also found to facilitate CA1 LTP (Matsumoto et al. 2001). However, this facilitation was blocked not only by GR113808 (a 5-HT_4 receptor antagonist) but also by scopolamine indicating an interaction between the serotonergic and cholinergic systems. This may indicate that any facilitation is due to 5-HT_4 mediated release of acetylcholine. Kulla & Managhan-Vaughan (2002) reported a lack of effect of 5-HT_4 receptor antagonism in nonstressed rats on dentate gyrus baseline synaptic transmission, LTP or depotentiation. They hypothesised that 5-HT_4 receptor activation plays a modulatory but not critical role in the elaboration of these processes. A number of hypotheses may be presented to explain the inability of RS67333 to overcome stress induced LTP inhibition in CA1. Firstly, it is possible that activation of 5-HT_4 receptors simply does not initiate a process which results in the relief of LTP inhibition. Secondly RS67333 is a partial 5-HT_4 receptor agonist and its effect would be expected to depend on levels of endogenous extracellular serotonin. As stress exposure elevates extracellular serotonin levels in the hippocampus it may be that in this situation RS67333 does not activate 5-HT_4 receptors. Finally 5-HT_4 receptor activation is hypothesised to facilitate CA1-LTP via acetylcholine release (Matsumoto et al. 2001). Acetylcholine facilitates neuronal firing in pyramidal neurons by means of muscarinic receptors (Bernardo & Prince 1982; Cole & Nicoll 1984; Halliwell & Adams 1982). The stress response itself induces acetylcholine release in the hippocampus (Fatranska et al. 1989; Gilad et al. 1985; Nilsson et al. 1992). Further 5-HT_4 mediated acetylcholine release may in fact be detrimental to LTP induction. This would be similar to the inverted U shape of the relationship between the effect of corticosterone and LTP. Jorgensen et al. (1998) suggested a possible role for 5-HT_4 receptor activation in stress induced ACTH release.

The NMDA receptor has been implicated in the pathogenesis of anxiety and depression (Petrie et al. 2000; Witkin 1995). Pre-stress treatment with NMDA receptor antagonists have been shown to block the effects of stress on synaptic plasticity (Kim et al. 1996). Indeed tricyclic antidepressants have been reported to block NMDA receptors (Reynolds & Miller, 1988). The finding that D-AP5 (100nmol) could significantly attenuate LTP in CA1 was as expected. AP5 was previously reported to impair LTP induction in CA1 and the dentate gyrus *in vitro* (Collingridge et al. 1983; Harris et al. 1984; Larson & Lynch 1988) and *in vivo* (Morris et al. 1986; Freir & Herron 2003). Unlike some authors i.c.v. administration of D-AP5 (100 nmol) did not completely block the induction of LTP. This is presumably due to the 200Hz protocol resulting in the induction of NMDA-receptor independent LTP via Ca^{2+} influx through voltage-dependent calcium channels (VDCCs) (Grover & Tyler 1990) or possibly an insufficient D-AP5 concentration at the time of HFS. Coadministration of (±)fenfluramine was found to relieve this NMDA-receptor mediated block of LTP induction at 60 minutes post HFS. This LTP had a slow onset.

The mechanism of action of (±)fenfluramine enabling it to alleviate an NMDA receptor dependent block in LTP is open to debate. Previously we summarised evidence how the metabolites of (±)fenfluramine are agonists of the 5-HT_2 receptor. It has been shown that activation of 5-HT_2 receptors facilitates TBS-LTP in the rat BLA. However, this process was blocked by the NMDA receptor antagonist AP5 indicating that it is dependent on NMDA receptor activation (Chen et al. 2003). Conversely, activation of the 5-HT_2 receptor family was found to inhibit LTP in the rat visual cortex (Edagawa et al. 2000) while administration of M100907, a 5-HT_{2A} receptor antagonist facilitated the induction of LTP in CA1 again in a NMDA receptor dependent mechanism (Wang & Arvanov 1998). The ability of (±)fenfluramine to enable LTP induction in the presence of D-AP5 may suggest that in some as yet undescribed way serotonin may facilitate VDCC mediated LTP rather than any NMDA receptor dependent process. Indeed, while the 5-HT_2 receptor agonist DOI facilitated NMDA receptor dependent calcium influx in the BLA (Chen et al. 2003) this may be unlikely in our system as we have blocked the NMDA receptors which should prevent any NMDA receptor-dependent

