Department of Physiology and Pharmacology
Karolinska Institutet, Stockholm, Sweden

STUDIES ON ANTIDEPRESSANT-MEDIATED REGULATION OF SIGNAL TRANSDUCTION

HONGSHI QI

Karolinska Institutet

Stockholm 2009

Published by Karolinska Institutet. Printed by US-AB.

ISBN 978-91-7409-604-0

To my family with love

ABSTRACT

Depression affects around 16% of the population at some point in their lives and major depressive disorder is a leading cause of disability worldwide. Depression is caused by an interaction of genetic and environmental factors, but the molecular and cellular mechanisms underlying its pathogenesis are not entirely clear. The “monoamine hypothesis” of depression, which involves imbalances in serotonergic, noradrenergic and possibly dopaminergic functions, has dominated notions and explanations of the pathophysiology of depression. Most clinically used antidepressants act by increasing the synaptic levels of these monoamines. While these treatments are beneficial in many cases, a large population of depressed patients do not respond, or respond suboptimal, to these current antidepressant treatments.

There is accumulating evidence that depression is associated with impairments of synaptic plasticity, dendritic arborization and cell proliferation/survival in hippocampus and frontal cortex and normalization of these processes could lead to antidepressant responses. The neurochemical mechanisms underlying these functional and structural impairments are poorly understood, but appear to involve changes in glutamate neurotransmission, neuropeptides, neurotrophins and intracellular signaling cascades.

The present studies used biochemical and behavioural techniques and found that the atypical antidepressant, tianeptine, which is a serotonin reuptake enhancer, increased phosphorylation of the GluA1 subunits of AMPA glutamate receptors both in brain slices and in intact animals. Antidepressant and stimulatory behavioral responses to tianeptine were attenuated in mice bearing point mutations at Ser^{831}-/Ser^{845}-GluA1. Acute elevated platform stress, known to inhibit synaptic plasticity in hippocampal→frontal cortex neurotransmission, was found to downregulate a putative BDNF/MEK/MAPK signaling cascade in frontal cortex which could be counteracted by tianeptine, imipramine and the glucocorticoid receptor antagonist mifepristone.

The serotonin receptors mediating the antidepressant effects of serotonin reuptake inhibitors, the currently most prescribed class of antidepressants, are not fully understood. Here, it is reported blockade of 5-HT_6 receptors with the antagonist SB271046 counteracts the potentiating actions of fluoxetine on cortical phospho-Ser^{845}-GluA1 and reduces its antidepressant-like behavioral action. Moreover, the 5-HT_6 receptor agonist EMDT mimics antidepressant-like biochemical and behavioral effects of fluoxetine.

In conclusion, these studies have shown that the atypical antidepressant tianeptine can potentiate signaling cascades associated with synaptic plasticity and add further evidence that tianeptine acts as an enhancer of AMPA glutamate transmission. These studies also suggest, for the first time, a role for 5-HT_6 receptors in mediating antidepressant responses.

List of Publications

I. Svenningsson P, Bateup H, **Qi H**, Takamiya K, Huganir RL, Spedding M, Roth BL, McEwen BS and Greengard P. (2007) Involvement of AMPA receptor phosphorylation in antidepressant actions with special reference to tianeptine. *European Journal of Neuroscience*. 26:3509-3517.

II. **Qi H**, Zhang X, Delagrange P, Spedding M and Svenningsson P. Tianeptine increases phosphorylation of AMPA receptors and potentiates MAPK signalling. *Manuscript*.

III. **Qi H**, Mailliet F, Spedding M, Rocher C, Zhang X, Delagrange P, McEwen B, Jay TM and Svenningsson P. (2009) Antidepressants reverse the attenuation of the neurotrophic MEK/MAPK cascade in frontal cortex by elevated platform stress; reversal of effects on LTP is associated with GluA1 phosphorylation. *Neuropharmacology*. 56:37-46.

IV. Mailliet F, **Qi H**, Rocher C, Spedding M, Svenningsson P and Jay TM. (2008) Protection of stress-induced impairment of hippocampal/prefrontal LTP through blockade of glucocorticoid receptors: Implication of MEK signaling. *Experimental Neurology*. 211:593-596.

V. Svenningsson P, Tzavara ET, **Qi H**, Carruthers R, Witkin JM, Nomikos GG and Greengard P. (2007) Biochemical and behavioral evidence for antidepressant-like effects of 5-HT6 receptor stimulation. *Journal of Neuroscience*. 27:4201-4209.

Additional publications during PhD studies

I. Smith DG, **Qi H,** Svenningsson P, Wade M, Davis RJ, Gehlert DR and Nomikos GG. (2008) Behavioral and biochemical responses to d-amphetamine in MCH1 receptor knockout mice. *Synapse.* 62:128-136.

II. Warner-Schmidt JL, Flajolet M, Maller A, Chen EY, **Qi H,** Svenningsson P and Greengard P. (2009) Role of p11 in cellular and behavioral effects of 5-HT4 receptor stimulation. *Journal of Neuroscience*. 29:1937- 1946.

CONTENTS

LIST OF ABBREVIATIONS

5-HT	5-Hydroxytryptamine, Serotonin
ADT	Antidepressant treatment
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
BDNF	Brain-derived neurotrophic factor
CamKII	Calcium⁄calmodulin-dependent kinase II
cAMP	Cyclic adenosine monophosphate
CREB	cAMP responsive element binding
DA	Dopamine
DARPP-32	Dopamine and cAMP regulated phosphoprotein MW 32kDA
ECT	Electroconvulsive therapy
EMDT	2 -ethyl-5-methoxy-N,N-dimethyltryptamine
GABA	Gamma-aminobutyric acid
GluA1	Glutamate receptor subunit 1
HFS	High frequency stimulation
HPA	Hypothalamic-pituitary-adrenal
LTP	Long-term potentiation
MAOB	Monoamine oxidase B
MAOIs	Monoamine oxidase inhibitors
MAPK	Mitogen-activated protein kinase
MEK	MAPK/ERK kinase
NA	Noradrenaline
NDRIs	Noradrenaline-dopamine reuptake inhibitors
NSSAs	Noradrenergic and specific serotonergic antidepressants
NMDA	N-methyl-D-aspartic acid

NRIs	Noradrenaline reuptake inhibitors
PDE4	Phosphodiesterase type 4
PFC	Prefrontal cortex
PKA	Protein kinase A
PKC	Protein kinase C
PP-1	Protein phosphatase-1
RIMA	Reversible inhibitor of monoamine oxidase A
RU486	Mifepristone
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS poly acrylamide gel electrophoresis
SNRIs	Serotonin-noradrenaline reuptake inhibitors
SSRE	Serotonin reuptake enhancer
SSRIs	Selective serotonin reuptake inhibitors
TCAs	Tricyclic antidepressants
TrkB	Tyrosine kinase receptor B

1 INTRODUCTION

1.1 DEPRESSION

Depression is a mental illness which affects around 16% of the population at some point in their lives and major depressive disorder is a leading cause of disability worldwide (Belmaker and Agam, 2008).

The clinical symptoms of major depression as outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) are depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful); Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day; Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day; Insomnia or hypersomnia nearly every day; Psychomotor agitation or retardation nearly every day; Fatigue or loss of energy nearly every day; Feelings of worthlessness or excessive or inappropriate guilt nearly every day; Diminished ability to think or concentrate, or indecisiveness, nearly every day; Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide. Thus, depression is a disorder with both physical and mental characteristics that negatively disrupts an individual's ability to function day to day in social and work environments. According to the DSM-IV, real depression is a condition of this nature that lasts for more than two weeks.

From a neurobiological standpoint, depression is a complex, heterogeneous disorder, and the mechanisms underlying its pathogenesis are not that clear and are the subject of intensive investigation using pharmacological and genetic tools in patients and in animal models. The “monoamine hypothesis” of depression, which involves

imbalances in serotonergic, noradrenergic and possibly dopaminergic functions, has dominated notions and explanations of the pathophysiology of depression since the empirical discovery of the antidepressant properties of monoamine oxidase inhibitors (MAOIs) and tricyclics about fifty years ago (Schildkraut, 1965). Although the monoaminergic neurotransmitters serotonin (5-HT), noradrenaline (NA) and dopamine (DA) are undoubtedly involved, it is now recognized that monoamine deficits are only part of the story and are not sufficient on their own to explain the mechanisms of action of antidepressants. It has, for example, been found that the antidepressant tianeptine exerts its actions with mechanisms which are clearly challenging the hypothesis of an immediate stimulatory modulation of monoamine axes for antidepressant actions, since this compound enhances serotonin reuptake. The efficacy and tolerability of tianeptine are clearly demonstrated in depressed patients (Kasper and McEwen, 2008). However, the monoamine hypothesis can obviously not explain these properties and studies on tianeptine can contribute to our realization of the complexity of the etiology of depression and to the complexity of central mechanisms triggered by antidepressants.

In extension to the chemical hypothesis of depression, contemporary theories suggest that major depressive disorders may be associated not only with an imbalance of neurotransmitters, neuropeptides, neurotrophins and neuromodulators but also with an impairment of glial function, neuroplasticity and cellular resilience, and that antidepressant medications act by normalizing thess impairments (Duman et al., 1999; Manji and Duman, 2001; Mathé et al., 2007; Pittenger and Duman, 2008). The term neuroplasticity describes the ability of the adult and differentiated brain to adapt functionally and structurally to internal and external stimuli and is considered today as a feature of depressive illness. Many aspects of neuroplasticity are regulated by glutamate, which is the major excitatory neurotransmitter in the brain controlling synaptic excitability and plasticity in most brain circuits. Glutamatergic mechanisms

play crucial roles in adaptive, neuronal and plasticity perturbed in depressed states (Bergink et al., 2004; Lowy et al., 1995; Paul and Skolnick, 2003; Sanacora et al., 2003; Skolnick et al., 2001; Zarate et al., 2003). There is accumulating evidence that direct modulation of synaptic plasticity, by for example glutamate Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor potentiation or N-methyl-D-aspartia acid (NMDA) receptor antagonism, has antidepressant actions (Alt et al., 2006; Zarate et al., 2003). In fact, acute treatment with the non-selective NMDA receptor antagonist causes a rapid and long-lasting antidepressant response (Zarate et al., 2003).

Brain regions which exhibit neuroplastic processes include the hippocampus, amygdala and prefrontal cortex (Duman and Monteggia, 2006), as they are reported to undergo structural changes in depression (Manji et al., 2003; Sheline, 2003). Alterations in these brain regions affect emotions, perceptions, memory, and cognitive function. Functional brain imaging studies also indicate that the regions most commonly found to be abnormal in major depression are the prefrontal cortex, the anterior cingulate gyrus and the temporal lobe, including hippocampus (Drevets et al., 2002; Mayberg et al., 1999). Interestingly, deep brain stimulation of the anterior cingulate gyrus (region Cg25) reverses immediately the signs of depression in some previously treatment resistant depressed patients (Mayberg et al., 2005).

1.2 STRESS AND DEPRESSION

Stress has been widely recognized as an important trigger in the expression of various clinical syndromes, particularly mood and anxiety disorders, like depression. Across the life span, stressful life events that involve threat, loss, humiliation, or defeat influence the onset and course of depression (Brown, 1998; Kendler et al., 1999; Kessler, 1997; Pine, 2002). Stress factors, such as early-life adverse events, have been

shown to interact with a variable background of genetic vulnerability (Caspi and Moffit, 2006), markedly increasing the risk for development of depression in adult life (Caspi et al., 2003). Moreover, stress profoundly affects cognitive functions and hippocampal synaptic plasticity (Kim and Diamond, 2002).

The dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been frequently reported in depressive patients (Holsboer and Barden, 1996; Nemeroff, 1996) pointing towards a new strategy of pharmacotherapy. Dysfunctional glucocorticoid receptor (GR)-mediated negative feedback regulation of cortisol levels has been implicated in over-activity of the HPA axis in patients with major depression (Pariante, 2004). Pharmacological intervention against the negative effects of stressors on neuroplasticity may turn out to be beneficial for the prevention and treatment of depression-related states (Agid et al., 2007). Indeed, hypercortisolemia has provided the rationale for pre-clinical and clinical studies that have indicated that the GR antagonist mifepristone (RU486) may be used to treat depression (Belanoff et al., 2001). Indeed, mifepristone is now in phase III clinical trials for psychotic major depression (Berton and Nestler, 2006).

Similar regulation of GRs occurs in laboratory animals and patients and recent evidence has shown that impaired negative feedback on the HPA axis may alter the function of the prefrontal cortex and the hippocampus (Aihara et al., 2007; Diorio et al., 1993; McEwen, 2005; Mizoguchi et al., 2003; Sullivan and Gratton, 2002). A powerful and valid method to study a depression-like phenotype in adult rodents is to expose them to maternal separation during their early development (e.g. Ryan et al., 2009). Professor Therese Jay and colleagues measured the hippocampal→limbic cortex synaptic connectivity in the rat (Rocher et al., 2004) and found that even a single stress exposure to an elevated platform causes a long-lasting alteration of long term potentiation (LTP) evoked in the prefrontal cortex by stimulation of the

hippocampal outflow (Cerqueira et al., 2007; Jay et al., 2004; Rocher et al., 2004) with a severe disruption of working memory and behavioral flexibility when rats were exposed to stress for a longer period (Cerqueira et al., 2007). The impairment of prefrontal LTP could be reversed by some antidepressants such as tianeptine and fluoxetine (Rocher et al., 2004).

1.3 ANTIDEPRESSANTS

An antidepressant is a psychiatric medication used to alleviate mood disorders, such as major depression and dysthymia. Drugs including the monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), tetracyclic antidepressants (TeCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin-noradrenaline reuptake inhibitors (SNRIs) are the most commonly used antidepressants. These medications are among those most commonly prescribed by psychiatrists and other physicians, and their effectiveness and adverse effects are the subject of many studies (Stahl and Felker, 2008; Rapaport, 2007; Preskorn et al., 1988; Barbui et al., 2007; Stahl et al., 2005; Hirschfeld 1999; Ikenouchi-Sugita A et al., 2007). Most typical antidepressants have a delayed onset of action (2–6 weeks) and are usually administered from months to years. Despite the name, antidepressants are often used to treat other conditions, such as anxiety disorders, obsessive compulsive disorder, eating disorders, chronic pain, and some hormone-mediated disorders such as dysmenorrhea.