alleviating mechanism. However, as D-AP5 is a competitive antagonist and the fact that we did elicit some LTP in its presence we therefore cannot entirely rule out a role for NMDA receptor activation in enabling the induction of LTP. A mechanism involving VDCCs to enable the induction of LTP in previously stressed animals is also unlikely as raised 5-HT levels inhibits Ca^{2+} channels (Foehring 1996; Chen & Lambert 1997; Sandler & Ross 1999). The most likely explanation for the ability of (±)fenfluramine to overcome the inhibition of LTP induction due to NMDA receptor antagonist exposure may involve protein kinase C (PKC). Previously, presynaptic PKC activation has been reported to rescue LTP from NMDA receptor blockade in the dentate gyrus (Kleschevnikov & Routtenberg 2001). Cortical synaptoneurosomes which had been incubated with D-fenfluramine showed a time and dose dependent increase in PKC activity (Giambalvo & Price 2003). This increase in PKC activity could be partially attenuated using the $5\text{-}HT_2$ receptor antagonist ketanserin suggesting that D-fenfluramine may act on PKC in part through $5\text{-}HT_2$ receptor activation. Also the increase in PKC activity mediated by SSRIs was reported to occur at the presynaptic terminal. Therefore it may be speculated that (±)fenfluramine administration enables the induction of LTP in the presence of D-AP5 by mediating increased PKC activity.

Among the many molecules affected by stress exposure is the neurotrophin brain-derived neurotrophic factor (BDNF). Acute stress exposure has been shown to decrease hippocampal BDNF concentrations (Smith et al. 1995). So too does corticosterone administration (Schaaf et al. 1998). BDNF has been reported to be required to elicit LTP in the hippocampus (Figurov et al. 1996). Of importance to the work presented in this thesis is the fact that BDNF and serotonin have been shown to display a co-dependent relationship (Mattson et al. 2004). Dysregulation of both has been reported in conditions of stress and anxiety. The effect of BDNF on LTP is supposedly mediated through the transcription factor cAMP-response element binding protein (CREB) (Ernfors & Bramham 2003). Activation of 5-HT receptors linked to cAMP formation and CREB activation results in the activation of BDNF (Mattson et al. 2004). SSRIs have been found capable of alleviating this stress induced BDNF decrease (Duman 2004). Blanquet & Lamour (1997) showed that BDNF stimulates both phoshorylation

of CaMKII and its kinase activity in rat hippocampal slices. CaMKII is of particular importance in LTP (Malinow et al. 1989; Lisman et al. 1997) and following its autophosphorylation can act independently of calcium concentration (Kennedy et al. 1987). Furthermore Blaquet et al. (1997) showed that BDNF required PLC to activate CaMKII. Interestingly, they also showed that this PLC-mediated activation of CaMKII required release of intracellular calcium and did not involve Ca^{2+} influx through Ca^{2+} channels. This enables the induction of LTP in the absence of an influx of extracellular Ca^{2+} which negates the reported inhibitory effect of raised 5-HT levels on Ca^{2+} channels . Therefore 5-HT_2 receptor agonists may overcome stress-induced inhibition of LTP by activating the PLC system, releasing intracellular Ca^{2+} and activating CaMKII which facilitates subsequent LTP induction.

In conclusion, activation of the serotonergic system, in particular activation of 5-HT_2 receptors seems able to overcome stress induced LTP inhibition by activating PLC to produce IP3 and DAG resulting in the release of Ca^{2+} from intracellular stores and the activation of PKC respectively.