1.3.1 Types of Antidepressants

Table 1. Types of antidepressants.

Types	e.g.
Tricyclic antidepressants (TCAs)	imipramine
Monoamine oxidase inhibitors (MAOIs)	moclobemide

Selective serotonin reuptake inhibitors (SSRIs)	fluoxetine
Serotonin reuptake enhancer (SSRE)	tianeptine
Noradrenaline reuptake inhibitors (NRIs)	reboxetine
Serotonin-noradrenaline reuptake inhibitors (SNRIs)	venlafaxine
Noradrenergic and specific serotonergic antidepressants (NSSAs)	mianserin
Noradrenaline-dopamine reuptake inhibitors (NDRIs)	bupropion

1.3.1.1 Tricyclic antidepressants (TCAs)

Tricyclic antidepressants are the oldest class of antidepressant drugs. Tricyclics block the reuptake of noradrenaline and serotonin. They are used less commonly now due to the development of more selective and safer drugs. Side effects include increased heart rate, drowsiness, dry mouth, constipation, urinary retention, blurred vision, dizziness, confusion, and sexual dysfunction. Toxicity occurs at approximately ten times normal dosages; these drugs are often lethal in overdoses, as they may cause a fatal arrhythmia. However, TCAs are still used because of their effectiveness, especially in severe cases of major depression in hospitalized patients. These include Tertiary Amine Tricyclic Antidepressants: Amitriptyline, Clomipramine, Doxepin, Imipramine, Trimipramine; and Secondary Amine Tricyclic Antidepressants: Desipramine , Nortriptyline, Protriptyline.

1.3.1.2 Monoamine oxidase inhibitor (MAOIs)

Monoamine oxidase inhibitors (MAOIs) may be used if other antidepressant medications are ineffective. Because there are potentially fatal interactions between this class of medication and certain foods (particularly those containing tyramine), red wine, as well as certain drugs, classic MAOIs are rarely prescribed anymore. MAOIs work by blocking MAO which breaks down the neurotransmitters dopamine, serotonin, and noradrenaline. MAOIs can be as effective as TCAs, although they can

have a higher incidence of dangerous side effects (as a result of inhibition of cytochrome P450 in the liver). A new generation of MAOIs has been introduced; moclobemide, known as a reversible inhibitor of monoamine oxidase A (RIMA), acts in a more short-lived and selective manner and does not require a special diet. One of the side effects is weight gain and could be extreme. These include: Isocarboxazid, Moclobemide, Phenelzine, Selegiline, Tranylcypromine.

1.3.1.3 Selective serotonin reuptake inhibitors (SSRIs)

Selective serotonin reuptake inhibitors (SSRIs) are a family of antidepressants which are currently the most prescribed drug treatment against depression. SSRIs are said to work by preventing the reuptake of serotonin by the presynaptic neuron, thus maintaining higher levels of 5-HT in the synapse. This family of drugs includes: Citalopram, Escitalopram, Fluoxetine, Fluvoxamine, Paroxetine, Sertraline.

These antidepressants typically have fewer adverse effects than the tricyclics or the MAOIs, although such effects as drowsiness, dry mouth, nervousness, anxiety, insomnia, decreased appetite, and decreased ability to function sexually may occur. Some side effects may decrease as a person adjusts to the drug, but other side effects may be persistent. Though safer than the first generation of antidepressants, SSRIs may not work on as many patients as previous classes of antidepressants, suggesting that the role of noradrenaline in depression is still important.

1.3.1.4 Selective serotonin reuptake enhancer (SSRE)

Selective serotonin reuptake enhancers are drugs which enhance the reuptake of the neurotransmitter serotonin and therefore decrease its synaptic concentrations. The only known drug on the market of this class is tianeptine (see 1.4.).

1.3.1.5 Noradrenaline reuptake inhibitors (NRIs)

Noradrenaline reuptake inhibitors (NRIs) act by increasing synaptic levels of noradrenaline. NRIs are thought to have a positive effect on concentration and

motivation in particular. These include: Atomoxetine, Mazindol, Reboxetine, Viloxazine.

1.3.1.6 Serotonin-noradrenaline reuptake inhibitors (SNRIs)

Serotonin-noradrenaline reuptake inhibitors (SNRIs) are a newer form of antidepressants that work on both noradrenaline and 5-HT. They typically have similar side effects to the SSRIs, though there may be a withdrawal syndrome on discontinuation that may necessitate dosage tapering. These include: Desvenlafaxine, Duloxetine, Milnacipram, Venlafaxine.

1.3.1.7 Noradrenergic and specific serotonergic antidepressants (NSSAs)

Noradrenergic and specific serotonergic antidepressant's (NSSAs) form a newer class of antidepressants which purportedly work to increase noradrenaline and serotonin neurotransmission by blocking presynaptic alpha-2 adrenergic receptors while at the same time certain serotonin receptors. Side effects may include drowsiness, increased appetite, and weight gain (Manev and Uz, 2006). Examples include: Mianserin, Mirtazapine.

1.3.1.8 Noradrenaline-dopamine reuptake inhibitors (NDRIs)

Noradrenaline-dopamine reuptake inhibitors inhibit the neuronal reuptake of dopamine and noradrenaline (Stimmel et al., 1997). They are also used for the treatment of nicotine dependence. These include: Bupropion.

1.4 TIANEPTINE

Tianeptine (Stablon, Coaxil, Tatinol) is a tricyclic antidepressant, but unlike conventional tricyclic antidepressants, tianeptine enhances the reuptake of serotonin (SSRE) instead of inhibiting it, opposite to the action of SSRIs.

Currently, tianeptine is approved in France under the trade name Stablon; it is also marketed in a number of other European countries under the trade name Coaxil as well as in Asia and Latin America as Tatinol.

1.4.1 The action of tianeptine

1.4.1.1 Clinical and Non-clinical antidepressant features

Compelling body of clinical data has demonstrated that the clinical efficacy of tianeptine in the treatment of depression is at least equivalent to those of selective serotonin reuptake inhibitors (SSRIs) (Kasper and Olie, 2002; McEwen and Olie, 2005). Tianeptine affords relief of depressive symptoms as the analysis of MADRS (Montgomery-Åsberg Depression Rating Scale) individual items shows that decreased ability of concentration and inner tension are more rapidly improved in tianeptine-treated than in fluoxetine-treated patients (Novotny and Faltus, 2003). Tianeptine is effective in reducing symptoms of depression in mild to moderate-to-severe major depression, while it alleviates anxious symptoms associated with depression without the need for concomitant anxiolytic therapy (Brion et al., 1996; Guelfi et al., 1989; Invernizzi et al., 1994; Lepine et al., 2001; Loo and Deniker, 1988; Ridout and Hindmarch, 2001; Szadoczky and Furedi, 2002;). The good tolerability of tianeptine is also established as the antidepressant lacks the sedative (Ridout and Hindmarch, 2001), autonomic, cardiovascular, and undesirable side effects on attention and memory of tricyclics (Wagstaff et al., 2001; Wilde and Benfield, 1995) and shows a low propensity to provoke sexual dysfunction and nausea as compared to SSRIs (Atmaca et al., 2003; Bonierbale et al., 2003).

There is a number of experimental studies demonstrating robust efficacies of tianeptine in rodent paradigms of antidepressant properties (Curzon et al., 1992; Kelly and Leonard, 1994; McEwen and Olie, 2005; Thiebot et al., 1992; Wagstaff et al., 2001). Further, tianeptine opposes not only the affective, but also the cognitive and

structural changes that characterize depressive states—at least in experimental models of depression based on chronic stress (McEwen and Olie, 2005; McEwen et al., 2002).

1.4.2 The emerging pharmacological profile of tianeptine

Tianeptine differs from other antidepressants in its pharmacological and neurochemical properties (Chamba et al., 1991; Kato and Weitsch, 1988). The first indication that tianeptine possesses a mechanism of action different to that of other classes of antidepressant was the finding that, upon acute and sustained administration, tianeptine decreased the extracellular levels of serotonin (5-HT) (Fattaccini et al., 1990; Mennini et al., 1987). This finding was hypothesized to be the consequence of a 5-HT re-uptake enhancement. It has been demonstrated that tianeptine also reduced both the number of transporter sites and their mRNA levels in the dorsal raphe nucleus (Watanabe et al., 1993). However, any facilitatory influence of tianeptine upon 5-HT re-uptake may be exerted indirectly rather than directly at 5-HT transporters, for which its affinity is low. Further, the validity of older data has been contested on the basis of technical limitations that could not be circumvented at that time (Malagie et al., 2000). More recent investigations have shown that acute and long-term administration of tianeptine does not elicit any marked alterations (neither increases nor decreases) in extracellular levels of 5-HT in corticolimbic structures of conscious rats (Malagie et al., 2000; Pineyro et al., 1995). From an electrophysiological point of view, sustained administration of tianeptine did not modify the spontaneous firing rate of serotonergic neurons in the dorsal raphe, nor did it modify the activity of postsynaptic 5-HT_{1A} receptors nor the effectiveness of the terminal 5-HT autoreceptor antagonist in increasing the efficacy of the stimulation of the 5-HT pathway, despite prolonged treatment (Pineyro et al., 1995). Thus, the role of 5-HT in the mechanism of antidepressant efficacy of tianeptine is doubtful.

Tianeptine shows no affinity for the noradrenaline or dopamine transporters or for several investigated neurotransmitter receptors: α_{1A}, α_{1B}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, 5-HT_1, 5-HT_2, 5-HT_3, 5-$HT_{4,}$ 5-HT_{5A}, 5-HT_6, 5-HT_7, D_1, D_2, D_3, $D_{4,}$ $D_{5,}$ NMDA, AMPA, kainate, benzodiazepine or Gamma-aminobutyric acid (GABA)-B receptors (Kato and Weitsch, 1988; Svenningsson et al., 2007). Tianeptine does not inhibit MAOa or MAOb activity in the cortex, hippocampus, and hypothalamus.

1.4.2.1 Neuroplasticity

As described above, stress paradigms have been used as models for depression in animal studies to investigate the actions of antidepressants on brain structure and neuroplasticity. In such models, tianeptine opposes the effects of chronic stress on brain structure and plasticity. For example, tianeptine prevented structural changes and modified neuronal metabolism and function in the hippocampus (Czeh et al., 2001). In tree shrews subjected to psychosocial stress, tianeptine reversed the stress-induced decreases in hippocampal volume, concentrations of cerebral metabolites such as *N*-acetyl-aspartate, and proliferation of the granule precursor cells in the dentate gyrus (Czeh et al., 2001). In an investigation of glucocorticoid induced remodeling of hippocampal CA3 dendrites, the therapeutic effects of tianeptine administration was further demonstrated after the shrinkage of dendrites had occurred, since tianeptine treatment was able to reverse these changes even while glucocorticoid administration was continuing (Magarinos et al., 1999).

1.5 SIGNAL TRANSDUCTION AND ANTIDEPRESSANTS

Cellular processes which include cell proliferation, neurogenesis and neurodegeneration have an important role for the mechanism of antidepressants. These processes are regulated by neuropeptides and neurotransmitters, of which glutamate via

its actions on AMPA and NMDA receptors are particularly important, as well as intracellular pathways including the Cyclic adenosine monophosphate (cAMP)/Protein kinise A (PKA), ras/Mitogen-activated protein kinase (MAPK) and Phosphoinositide 3-kinase (PI-3K) cascades. Moreover, regulation of CREB mediated genes coding for neurotrophic proteins such as Brain-derived neurotrophic factor (BDNF) appears also to be involved.

AMPA receptors play a crucial role in regulating glutamate transmission and plasticity at excitatory synapses. These effects involve alterations in phosphorylation of the different subunits of the AMPA receptor. Then nomeuclature of AMPA receptor subunits has recently been raised and GluR1 subunits are now referred to as GluA1 subunits (Spedding et al., 2009). It has, for example, been shown that phosphorylation by PKA at Ser^{845}-glutamate receptor subunit 1 (GluA1) is required for PKA-mediated potentiation of peak current by homomeric GluA1 channels (Roche et al., 1996; Collingridge et al., 2009) and membrance insertion of the AMPA receptor (Man et al., 2007). Phosphorylation of Ser^{831}-GluA1 by calcium/calmodulin-dependent kinase II (CaMKII) or protein kinase C (PKC) potentiates AMPA currents in hippocampal neurons (Barria et al., 1997). Fluoxetine and imipramine increases phospho-Ser^{845}-GluA1 (Du et al., 2007; Svenningsson et al., 2002). Recent studies using allosteric potentiators of AMPA receptors have provided further evidence for the possible involvement of AMPA receptors in antidepressant actions as it has been shown that such compounds have robust antidepressant-like actions in two tests of 'learned helplessness', the forced-swim test and the tail-suspension test (Alt et al., 2006). It has also been shown that chronic treatment with an allosteric AMPA potentiator, LY451646, like fluoxetine and other classical antidepressants, increases hippocampal neurogenesis (Bai et al., 2003; Santarelli et al., 2003).

The cAMP-CREB (cAMP response element binding protein) signal transduction

cascade contributes to the action of antidepressant treatment (ADT). This is supported by studies demonstrating that drugs that activate the cAMP-CREB cascade, by inhibition of the cAMP phosphodiesterase type 4 (PDE4), produces an ADT-response in rodent models and in humans (Fleischhacker et al., 1992; Fujimahi et al., 2000). One target gene of the cAMP-CREB of this pathway is BDNF. Indeed, expression of BDNF and its receptor tyrosine kinase receptor B (TrkB) are increased in rat hippocampus by repeated administration of antidepressant drugs as well as by electroconvulsive seizure (Nibuya et al., 1995; Russo-Neustadt et al., 2000). Furthermore, BDNF is increased in postmortem brain of human subjects who had been treated with antidepressants (Chen et al., 2001). Conversely, downregulation of BDNF is associated with stress exposure and depression (Karege et al., 2002). Immobilization stress results in decreased BDNF in the hippocampus, an effect that is blocked by antidepressant treatment (Nibuya et al., 1995; Smith et al., 1995). There is also evidence that BDNF can regulate functions relevant to antidepressant-responsive behavior in rats. Chronic infusion of BDNF into posterior midbrain nuclei results in effects that are similar to those of antidepressants in forced swim and learned helplessness models of depression (Siuciak et al., 1997) and administration of BDNF directly into localized regions of the hippocampus similarly results in antidepressant-like effects in these behavioral models (Shirayama et al., 2002). This work has resulted in the neurotrophic hypothesis of depression (Duman et al., 1997), suggesting that CREB and BDNF are necessary, as well as sufficient for an ADT-response. However, more recent studies have demonstrated that BDNF in the mesolimbic dopamine system produces pro-depressive effects in behavioral models, demonstrating region-specific actions of this neurotrophic factor (Berton et al., 2006; Eisch et al., 2003). A major revision on the role of BDNF in depression is needed.

One of the best-studied BDNF-regulated signaling cascades is activation of the mitogen-activated protein kinase (MAPK) (also called extracellular signal-regulated kinase (ERK)) pathway (Huang and Reichardt, 2003). The MAPK pathway is an intracellular signaling cascade implicated in several forms of learning, memory, and neuroplasticity (Mazzucchelli and Brambilla, 2000; Rodrigues et al., 2004). MAPK is activated by BDNF binding of TrkB via the Ras-Raf-MEK cascade, inducing nuclear translocation and phosphorylation of target transcription factors. As such, MAPK translates extracellular events, such as neurotransmitter and neurotrophin receptor binding, into gene transcription, synaptic remodeling, and behavioral events (Roberson et al., 1999; Sweatt, 2001). For example, BDNF infusion into the dentate gyrus has ADT-like consequences that can be blocked by inhibiting MAPK phosphorylation (Shirayama et al., 2002). Acute restraint or swim stress has been shown to increase p42/44-MAPK phosphorylation in cortex (Meller et al., 2003; Shen et al., 2004), whereas chronic restraint stress decreased, or had no effect on, p42/44-MAPK phosphorylation (Lee et al., 2006; Meller et al., 2003). In the hippocampus, chronic restraint stress and chronic unpredictable and inescapable tail-shock stress increased the phosphorylation states of p42/44-MAPK (Lee et al., 2006; Yang et al., 2004). Peripheral injection of MEK inhibitors produces helplessness-like behaviors and eliminates response to ADTs in standard assays of inescapable stress (Duman et al., 2007).

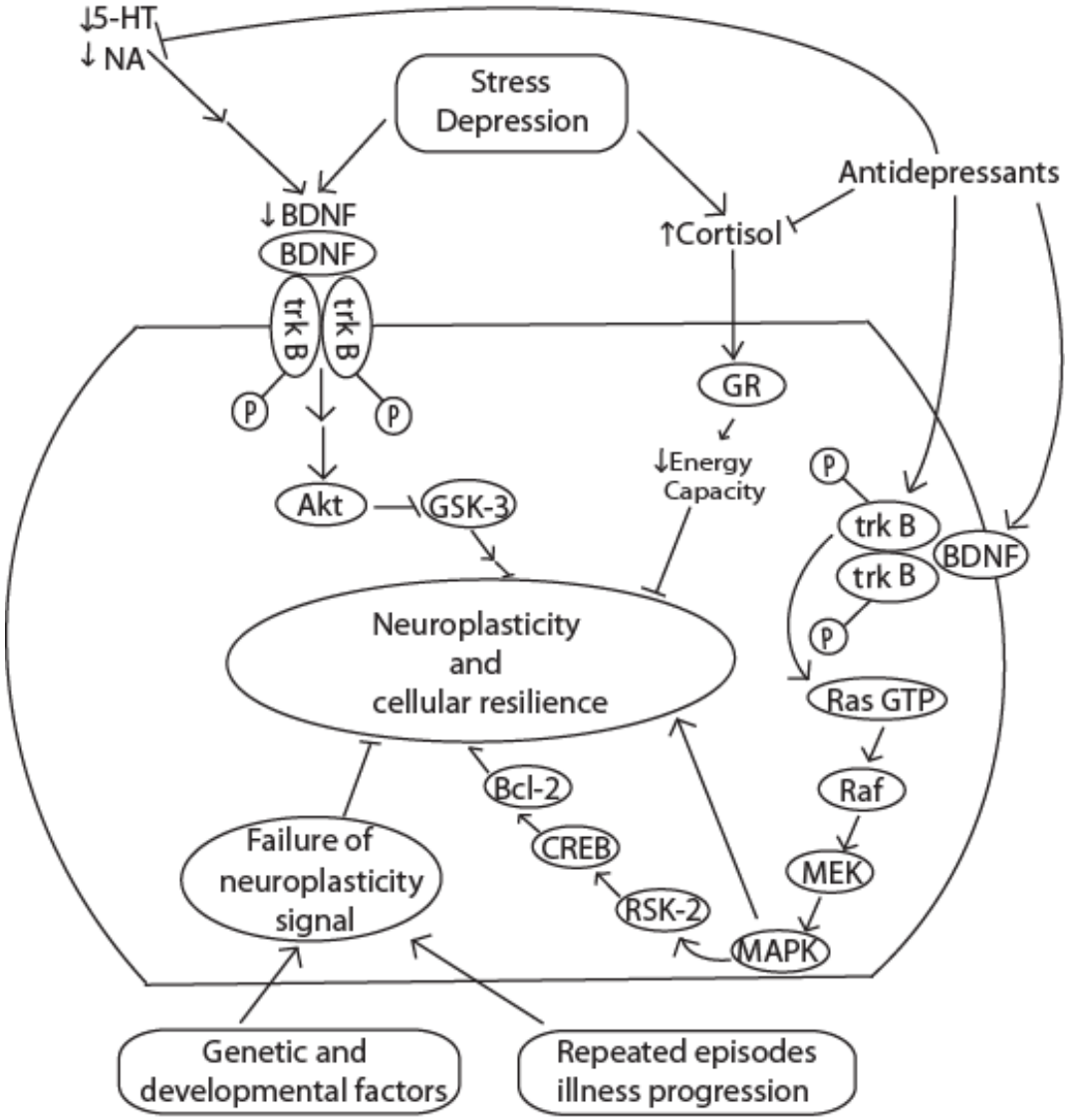


Fig. 1. Neuroplasticity and cellular resilience in mood disorders. This figure depicts the multiple influences on neuroplasticity and cellular resilience in mood disorders. Genetic/neurodevelopmental factors, repeated affective episodes and illness progression might all contribute to the impairments of cellular resilience, volumetric reductions and cell death/atrophy observed in mood disorders. Stress and depression likely contribute to impairments of cellular resilience by a variety of mechanisms, including reductions in the levels of BDNF and increases of cortisol. Neurotrophic factors such as BDNF enhance cell survival by activating two distinct signaling pathways: the PI-3–kinase pathway, and the MAP-kinase pathway. One of the major mechanisms by which BDNF promotes cell survival is by increasing the expression of the major cytoprotective protein, Bcl-2. The chronic administration of a variety of antidepressants increases the expression of BDNF, and its receptor TrkB. BDNF, brain derived neurotrophic receptor; trkB, tyrosine kinase receptor for BDNF; Raf, MEK, components of the MAP kinase pathway; CREB, cyclic AMP responsive element binding protein; Rsk-2, Ribosomal S-6 kinase; GR, glucocorticoid receptor; GSK-3, glucogen synthase kinase. Adapted from Manji HK et al., 2001.

2 GENERAL AIMS

1. To examine whether tianeptine regulates glutamate AMPA receptor phosphorylation in brain slices and in intact animals, by measuring P-Ser^{831}- and P-Ser^{845}-GluA1.
2. To examine the functional relevance of AMPA receptor phosphorylation in behavioural and biochemical responses to chronic treatment with saline or tianeptine using mice bearing point mutations at both Ser^{831}- and Ser^{845}-GluA1.
3. To use hippocampal brain slices and study mechanisms whereby tianeptine increases phosphorylation of GluA1 with an emphasis on the role of MEK/MAPK signaling.
4. To examine the phosphorylation state of a number of receptors, signaling proteins and transcription factors in frontal cortex that could be compromised by acute elevated platform stress.
5. To examine the effects of the structurally similar, but pharmacologically distinct, antidepressants, tianeptine and impramine, on stress-induced alterations in the phosphorylation state of receptors, signaling proteins and transcription factors.
6. To examine whether blockade of the glucocorticoid receptor through mifepristone could prevent the stress-induced down-regulation of MEK phosphorylation.
7. To examine the role of 5-HT$_6$ receptors in mediating biochemical and behavioral actions of fluoxetine indicative of its antidepressive properties. Specifically, we assessed the ability of the 5-HT$_6$ receptor antagonist, SB271046, to modify fluoxetine-induced phospho-Ser^{845}-GluA1 and antidepressant-like behavioral effects in mice.
8. To examine the effects of the 5-HT$_6$ receptor agonist 2-ethyl-5-methoxy-*N*,*N*-dimethyltryptamine (EMDT) on PKA-mediated signaling and antidepressant-like effects in mice.

3 METHODOLOGY

Table 2. Methods used in the present work.

METHODS	PAPERS
SDS-PAGE and Western blotting	I, II, III, IV, V
Studies of intracellular protein phosphorylation	I, II, III, IV, V
Pharmacological stimulation of striatal slices	II, V
Elevated platform stress model	III, IV
Tail suspension test	I, V
Open field test	I, V
In situ hybridization	I
Electrophysiological recordings	III, IV

Pharmacological Treatments

In the various studies adult male mice (C57BL/6 mice) or rats (Sprague-Dawley rats) were given intraperitoneal injections of saline, tianeptine, imipramine, mifepristone (RU486), fluoxetine, SB271046 or EMDT. The doses and duration of treatment is indicated in the individual papers.

Mutant animal models

GluA1 mutant mice (on a C57BL/6 background) bearing point mutations at Ser^{831}-/Ser^{845}-GluA1 were used in Paper I. The generation and basal characterization of these mice have been described elsewhere (Lee et al., 2003).

DARPP-32 knockout and D1 receptor knockout mice (on C57BL/6 background) were used in Paper V. The generation and basal characterization of these mice have been described elsewhere (Fienberg et al., 1998; Xu et al., 1994).

Stress protocol and pharmacological treatments.

The behavioral stress protocol was adapted from Xu et al. (1998) and performed as previously described (Rocher et al., 2004) in Professor Therese Jay´s laboratory. Briefly, rats were placed during 30 min on an elevated and unsteady platform positioned 1 m above the ground. The animals showed behavioral ‘‘freezing’’ (piloerection, immobility for up to 10 min, defecation, urination) while on the platform. At the end of stress, rats were anaesthetized with sodium pentobarbital on the platform and immediately after, placed in their home cage until being sacrificed. Control rats (non-stressed rats) were anaesthetized immediately after transfer from the animal cage. Body temperature was maintained at 37 °C by a homeothermic warming blanket. Groups of stressed and non-stressed rats were also put back in their home cage without being anesthetized. The effect of stress on different phosphorylation sites in anesthetized vs non-anesthetized rats sacrificed 30 min after the end of the stress was examined in a first experiment. In all subsequent experiments, all rats were anesthetized at the end of the stress. In a time course experiment, rats were sacrificed 30, 60, 140 and 180 min after the end of the stress. In a biochemical experiment measuring effects of antidepressants, tianeptine and imipramine were injected intraperitoneally just after the end of stress and the rats sacrificed after an additional 30 min.

In vivo *whole animal studies to measure protein phosphorylation*.

Mice were briefly immobilized in a holder and their skulls exposed to focused microwave irradiation (4.5–5 kW for 1.4 s) using a small animal microwave (Muromachi Kikai, Tokyo, Japan). The brain tissue was then rapidily frozen and stored at -80 °C until analysed.

In vitro *brain slice experiments to measure protein phosphorylation*.

Striatal slices (300µm) were prepared from mice using a Leica Vibratome. The slices were preincubated in Krebs buffer (126mM NaCl, 2.5mM KCl, 1.2mM NaH_2PO_4,

1.3mM $MgCl_2$, 2.4mM $CaCl_2$, 10mM glucose and 26mM $NaHCO_3$, pH 7.4) at 30°C under constant oxygenation (95%O2/5%CO2) for 60 min, with a change of buffer after 30 min. The slices were then treated with different drugs for different time periods. After drug treatment, the buffer was removed and the slices were rapidly frozen on dry ice and stored at –80°C until assayed.

Immunoblotting.

Frozen tissue samples from the *in vitro* and *in vivo* experiments were sonicated in 1% SDS and boiled for 10 min. Small aliquots of the homogenate were retained for protein determination by the bicinchoninic acid protein assay method (Pierce, Stockholm, Sweden). Equal amounts of protein were processed using 12% acrylamide gels. Immunoblotting was performed with phosphorylation state-specific antibodies against Ser^{831}- or Ser^{845}-GluA1, Ser^{896}- or Ser^{897}-NR1, Ser^{1303}-NR2B, $Tyr^{490/515}$-TrkA/B, $Ser^{217/221}$-MEK, Thr^{183}/Tyr^{185}-p42MAPK, Thr^{202}/Tyr^{204}-p44MAPK, Thr^{180}/Tyr^{182}-p38MAPK, Thr^{218}/Tyr^{220}-ERK5, Thr^{183}/Tyr^{185}-SAPK, Ser^{383}-ELK-1, Thr^{308}-AKT, Ser^{473}-AKT, Ser^{9}-GSK3b, Thr^{286}-CamKIIa, Thr^{286}-CamKIIb, Ser^{133}-CREB, Ser^{63}-ATF-1, Thr^{34}-DARPP-32, Thr^{75}-DARPP-32 or antibodies that are not phosphorylation state specific against total BDNF, GluA1, NR1, NR2B, TrkB, MEK, ERK5, SAPK, p38, p42/44-MAPK, ELK-1, AKT, GSK3b, CamKII, CREB, ATF-1, DARPP-32. Antibody binding was detected by enhanced chemiluminescence and quantified by densitometry using NIH Image 1.61 software. Data on protein phosphorylation are expressed as percentage of control.