4.2 Flinders Model of Depression and LTP.

The present study found that Flinders Sensitive Line (FSL) rats (an accepted genetic animal model of depression) had significantly less LTP in the CA1 area *in vivo* than the control strain, the Flinders Resistant Line (FRL) rats. As LTP induction is a putative memory mechanism this suggests that FSL animals may have impaired cognitive performance. Such a deficit might model cognitive impairment symptoms which are seen in human depression sufferers. However, as yet using behavioural paradigms there is no evidence for any difference in cognitive performance between FRL and FSL groups (Bushnell et al. 1995). The reason for the reduced level of potentiation in FSL rats when compared to FRL rats is unclear. The Flinders Sensitive rats have abnormalities in numerous neurotransmitter systems which may account for this difference in LTP induction. Differences between FSL and FRL groups have been reported for the cholinergic, serotonergic, noradrenergic and dopaminergic systems. For example FSL rats are supersensitive to cholinergic agonists (Overstreet & Russell 1982; Overstreet 1993, 2002). FSL rats also have abnormalities in their serotonergic functioning (Overstreet et al. 1994b; Wallis et al. 1988; Zangen et al. 1997). FSL rats have significantly higher levels of serotonin in the hippocampus than do control Sprague Dawley animals (Zangen et al. 1997). Serotonin has been shown to inhibit LTP in CA1 *in vitro* (Corradetti et al. 1992). In addition, FSL rats have been reported to be supersensitive to serotonin (Schiller et al. 1991; Overstreet et al. 1992). Important molecules which may account for a difference in LTP induction between groups include PKC or CaMKII. Unfortunately to date there is no available literature in respect to FSL rats.

Chronic treatment with escitalopram, a clinically effective anti-depressant (SSRI) inhibited HFS induced LTP in FRL rats. In addition, this drug regimen had a similar inhibitory effect on LTP induction in FSL animals. As FRL animals represent the 'normal' control animals for this study, and display normal LTP it is perhaps not surprising that chronic drug treatment should negatively impact upon LTP induction. It is likely that chronic SSRI treatment results in inappropriate increased serotonin levels

in the 'normal' control animal. Stewart et al. (2000) reported that repeated fluoxetine treatment significantly reduced LTP in the dentate gyrus of male Hooded Lister rats. These authors suggested that this may be due to a fluoxetine-induced increase in baseline synaptic transmission thereby occluding/saturating HFS induced LTP. Acute treatment with SSRIs has been previously shown to be inhibit LTP induction in CA1 (Kojima et al. 2003; Shakesby et al. 2002) in 'normal' control animals. The inhibitory effect of chronic escitalopram treatment on LTP induction in FRL animals may just be an extension of the inhibitory effect of acute SSRI treatment. The lack of a positive effect in the present study of this drug regimen on FSL animals was more surprising. It had been hypothesised that chronic escitalopram treatment would reverse any reduction of LTP in FSL rats, since SSRIs were effective antidepressants in the FSL depression model (Yadid et al. 2000a). Moreover chronic milnacipran (a SNRI) treatment was found to reverse the impairment of synaptic plasticity induced by conditioned fear stress (Matsumoto et al. 2005). The reason why escitalopram did not enable LTP in the FSL animals may be that the duration of treatment was not sufficient. It is well documented that SSRI treatment in humans has a lag period before behavioural recovery is apparent. The two week chronic treatment may not be sufficient in duration to have a positive effect on synaptic plasticity. It should be noted however that this dose regimen is not without effect as it was capable of blocking LTP induction in the FRL animals. Another explanation for the lack of a positive effect was the possibility that the dose was not optimal. Finally it may be that escitalopram simply has no beneficial effect on LTP induction in the FSL model.

Maternal separation (neonatal isolation) is an early life stressor. This experience may be considered stressful as it activates the HPA axis (Hofer et al. 1993; Staunton et al. 1988), induces analgesia (Spear et al. 1985), activates the opioid system (Harvey et al. 1994) and increases dopamine and noradrenalin release (Kehoe et al. 1996; Harvey et al. 1994). Moreover the effects of maternal separation are long lasting and endure into adulthood. For example, maternal separation has been reported to result in alterations in behavioural, neuroendocrine, neurochemical responses as well as neuronal plasticity in the adult rat (Cirulli et al. 2003). As exposure to acute stress was found to inhibit the

induction of LTP in CA1 (Shakesby et al. 2002) we hypothesised that stress exposure in the form of maternal separation may also result in the inhibition of LTP in CA1 in adult rats.

We found that exposure to maternal separation resulted in reduced though still statistically significant LTP in FRL animals. This reduction in LTP at 60 minutes post HFS was not statistically significant when compared to non-maternally separated FRL animals. Maternal separation resulted in a statistically non-significant increase in synaptic transmission in FSL animals. Previously, Kehoe et al. (1995) had reported a facilitation in both duration and size of LTP after 5 hours post-tetanus in the dentate gyrus of freely moving juvenile Wistar rats which had been maternally separated. However, there was no difference at 1 hour post tetanization in EPSP slope between isolated and non-isolated groups. This is similar to our findings in FRL animals. Furthermore, Bartesaghi (2004) reported that dentate LTP is unaffected in the guinea pig following 80-90 days of isolation. Our results suggest that maternal separation may have a greater negative effect on LTP in CA1 in FSL animals than FRL animals. Exposure to our maternal separation protocol was not sufficient to induce an inhibition of LTP in FRL rats in adulthood. In adult FSL animals that underwent the same maternal separation protocol HFS failed to induce a statistically significant LTP ($p=0.066$) but there was no statistical difference in the level of potentiation between these animals and control FSL animals ($p>0.1$). Therefore, the finding that exposure to maternal separation results in a lack of a statistically significant LTP in maternally separated adult FSL animals should be interpreted with caution.