Statistics.

When two groups were compared, Student´s T-test was used. When more than two groups were compared, one-way ANOVA was used. When a statistical difference was found, pairwise comparisons were made with Newman-Keul´s, Duncan or Dunnett´s tests. Statistical difference was set at $p<0.05$.

4 RESULTS

4.1 PHOSPHORYLATION STUDIES IN GLUA1-PHOSPHOMUTANT MICE UNDER BASELINE CONDITIONS AND IN RESPONSE TO ACUTE OR CHRONIC TREATMENT WITH TIANEPTINE (PAPER I)

4.1.1 Regulation of AMPA receptor phosphorylation in vivo by acute or chronic treatment with tianeptine

In paper I, we investigated whether tianeptine can regulate the phosphorylation state of the GluA1 subunit of the AMPA receptor at two different phosphorylation sites known to affect the functional properties of AMPA receptors, namely Ser^{831}-GluA1, a PKC / CamKII site, and Ser^{845}-GluA1, a PKA site. The results shown in Fig. 2 demonstrate that acute treatment with tianeptine tended to increase GluA1 phosphorylation at Ser^{831} or Ser^{845} (Fig. 2A and B). However, following chronic administration, tianeptine significantly increased phospho-Ser^{831}-GluA1 and subsequent pairwise analyses showed significant changes in frontal cortex and in the CA3 region of hippocampus (Fig. 2C). Tianeptine also significantly increased phospho-Ser^{845}-GluA1 and subsequent pairwise analyses showed significant changes in the CA3 region of hippocampus (Fig. 2D).

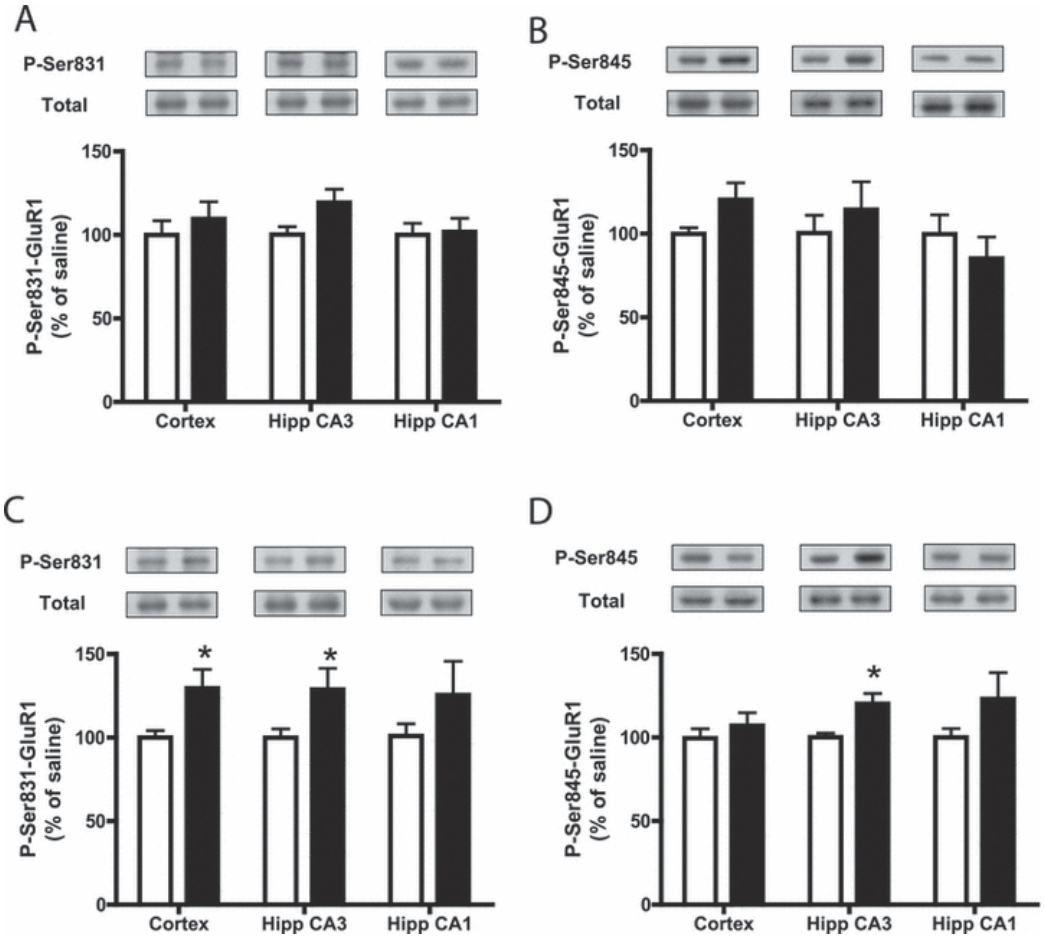


Fig. 2. Regulation of AMPA receptor phosphorylation by tianeptine. The amounts of (A and C) phospho-Ser^{831}-GluR1 and (B and D) phospho-Ser^{845}-GluR1 were measured in the frontal cortex and in the CA3 and CA1 regions of hippocampus from animals treated (A and B) acutely or (C and D) chronically with saline (white bars) or tianeptine (black bars). Chronic treatment with tianeptine increased phospho-Ser^{831}-GluR1 in both the frontal cortex and the CA3 region of hippocampus and phospho-Ser^{845}-GluR1 in the CA3 region of hippocampus. Data are means ± SEM for n=8–16 animals. ANOVA followed by Newman–Keul's test for pairwise comparisons; *P < 0.05 compared with saline-treated animals.

4.1.2 Behavioural effects of saline and tianeptine in wild-type and GluA1-phosphomutant mice

As chronic treatment with tianeptine had a significant effect on phosphorylation of GluA1 subunits, we studied the behavioural effects of chronic treatments with saline or tianeptine. It was found that saline-treated phosphomutant GluA1 mice exhibited significantly longer latency to enter the centre of the open field (Fig. 3A), results

which can be interpreted as an anxiety-like phenotype in the phosphomutant GluA1 mice. This phenotype could not be explained by a general reduction in locomotion as saline-treated wild-type mice and phosphomutant GluA1 mice showed similar locomotor behaviour (Fig. 3B). Immobility in an escapeable stress situation, as measured in the tail-suspension test, was significantly increased in the saline-treated phosphomutant GluA1 mice (Fig. 3C), indicating a depression-like phenotype. Like the saline-treated phosphomutant GluA1 mice, tianeptine-treated phosphomutant GluA1 mice exhibited an increased latency to enter the centre of the open field (Fig. 3A). The tianeptine-treated wild-type mice showed an increased locomotion in the open field (Figs. 3B, D and E). The stimulatory effect of tianeptine was significantly attenuated in the phosphomutant GluA1 mice (Fig. 3B, D and E), demonstrating an involvement of phosphorylated Ser^{831}-/Ser^{845}-GluA1 residues in mediating this action of tianeptine. The tianeptine-treated wild-type mice also showed a reduced immobility in the tail-suspension test compared to the saline-treated mice (Fig. 3C).

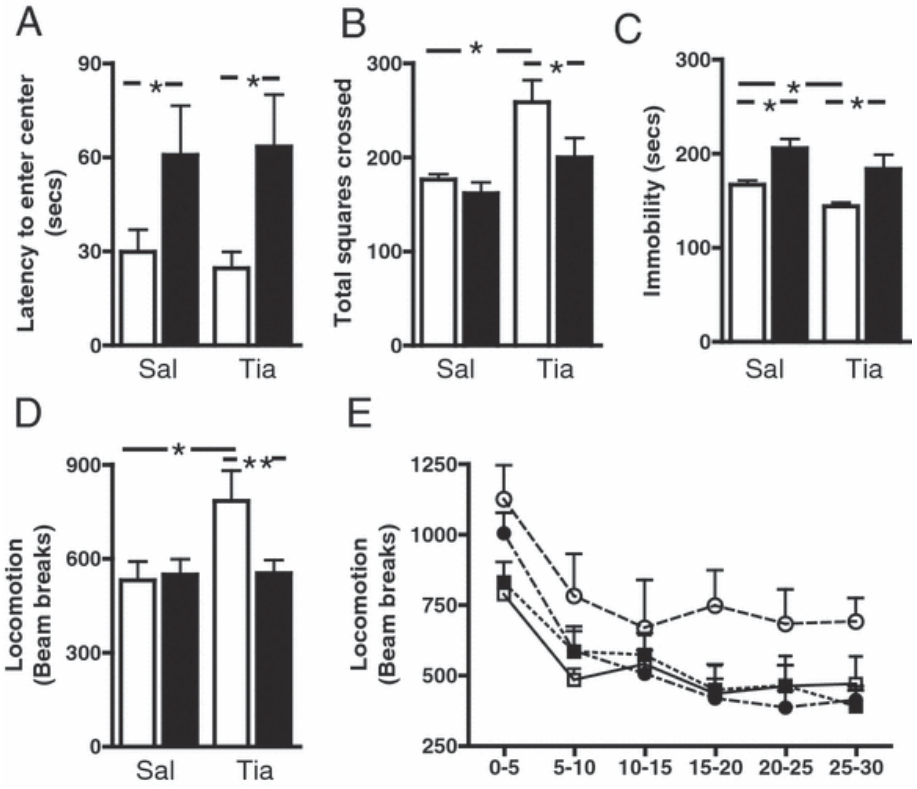


Fig. 3. Behavioural effects of wild-type mice (white bars) and the GluA1-phosphomutant counterparts

(black bars) treated with saline and following chronic tianeptine treatment. (A and B) In the open-field experiments, the latency of the mice to enter the centre of the arena (A) and the overall locomotion (B) were measured manually. (C) In the tail-suspension test, the immobility was scored manually. (D and E) Open-field locomotion was also studied using an automated procedure in locomotor boxes. GluA1-phosphomutant mice treated with saline exhibited increased latency to enter the centre of an open field and increased immobility in the tail-suspension test compared to their wild-type counterparts. Chronic tianeptine treatment increased open-field locomotion and reduced immobility in wild-type mice, but not in phosphomutant GluR1 mice. Data are means ± SEM for n=9–12 animals. ANOVA followed by Newman–Keul's test for pairwise comparisons; *P < 0.05, **P < 0.01 between indicated groups.

4.1.3 Phosphorylation of CREB and CamKII in GluA1-phosphomutant mice under baseline conditions and in response to tianeptine

We compared the phosphoryation states of CREB and CamKII in wild-type and GluA1-phosphomutant mice under baseline conditions. An increased level of P-Ser^{133}-CREB, but not of P-Thr^{286}-CamKII, was found in extracts from frontal cortices in phosphomutant GluA1 mice compared to wild-type mice (Fig. 4B and C). There were no significant effects on phosphorylation of CREB or CamKII in extracts from hippocampus or striatum from phosphomutant GluA1 mice.

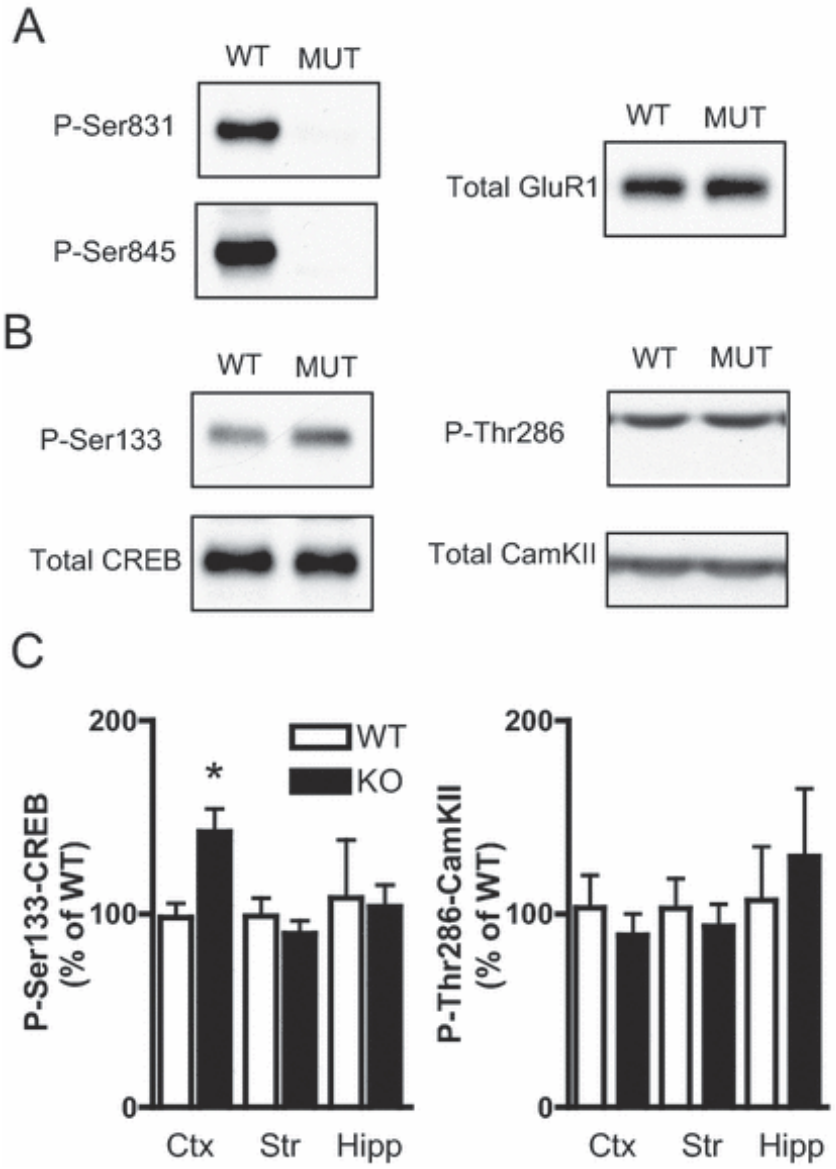


Fig. 4. Levels of phosphorylated and total GluR1, CREB and CamkII in wild-type mice (white bars) and their GluR1-phosphomutant counterparts (black bars). (A) Phospho-Ser^{831}-GluR1 and phospho-Ser^{845}-GluR1 were not detected in the GluR1-phosphomutant mice, but normal levels of total GluR1 were found (B and C). Increased levels of phospho-Ser^{133}-CREB, but not total CREB, were found in samples from frontal cortex. No changes in phospho-Thr^{286}-CamKII were found. Data are means ± SEM for n=6 animals. Unpaired, two tailed, Student's t-test; *P < 0.05 compared with wild-type mice.