HFS failed to elicit LTP in either FSL maternally separated or FRL maternally separated groups that received chronic escitalopram treatment. Escitalopram treatment of the FRL maternally separated animals further reduced the level of potentiation induced in the FRL maternally separated group. This shows that escitalopram treatment (at least by the regimen used here) does not enhance LTP induction in maternally separated FRL rats. Similarly, escitalopram treatment had no facilitatory effect on LTP induction in FSL maternally separated rats.

The FRL control group had altered baseline synaptic transmission. This group required significantly less current to elicit a 50% maximal EPSP response. However, the average magnitude of the test EPSP for the FRL control group was not significantly different from any other group. This suggests that FRL CA1 neurons are more excitable than those of FSL animals and this may contribute to the difference in LTP induction between these sets of animals.

Paired-pulse facilitation is a short-term form of synaptic plasticity sensitive to changes in presynaptic glutamate release probability (Zucker 1989). Blatow et al. (2003) suggested that facilitation in Schaffer collaterals is most likely due to accumulation of free Ca^{2+}. All groups in the Flinders study showed PPF at all interstimulus intervals tested. Compared with that of the controls (FRL), the paired-pulse facilitation at 40ms for the FSL group was significantly depressed. The reason for this decrease in PPF is unclear. There is no report to our knowledge of any difference in presynaptic calcium between FRL and FSL animals. This result showing that FSL animals have reduced short-term plasticity compared with FRL animals coincides with our findings with respect to LTP. We did not identify any difference between any FSL treated group and their respective FRL counterpart with regards to PPF. There was a strong positive correlation between the level of initial PPF and subsequent LTP in some groups. Most interesting was the finding that both the untreated FRL and FSL rats showed this positive correlation. This would suggest that the initial level of PPF is an indicator of subsequent ability to induce LTP in both FRL and FSL treatment naive animals.

In conclusion, it would appear that FSL animals have altered synaptic properties which may contribute to their depressive-like state and which warrant further scientific investigation.

4.3 Learned Helplessness Model of Depression and LTP.

Learned Helplessness is an accepted model of depression (Overmier & Seligman 1967). Acute stress exposure results in an inhibition of LTP in brain areas such as the CA1 region of the hippocampus (Xu et al. 1997). Therefore, it was hypothesised that there may be differences in the plasticity properties between different specially bred subgroups of rats that were differentially susceptible to the learned helplessness model of depression. It was hypothesised that animals that were resistant to learned helplessness (cNLH) would have normal LTP while those animals which have an inherent tendency to learned helplessness, exhibiting a helpless phenotype even in the absence of uncontrollable shock (cLH) would show impaired LTP induction. Upon investigation, it was discovered that animals which were congenital non-learned helpless and expressed a non-learned helpless phenotype elicited a small albeit significant amount of potentiation post HFS. Interestingly, congenital learned helpless animals, expressing either a learned or non-learned helpless phenotype elicited numerically greater LTP post HFS than did the learned helpless resistant group. This increase bordered on statistical significance (cLH-lh/nlh vs cNLH-nlh, $p=0.057$). This finding is somewhat surprising and the reasons for it are unclear. This animal model is based on selective breeding and therefore it may be that the genetic difference between strains may account for this tendency for a difference in LTP induction. Diversity of the gene pool is ensured by using repeated back crosses to the paternal strain which should reduce the likelihood of incidental co-selection of genes not related to learned helplessness. Interestingly, the level of LTP does not seem to be dependent on the behavioural phenotype expressed as there was no statistical difference in LTP induced between cLH-lh and cLH-nlh animals ($p>0.5$). In addition, neither cLH nor cNLH rats show any hippocampal-dependent learning deficits (Vollmayr et al. 2004).