Following treatment with tianeptine, significant changes between the treatment groups on P-Ser^{133}-CREB were found in frontal cortex (Fig. 5A), the CA3 region of hippocampus (Fig. 5C) and the CA1 region of hippocampus (Fig. 5E). No significant changes were found in total CREB levels (Figs. 5B, D and F). Phosphomutant GluA1 mice treated with saline exhibited higher P-Ser^{133}-CREB levels in frontal cortex than

their wild-type counterparts (Fig. 5A). Chronic tianeptine treatment had no effect on P-Ser^{133}-CREB in the frontal cortex in wild-type mice but counteracted the increase in P-Ser^{133}-CREB found in the saline-treated phosphomutant GluA1 mice (Fig. 5A). Phosphomutant GluA1 mice treated with saline exhibited lower P-Ser^{133}-CREB levels in the CA3 region of hippocampus than their wild-type counterparts (Fig. 5C). Chronic tianeptine treatment decreased P-Ser^{133}-CREB in the CA3 region of hippocampus in wild-type, but not phosphomutant GluA1, mice (Fig. 5C). Chronic tianeptine treatment increased P-Ser^{133}-CREB in the CA1 region in wild-type mice (Fig. 5E) but not in the phosphomutant GluA1 mice (Fig. 5E).

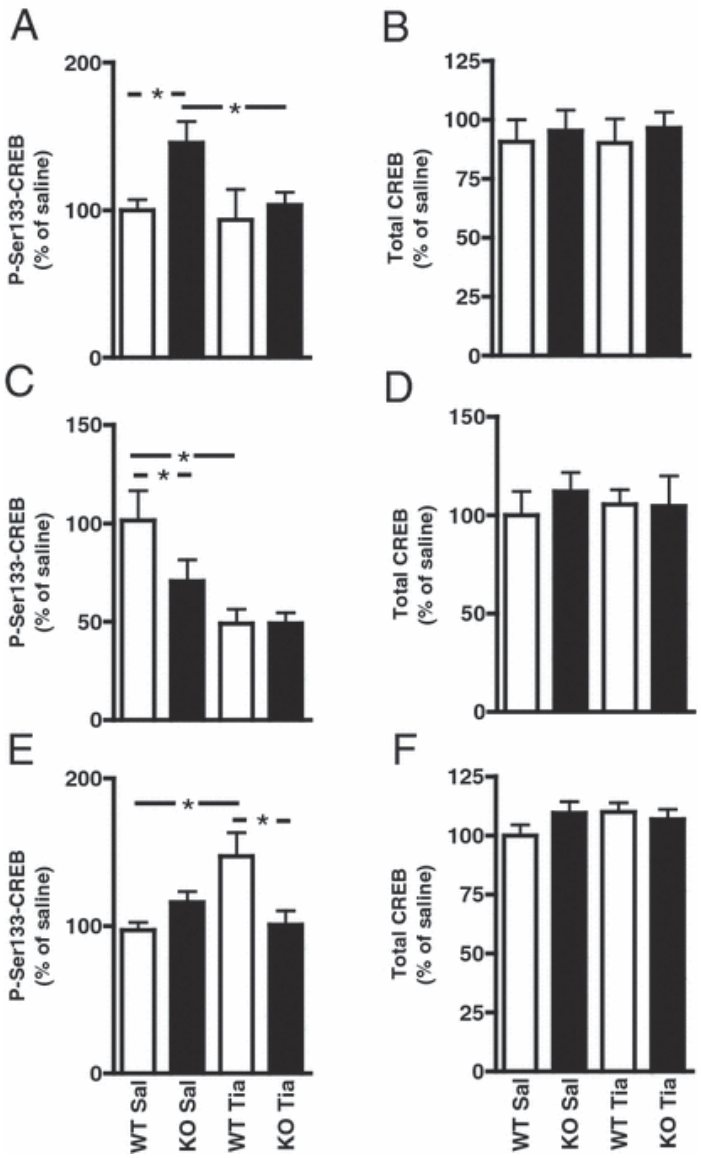


Fig. 5. Levels of phosphorylated and total CREB in wild-type mice (white bars) and their GluR1-

phosphomutant counterparts (black bars) in response to chronic treatment with saline or tianeptine. The amounts of (A, C and E) P-Ser^{133}-CREB and (B, D and F) total CREB were measured in (A and B) the frontal cortex and (C and D) the CA3 and (E and F) the CA1 regions of hippocampus. In saline-treated phosphomutant GluA1 mice, higher levels of P-Ser^{133}-CREB were found in frontal cortex but lower levels in the CA3 region of hippocampus. Treatment with tianeptine decreased P-Ser^{133}-CREB in the CA3 region of hippocampus and increased P-Ser^{133}-CREB in the CA1 region in wild-type mice but not in phosphomutant GluA1 mice. Data are means ± SEM for n=7–10 animals per group. One-way ANOVA followed by Newman–Keul's test for pairwise comparisons; *P < 0.05 between indicated groups.

4.2 TIANEPTINE INCREASES PHOSPHORYLATION OF AMPA RECEPTORS AND POTENTIATES MAPK SIGNALLING IN HIPPOCAMPAL SLICES (PAPER II)

4.2.1 Effects of tianeptine on phosphorylation at Ser^{831}-GluA1, Ser^{845}-GluA1, Ser^{896}-NR1 and Ser^{897}-NR1 in hippocampal brain slices

In agreement with previous data found in whole animals (Svenningsson et al., 2007; Qi et al., 2009), we found that tianeptine caused a rapid and significant increase in the phosphorylation of Ser^{831}- and Ser^{845}-GluA1 (Fig. 6A). Meanwhile, tianeptine had no effects on the phosphorylation state of the other major ionotropic glutamate receptor, the NMDA receptor, at the Ser^{896}- and Ser^{897}-NR1 residues (Fig. 6B). Tianeptine had no effects on the levels of total GluA1 or NR1 at any of the timepoints studied (Figs 6A and B).

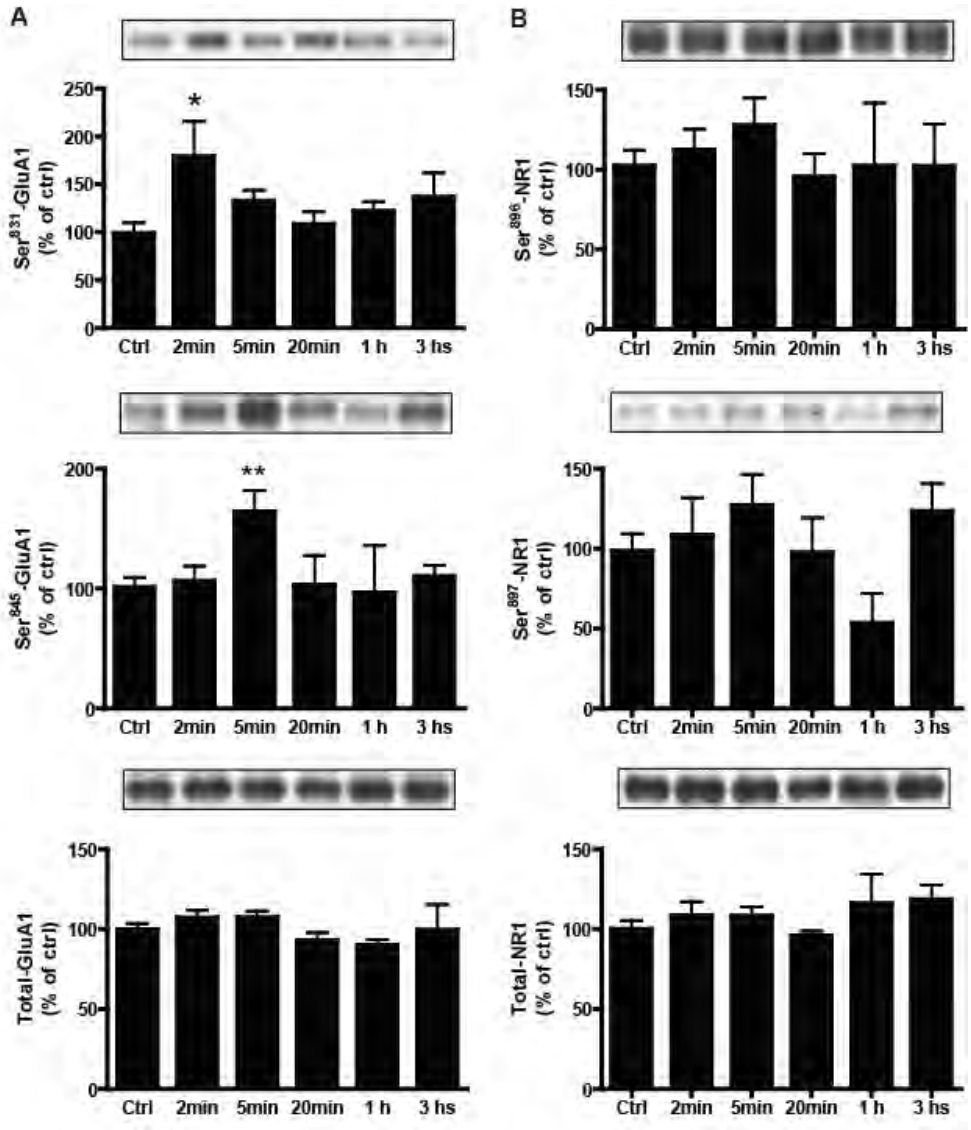


Fig. 6. Time-course study on the effects of tianeptine on P-GluA1 and P-NR1. (A) Immunoblots against P-Ser^{831}-GluA1, P-Ser^{845}-GluA1 and total GluA1 and (B) P-Ser^{896}-NR1, P-Ser^{897}-NR1 and total NR1 in control slices and in hippocampal slices treated with tianeptine (50 μM) for 2, 5, 20, 60 and 180 mins. Histograms show the quantifications of P-Ser^{831}-GluA1, P-Ser^{845}-GluA1, total GluA1, P-Ser^{896}-NR1, P-Ser^{897}-NR1 and total NR1 levels, respectively. Data were normalized to total levels for each of these proteins. *$P < 0.05$, **$P < 0.01$ vs control; one-way ANOVA followed by Dunnett´s test.

4.2.2 Effects of tianeptine on phosphorylation at Ser$^{217/221}$-MEK, Thr^{183}/Tyr^{185}-p42MAPK, Thr^{202}/Tyr^{204}-p44MAPK, Thr^{286}-CamKII and Ser^{133}-CREB in hippocampal brain slices

In accordance with the data we previously found in whole rats (Qi et al., 2009), tianeptine caused rapid and significant increases in the phosphorylation of Ser$^{217/221}$-MEK and Thr^{183}/Tyr^{185}-p42MAPK (Figs. 7A and C). We also found a delayed decrease of Thr^{202}/Tyr^{204}-p44MAPK after 180 mins (Fig. 7B).

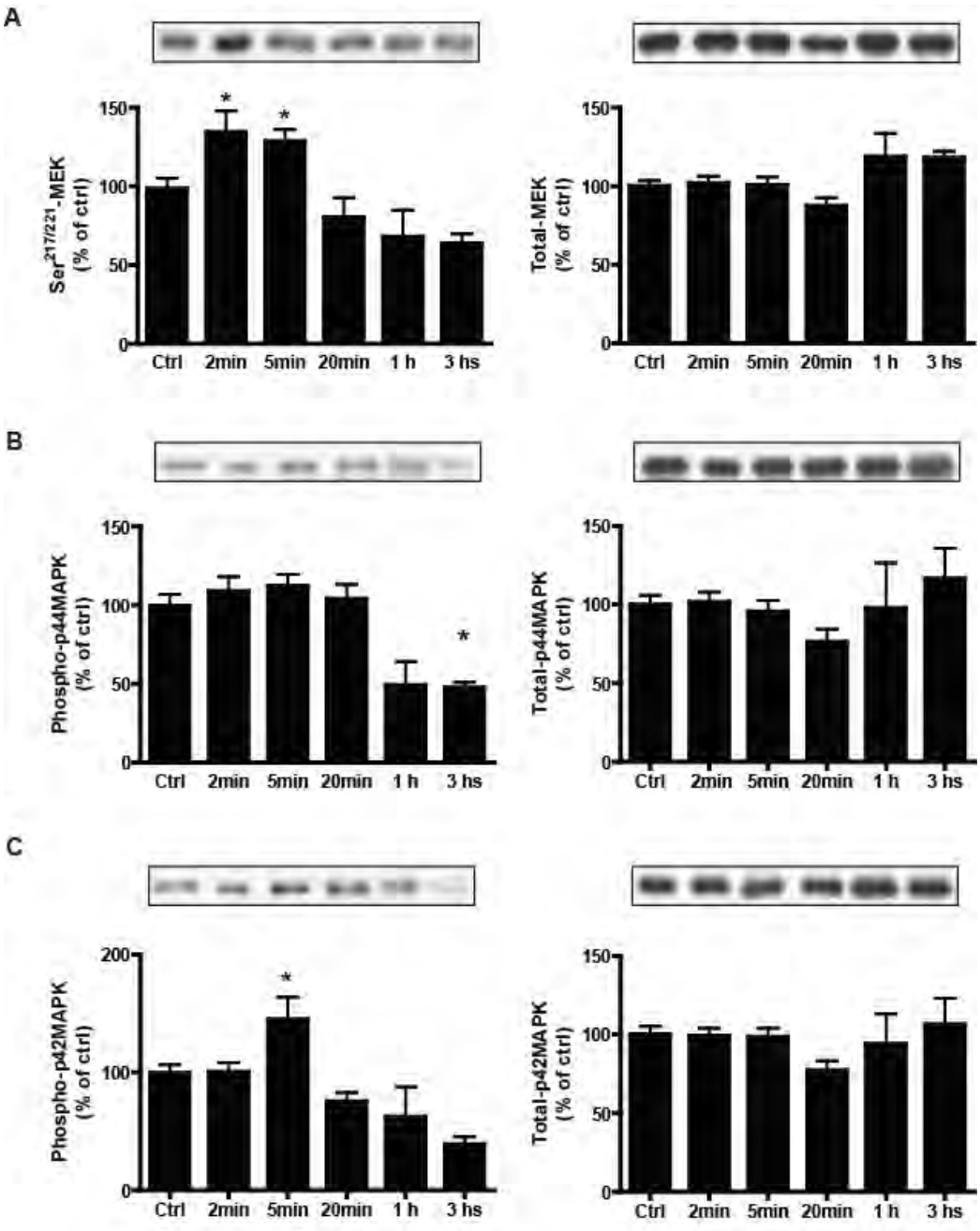


Fig. 7. Time-course study on the effects of tianeptine on P-MEK and P-p42/44MAPK. (A) Immunoblots against P-Ser$^{217/221}$-MEK, and total MEK, (B) P-p42MAPK and total p42MAPK and (C) P-p44MAPK and total p44MAPK in control hippocampal slices and in slices treated with tianeptine (50 μM) for 2, 5, 20, 60 and 180 mins. Histograms show the quantifications of P-Ser$^{217/221}$-MEK, total MEK, P-p42MAPK, total p42MAPK, P-p44MAPK and total p44MAPK levels, respectively. Data were normalized to total levels for each of these proteins. *P < 0.05 versus control; one-way ANOVA followed by Dunnett´s test.

In addition to GluA1 subunits and MEK/MAPK signaling, CamKII and CREB are known to modulate synaptic plasticity. We found that tianeptine caused a no significant increase in the phosphorylation of Thr^{286}-CamKII neither at the alpha or

beta subunits (Fig. 8A). A more delayed regulation of CREB by tianeptine was found as this compound caused a significant decrease in phosphorylation of Ser^{133}-CREB after 20 mins (Fig. 8B). This regulation of CREB is consistent with the fact that CREB is a transcription factor that regulates long-term effects on synaptic plasticity.

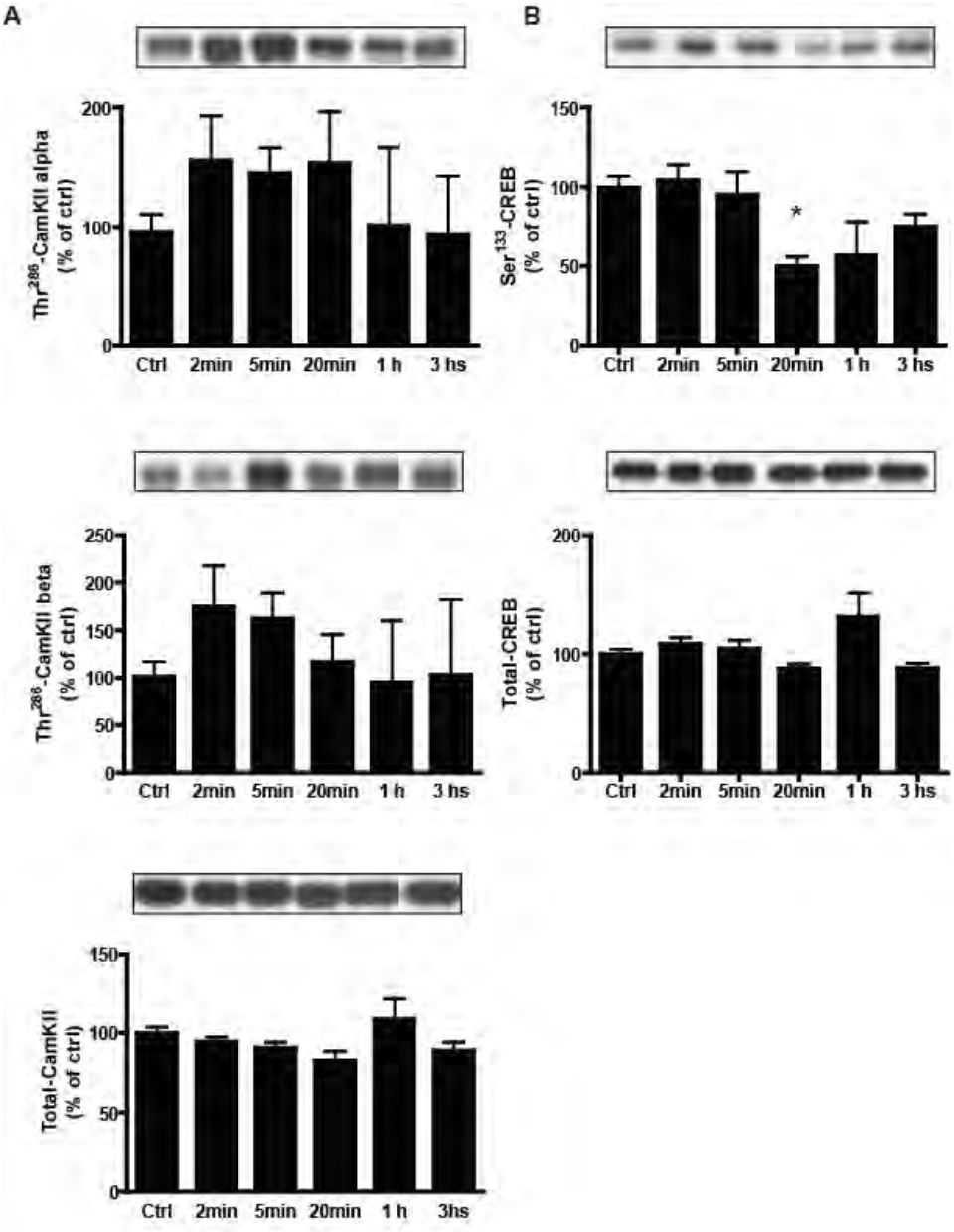


Fig. 8. Time-course study on the effects of tianeptine on P-CamKII and P-CREB. (A) Immunoblots against P-Thr^{286}-CamKII alpha, P-Thr^{286}-CamKII beta and total CamKII and (B) P-Ser^{133}-CREB and total CREB in control hippocampal slices and in slices treated with tianeptine (50 μM) for 2, 5, 20, 60 and 180 mins. Histograms show the quantifications of P-Thr^{286}-CamKII alpha, P-Thr^{286}-CamKII beta, total CamKII, P-Ser^{133}-CREB and total CREB levels, respectively. Data were normalized to total levels for each of these proteins. *P < 0.05 versus control; one-way ANOVA followed by Dunnett´s test.

4.2.3 Effects of the MEK inhibitor, PD184161, and subsequent treatment of tianeptine on the phosphorylation state of Thr^{183}/Tyr^{185}-p42MAPK in hippocampal brain slices

As expected, treatment with the MEK inhibitor, PD184161, caused a reduction in the basal phosphorylation state of Thr^{183}/Tyr^{185}-p42MAPK (Fig. 9) and Thr^{202}/Tyr^{204}-p44MAPK (data not shown). Moreover, the ability of tianeptine to increase phosphorylation at Thr^{183}/Tyr^{185}-p42MAPK was abolished (Fig. 9).

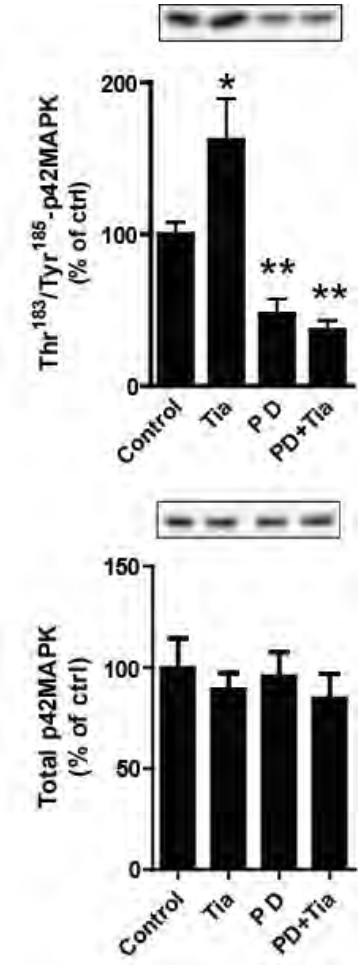


Fig. 9. Effects of MEK inhibition on basal and tianeptine-induced regulation of P-p42MAPK. Immunoblots and histograms showing P-p42MAPK and total p42MAPK in control hippocampal slices and in slices treated with PD184161 (10 μM) and/or tianeptine (50 μM). *$P < 0.05$, **$P < 0.01$ vs control; two-way ANOVA followed by Bonferroni´s test.

4.2.4 Effects of PD184161 and subsequent treatment of tianeptine on the phosphorylation state of Ser^{831}-GluA1, Ser^{845}-GluA1, Ser^{896}-NR1 and Ser^{897}-NR1 in hippocampal brain slices

Treatment with the MEK inhibitor, PD184161, had no effects on the basal phosphorylation levels of neither Ser^{831}-GluA1, Ser^{845}-GluA1, Ser^{896}-NR1 nor Ser^{897}-

NR1 (Fig. 10). Moreover, PD184161 did not significantly affect the ability of tianeptine to increase Ser^{831}-GluA1 phosphorylation (Fig. 10).

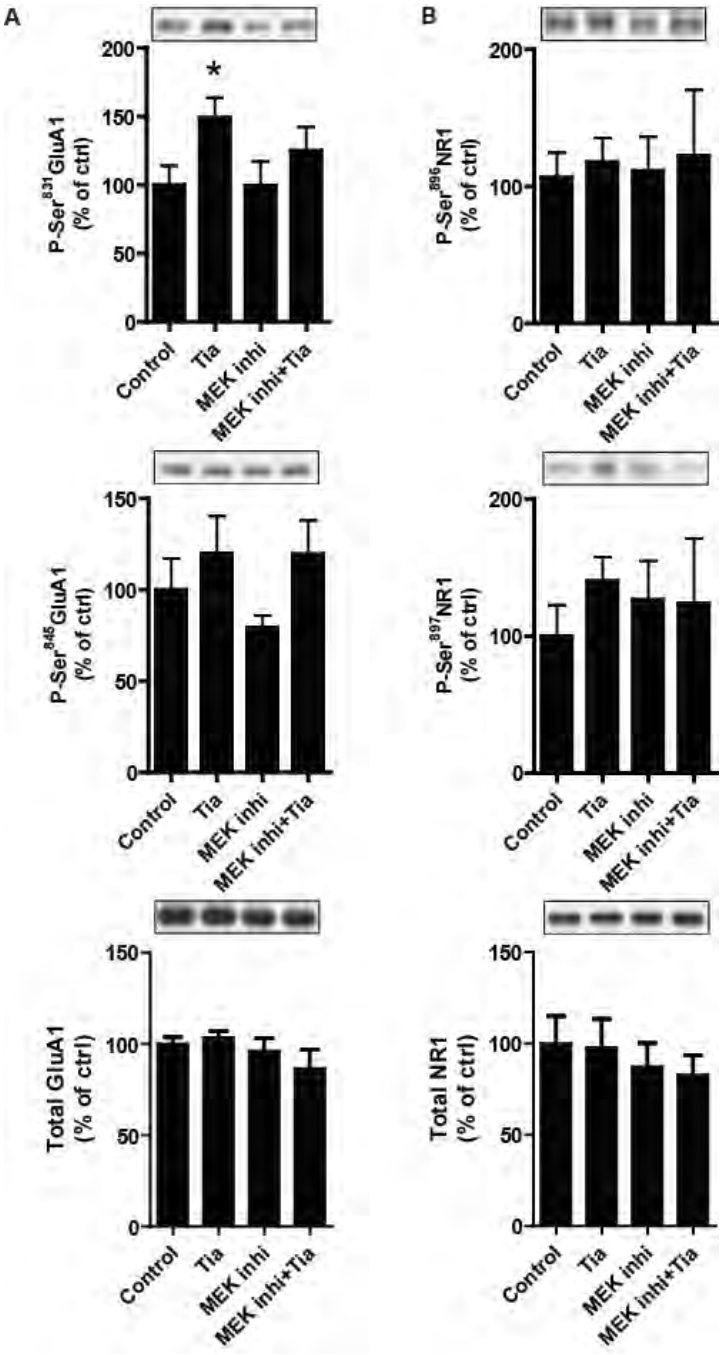


Fig. 10. Effects of MEK inhibition on basal and tianeptine-induced regulation of P-GluA1 and P-NR1. Immunoblots showing (Left side) P-Ser^{831}-GluA1, P-Ser^{845}-GluA1, total GluA1 and (Right side) P-Ser^{896}-NR1, P-Ser^{897}-NR1, total NR1 in control hippocampal slices and in slices treated with PD184161 (10 μM) and/or tianeptine (50 μM). *P < 0.05 vs control; two-way ANOVA followed by Bonferroni´s test.

4.3 ANTIDEPRESSANTS REVERSE THE ATTENUATION OF THE NEUROTROPHIC MEK/MAPK CASCADE IN FRONTAL CORTEX BY ELEVATED PLATFORM STRESS (PAPER III)

As described above the behavioural stress protocol used in this thesis was adapted from Xu et al. (1998) and performed in Professor Therese Jay´s laboratory. Rats were placed on an elevated and unsteady platform (21 × 20 cm^2, 1 m above ground level) for 30 min (Fig. 11). The animal showed behavioural 'freezing' — i.e. piloerection, immobility for up to 10 min, defecation and sometimes urination — while on the platform. At the end of stress, rats were anaesthetized (sodium pentobarbital) on the platform. This was done so that animals could be placed in a stereotaxic frame and electrophysiological recordings could be made. LTP was induced within 180 min after the end of stress. Control nonstressed rats were anaesthetized immediately after transfer from the animal house.