As expected, HFS induced LTP in outbred Sprague Dawley control rats that were not behaviourally tested. In contrast, statistically significant LTP was not induced in outbred Sprague Dawley animals exposed to the 'training and testing' protocol for learned helplessness. Thus the magnitude of potentiation in the Sprague Dawley

outbred control animals was significantly greater than in those animals that underwent the 'training and testing' procedure. This possibly suggests an explanation for the unexpected results of the congenital learned helplessness study. The training session involves exposure to 20 minutes of unpredictable and uncontrollable shock within a 40 minute period. The testing procedure involves the delivery of 15 controllable shocks each of a maximum duration of 60 seconds terminable using a lever by the animal. Previously, Shors et al. (1989) reported that LTP in hippocampal slices of animals exposed to inescapable shock was impaired compared to that of animals exposed to the same amount of controllable shock. This suggests that the context of stress exposure (controllable vs uncontrollable) in relation to its effect on subsequent LTP induction may be of greater importance than the absolute amount of shock delivered. cNLH animals received both the training and testing. Therefore they received both controllable and uncontrollable shock. cLH animals of either behavioural phenotype (lh or nlh) received testing only, i.e. they received only controllable shock. However there was no significant correlation between LTP induced and amount of escapable shock exposure. Taken together, this suggests that the unexpected result of a tendency for reduced level of LTP in cNLH animals is possibly a result of exposure to uncontrollable shock (as part of the training procedure) and may not necessarily indicate a real difference between the cLH and cNLH animals with respect to LTP induction. The results in the outbred Sprague Dawley rats suggest that the exposure to uncontrollable shock results in prolonged inhibition of the induction of LTP at synapses in the CA1 area. Indeed, there was a significant reduction in LTP between all animals that received 'training and testing' both outbred and congenital animals and those outbred and congenital animals that received testing only ($p<0.05$). Our finding is consistent with Vollmayr et al. (2004) who reported that cLH and cNLH animals having underwent the same 'training and testing' procedure as outlined in our experiments showed no difference in their performance in the Morris Water Maze (a hippocampal-dependent learning paradigm). It may be that the tendency for reduced LTP in the CA1 area of cNLH animals is simply a result of plasticity being more sensitive to the differences between controllable and uncontrollable shock than is the Morris Water Maze task. However, King et al. (2001) reported that in naive cLH and

cNLH animals (i.e. no training or testing), cNLH animals showed a significantly reduced latency to find the platform in the Morris Water Maze when compared to cLH animals. Furthermore following exposure of both groups to the same amount of uncontrollable stress cNLH animals showed enhanced performance in this task as opposed to the further compromised performance of the cLH animals. Therefore, it would be interesting in future studies to investigate naive cLH and cNLH animals (i.e. no training or testing) with respect to LTP induction in CA1.

Alternatively, differences in various transmitter systems between learned helpless and non-learned helpless animals may contribute to the tendency for reduced LTP induction in cNLH animals and should be investigated. Hippocampal slices from Sprague Dawley rats in which learned helplessness was induced by uncontrollable shock exposure show a significant increase in both endogenous and K^+-stimulated serotonin release (Edwards et al. 1992). Also such learned helpless animals have upregulation of beta-adrenoceptors in the hippocampus (Martin et al. 1990). Moreover learned helpless rats show up regulated 5-HT_{1B} receptors in the hippocampus, cortex and septum (Edwards et al. 1991). Similar alterations in monoaminergic receptors are observed in cLH animals (Henn et al. 1993). cLH animals also have upregulated μ opioid receptors in the hippocampus and cortex. These receptors are down regulated in the hypothalamus (Henn et al. 1993). cLH animals also have altered intracellular signalling when compared to Spague Dawley or cNLH animals. Vollmayr et al. (2001) reported that there was no difference in BDNF mRNA levels in either the dentate or CA3 region of the hippocampus. They further reported that following exposure to restraint stress only the cLH group failed to show a decrease in BDNF levels. BDNF itself is important for the expression of LTP in the hippocampus (Patterson et al. 1996) and alterations in its expression may account for the tendency for larger LTP in the cLH groups than in cNLH groups.

There was no significant difference in the current required to elicit a 50% maximum EPSP between groups. Furthermore there was no statistically significant difference

between any group with respect to test EPSP amplitude. Therefore, it appears that there were no basal differences between groups in respect of baseline synaptic transmission.