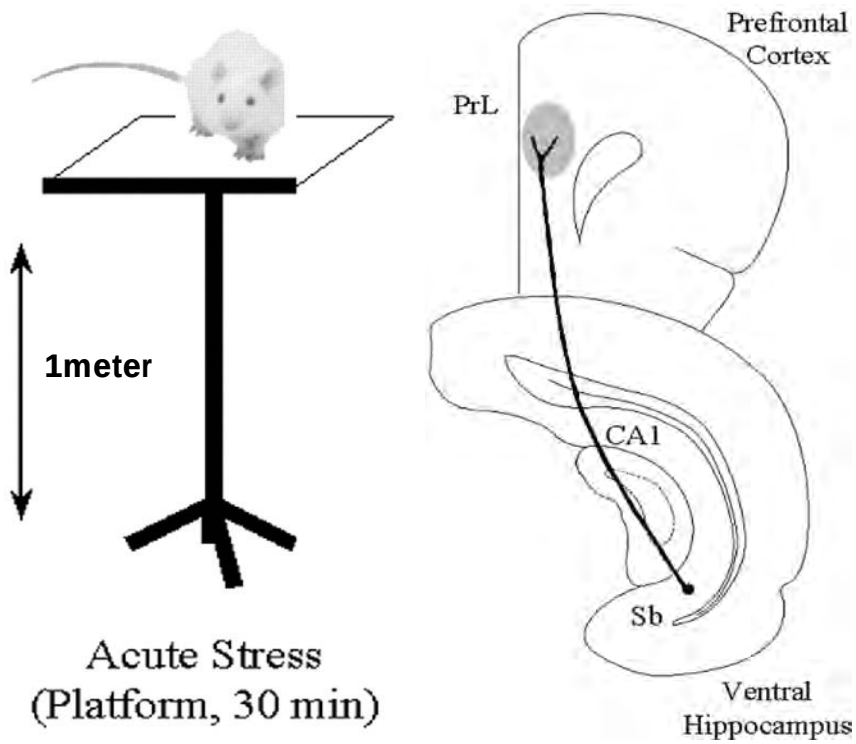


Fig. 11. Illustration of the acute stress protocol and schematic representation of the direct hippocampal projection to the PFC in rats. Glutamatergic neurons of the CA1/subicular region in the ventral hippocampus project directly to the prelimbic area (PrL) of the PFC. Sb, subiculum.

4.3.1 Influence of pentobarbital anesthesia on stress-induced changes in protein phosphorylation

Since the electrophysiological experiments were performed in anesthetized rats, and we wanted to compare our biochemical data with the electrophysiological data, we investigated whether pentobarbital anesthesia would influence stress-induced alterations in protein phosphorylation in rats sacrificed 30 min after stress. Reductions of $Ser^{217/221}$-MEK and Ser^{1303}-GluN2B were found in both anesthetized and non-anesthetized rats (Fig. 12). There were also reductions in the levels of Thr^{183}/Tyr^{185}-p42MAPK, Thr^{202}/Tyr^{204}-p44MAPK and Thr^{286}-CamKIIb in the non-anesthetized, and with trends at least for Thr^{183}/Tyr^{185}-p42MAPK and Thr^{202}/Tyr^{204}-p44MAPK, to be reduced also in anesthetized rats (Fig. 12).

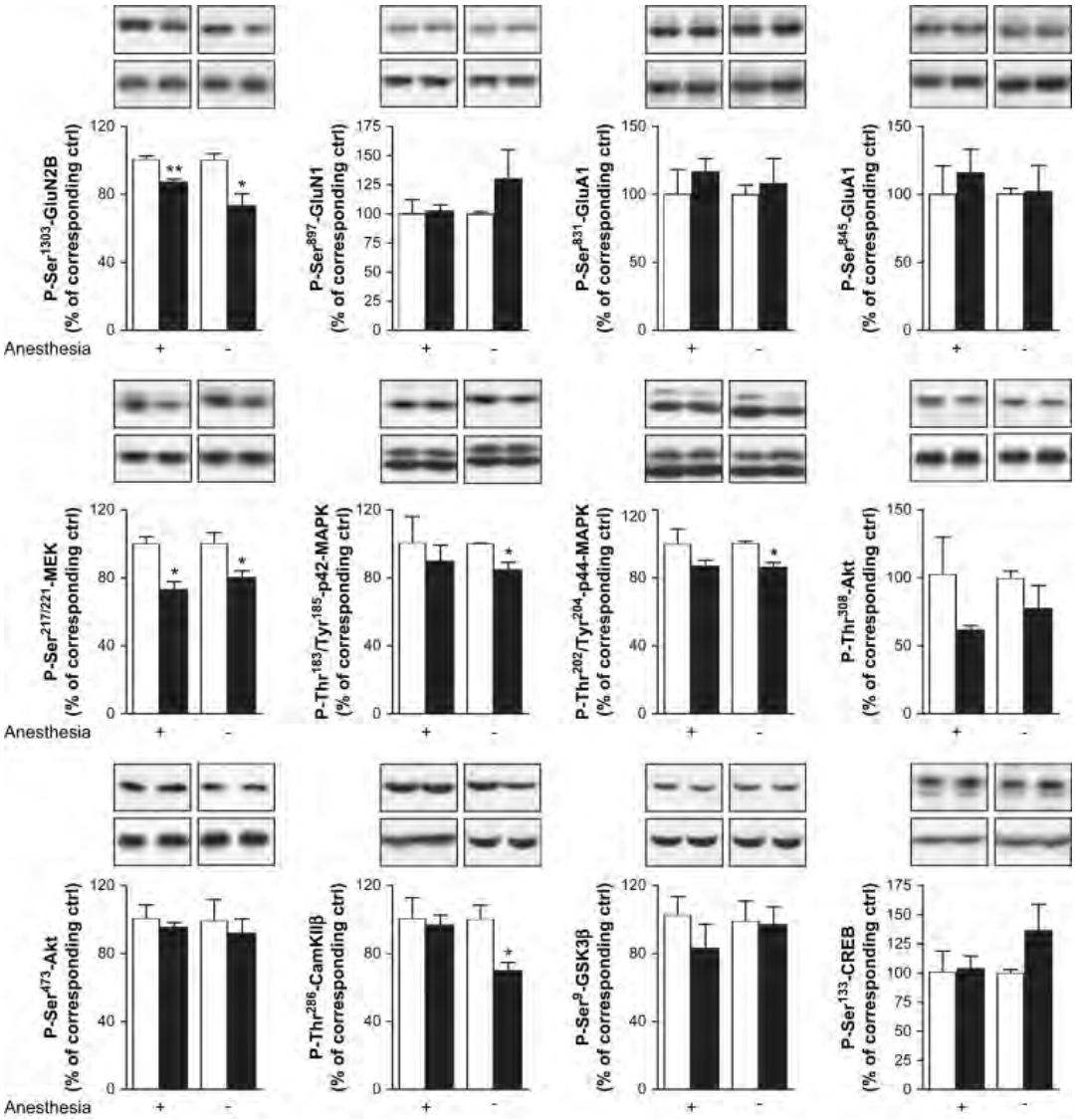


Fig. 12. Immunoblots and histograms showing the regulation of multiple phosphorylation pathways after 30 min in anesthetized (right) and non-anesthetized (left) rats after control conditions (white bars) or elevated platform stress (black bars). Upper immunoblots illustrates the phosphorylated form of the protein, whereas lower immunoblots illustrate the total protein. The number of animals per group is 5. Unpaired, two-tailed, Student's T-test. *$p < 0.05$, compared with corresponding control group.

4.3.2 Regulation of multiple phosphorylation pathways at different timepoints after elevated platform stress

To identify biochemical alterations induced by elevated platform stress, a time course experiment was performed. In this experiment, rats were sacrificed 30, 60, 140 or 180 min after the end of exposure to the elevated platform stress.

Thirty minutes after the end of the stress there was a reduction in the

phosphorylation state of Ser^{1303}-GluN2B that thereafter returned to control levels (Fig. 13). A total of 140 min after the end of the stress there was a reduction in the levels of P-Tyr$^{490/515}$-TrkA/B. No significant changes in the phosphorylation states of Ser^{831}- or Ser^{845}-GluA1, Ser^{896}- or Ser^{897}-GluN1 could be detected at any of the studied timepoints.

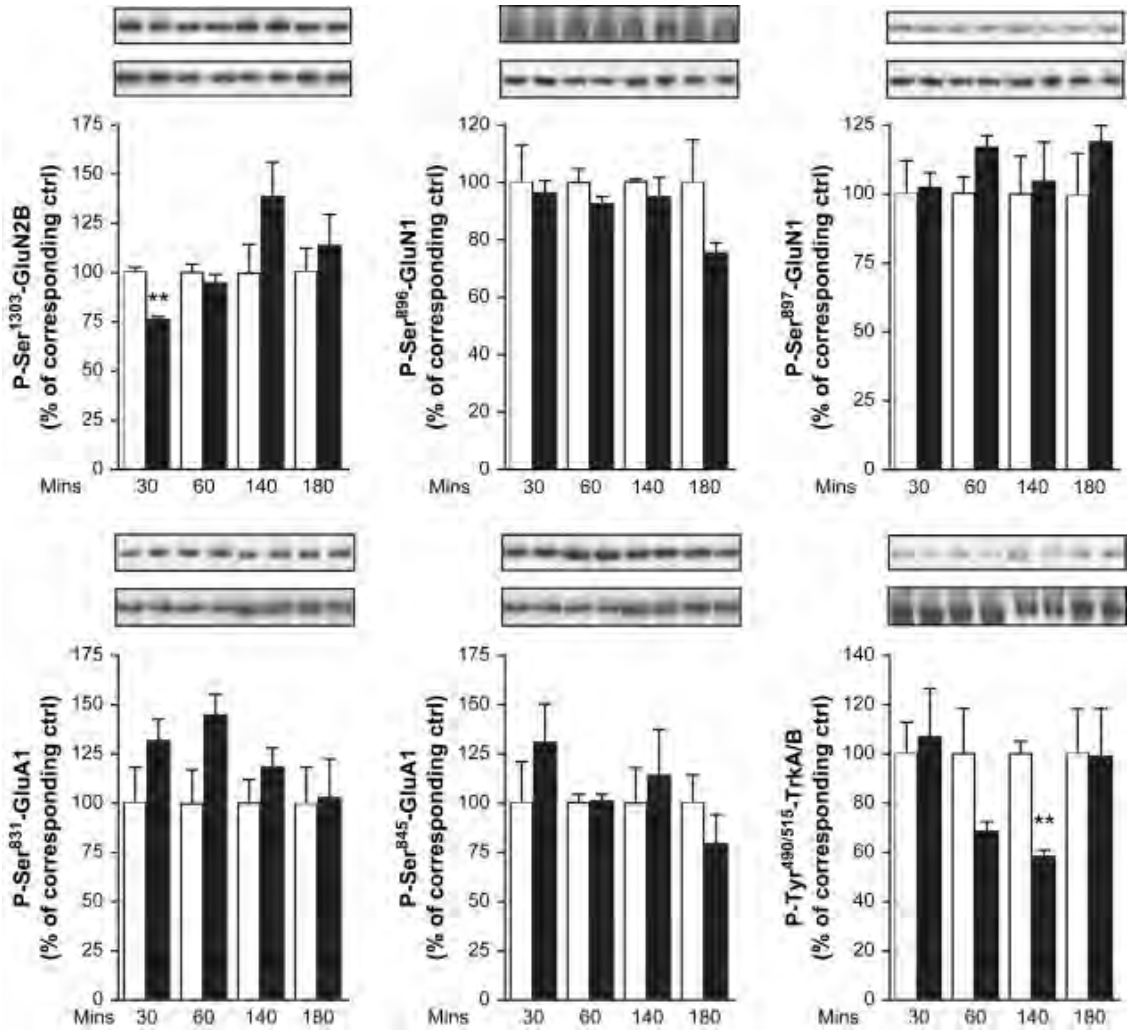
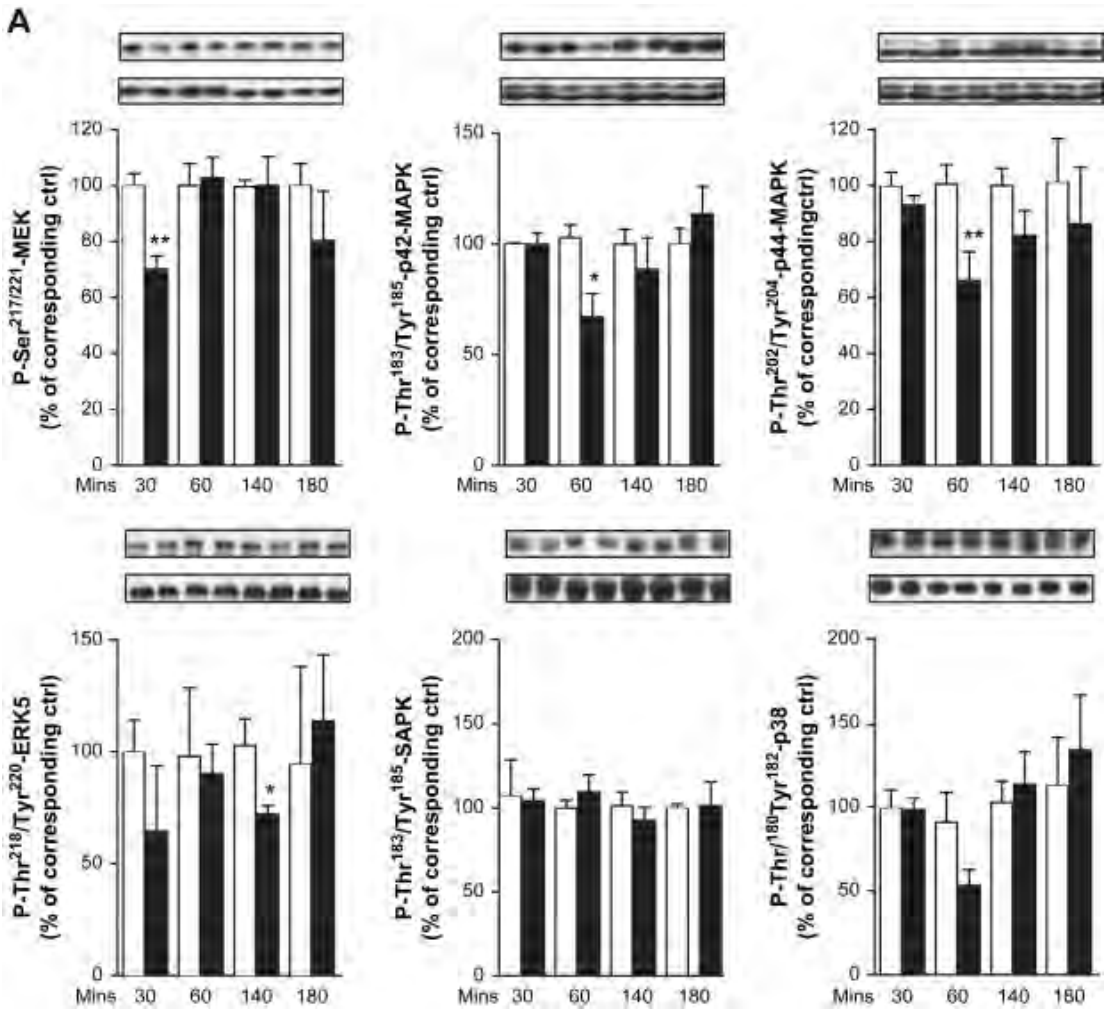


Fig. 13. Immunoblots and histograms showing the phosphorylation state at multiple receptor subunits 30, 60, 140 and 180 min after control conditions (white bars) or elevated platform stress (black bars). Upper immunoblots illustrates the phosphorylated form of the protein, whereas lower immunoblots illustrate the total protein. The number of animals per group is 5. Unpaired, two-tailed, Student's T-test. **p < 0.01, compared with corresponding control group.