All groups elicited PPF at all interstimulus intervals tested. There was no significant difference between groups. As PPF is sensitive to differences in glutamate release this suggests that there is no difference in the presynaptic properties of the synapses in the CA1 area between these groups.

V. Conclusion

5.0 Conclusion

Elevated platform stress, a mild behavioural stressor has the ability to inhibit the induction of LTP in the CA1 region of the hippocampus of urethane anaesthetised rats. There is evidence to suggest that manipulation of the serotonergic system has the ability to affect this stress-induced LTP blockade (Shakesby et al. 2002).

Adminstration of (±)fenfluramine, a serotonin releasing agent enabled the induction of LTP in previously stressed animals. Cinanserin, a non-subtype selective 5-HT_2 receptor antagonist prevented (±)fenfluramine enabling the induction of LTP in previously stressed animals. This result suggests that the ability of (±)fenfluramine to alleviate a stress-induced block in LTP may involve activation of 5-HT_2 receptors. Indeed administration of 5-HT_2 receptor agonists alleviated the stress-induced block in LTP induction further indicating a role for 5-HT_2 receptor activation in enabling LTP induction in stressed animals. We found no experimental evidence to suggest that administration of a 5-HT_4 receptor partial agonist enabled the induction of LTP in previously stressed animals. Interestingly, we showed that (±)fenfluramine administration significantly enhanced LTP induction in the presence of D-AP5.

Animal models of depression were also investigated. Flinders Sensitive Line (FSL) rats (an accepted genetic animal model of depression) had significantly reduced LTP induction in the CA1 area of the hippocampus when compared with their control animals (Flinders Resistant Line, FRL). Chronic treatment with escitalopram, a selective serotonin reuptake inhibitor failed to enhance the induction of LTP in either FRL or FSL groups. Indeed, escitalopram treatment resulted in reduced potentiation post-HFS in both groups mentioned. While maternal separation failed to inhibit LTP induction in adult FRL animals, it did result in the failure to elicit significant LTP in FSL animals. However this effect on FSL animals should be interpreted with caution. Chronic treatment with escitalopram in FRL maternally separated animals failed to enhance LTP induction. It also failed to enable LTP induction in FSL maternally separated animals. As FRL animals were found to have more excitable CA1 neurons

when compared with FSL animals we suggest that this fact may account for subsequent differences in LTP induction.

A second accepted animal model of depression, the congenital Learned Helplessness model was also investigated. It was found that, surprisingly, congenital learned helpless (cLH) animals which expressed either a learned helpless or non-learned helpless phenotype had a tendency for greater LTP than congenital non-learned helpless animals (cNLH). This may be as a result of differential exposure to the training protocol between groups. Furthermore outbred Sprague Dawley animals which underwent the 'training and testing' protocol showed significantly less LTP than control non 'trained and tested' animals. There was no apparent difference in short term plasticity in CA1 between cLH and cNLH animals.

In conclusion, we suggest that manipulation of the serotonergic system does affect the induction of LTP in previously stressed animals. In particular it seems that activation of 5-HT_2 receptors may play a particular role in enabling the induction of LTP in acutely stressed rats. As hippocampal plasticity is thought to be negatively affected in disorders such as depression and anxiety it may be of interest to investigate 5-HT_2 receptor agonists with regard to their therapeutic benefit in individuals suffering from acute stress disorders. Flinders Sensitive Line rats have compromised LTP in CA1 when compared to their controls. However, the subsequent inability of a known antidepressant (escitalopram) to ameliorate this reduction in LTP induction may lead one to question the predictive validity of the model itself or the suggested presence of impaired LTP in depression. Certainly, lack of LTP recovery following drug treatment should necessarily be interpreted as indicative of an ineffective drug with respect to antidepressant treatment. While the results of the Learned Helplessness study were opposite to those predicted this may be accounted for by the differential exposure of uncontrollable shock between groups. It would be of interest to investigate non 'trained and tested' animals with respect to LTP induction in CA1. However it may simply be the case that no difference exists with respect to LTP induction between the constituent members of the congenital Learned Helplessness depression model. It may be

suggested that congenital Learned Helplessness does not model the supposed negative impact of depression on synaptic plasticity. However as congenitally Learned Helpless animals are resistant to antidepressant therapy i.e. model refractive depression it is maybe not surprising that their synaptic plasticity properties should be different to antidepressant amenable depression models.

VI. Bibliography

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