We also studied the phosphorylation state of additional member of the MAPK signaling family representing other signaling cascades. It was found that elevated

platform stress reduced Thr^{218}/Tyr^{220}-ERK5 after 140 min, but had no significant effects on Thr^{180}/Tyr^{182}-p38MAPK or Thr^{183}/Tyr^{185}-SAPK (Fig. 14A). In contrast to the reductions in the phosphorylation of proteins involved in the MAPK signaling cascade, there was increased phosphorylation levels of Thr^{308}-AKT 140 and 180 min after the end of the stress (Fig. 14B). No significant changes of Ser^{473}-AKT, Ser^{9}-GSK3b or Thr^{286}-CamKIIb were found at any of the studied timepoints (Fig. 14B).



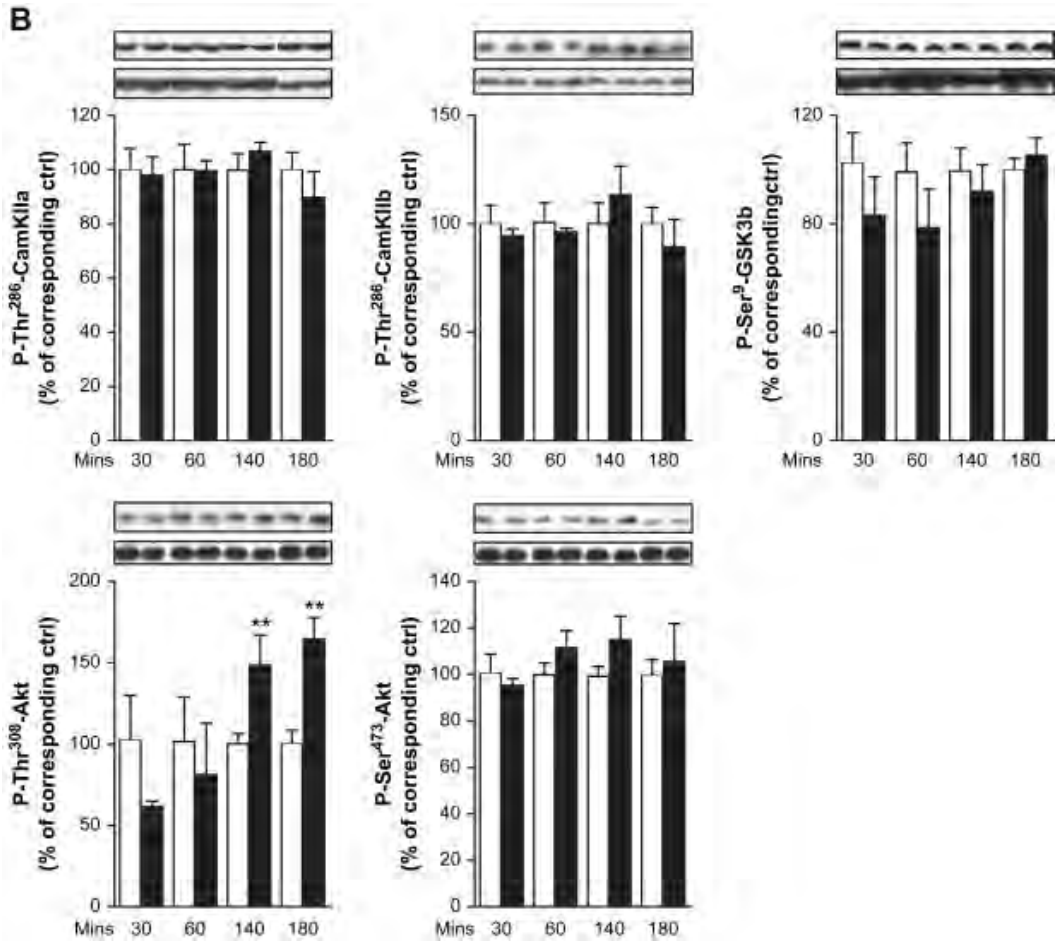


Fig. 14. (A, B) Immunoblots and histograms showing the phosphorylation state at different members of the MAPK signaling pathway (A) and CamKII, GSK-3 and AKT (B) 30, 60,140 and 180 min after control conditions (white bars) or elevated platform stress (black bars). Upper immunoblots illustrates the phosphorylated form of the protein, whereas lower immunoblots illustrate the total protein. The number of animals per group is 5. Unpaired, two-tailed, Student's T-test. *p < 0.05; **p < 0.01, compared with corresponding control group.

There was an increase Ser^{63}-ATF-1 140 min after the end of the stress (Fig. 15). No changes in the phosphorylation state of Ser^{133}-CREB after the elevated platform stress was found.

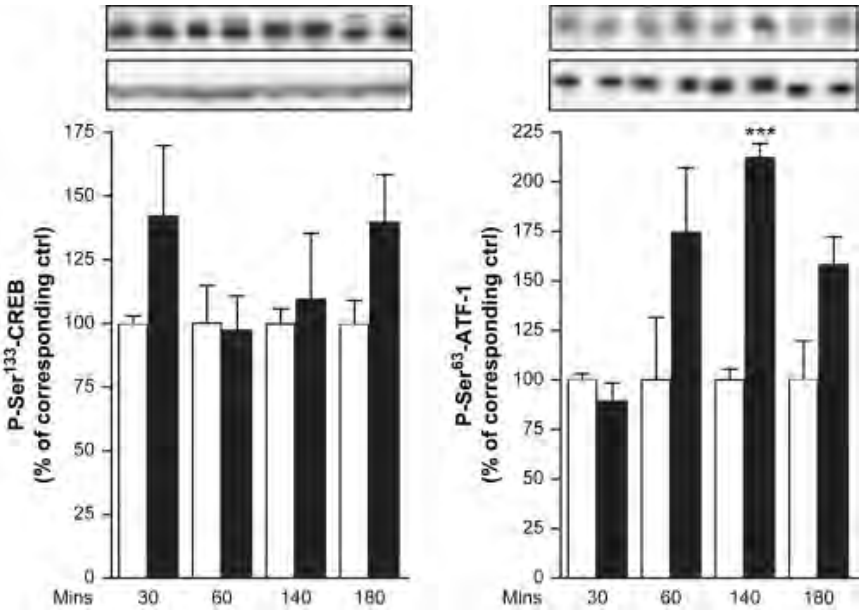


Fig. 15. Immunoblots and histograms showing the phosphorylation state at Ser^{133}-CREB and Ser^{63}-ATF1, 30, 60, 140 and 180 min after control conditions (white bars) or elevated platform stress (black bars). Upper immunoblots illustrates the phosphorylated form of the protein, whereas lower immunoblots illustrate the total protein. The number of animals per group is 5. Unpaired, two-tailed, Student's T-test. ***$p < 0.001$, compared with corresponding control group.

4.3.3 Tianeptine but not imipramine reverses stress-induced impairment of hippocampus→prefrontal cortex (H-PFC) LTP after a 30 min exposure to the platform stress

As previously reported (Rocher et al., 2004), acute elevated platform stress fully inhibited LTP in H-PFC synapses, measured after the animals had been anesthetized (Fig. 16). LTP was impaired for the whole 120 min of recording after high frequency stimulation (HFS) in stress-saline treated rats compared to control-saline treated rats. Administration of tianeptine (10 mg/kg i.p.) after the stress (so that the drug could not modify the stress, but only subsequent changes in plasticity) fully reversed the stress-induced suppression of LTP when compared to stressed rats injected with saline (Fig. 16). In contrast, administration of imipramine (10 mg/kg i.p.) after the stress did not reverse the effects of stress on LTP when compared to stress rats injected with saline (Fig. 16).

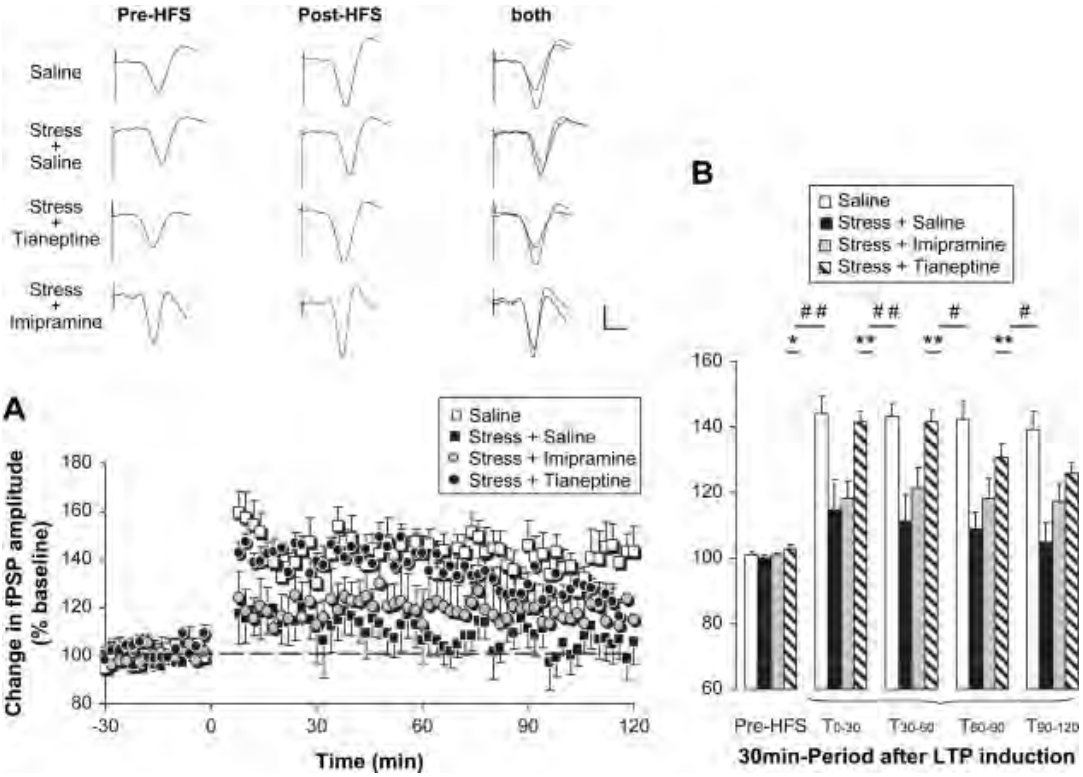


Fig. 16. Tianeptine but not imipramine reverses stress-induced impairment of H-PFC LTP after a 30 min exposure to the platform stress. A. Hippocampal HFS failed to induce LTP in the PFC of anaesthetized rats within 180 min following the end of the stress period (black squares, n=6). Tianeptine (10 mg/kg) when administered 40 min prior to HFS enables LTP in stressed rats (black circles; n=9) which not significantly different from controls (open squares, n=6). In contrast, imipramine (10 mg/kg) did not reverse the effects of stress on LTP (gray circles, n=6). Values are mean±SEM of the normalized H-PFC post-synaptic response amplitude. HFS is represented by arrows. B. LTP in saline, imipramine and tianeptine-treated stressed rats compared to controls is represented during different stages after HFS. Columns represent respectively 30 min periods of mean ±SEM of the normalized fPSP amplitude before, and after HFS. ANOVA: *$p < 0.05$ and ** $p < 0.01$ control vs saline treated stressed rats. #$p < 0.05$ and ##$p < 0.01$ tianeptine vs saline treated stressed rats. Top tracings, representative fPSP responses recorded in the medial PFC after stimulation of CA1/subicular region during pre-HFS, post-HFS and both illustrate the amplitude differences between the experimental groups. Scale bars: 10 ms (horizontal), 200 mV (vertical).

4.3.4 Regulation of multiple phosphorylation pathways by elevated platform stress and the antidepressants tianeptine and imipramine

To study how the antidepressants tianeptine and imipramine affected the stress-induced alterations in protein phosphorylation, these compounds were administered in a separate group of animals using a similar injection protocol after stress exposure.

Rats were sacrificed 30 min after the end of the stress. Based on the results previously obtained in the time course experiment, analyses were limited to a select number of phosphoproteins.

In agreement with the time course experiment, stress decreased Ser^{1303}-GluN2B and this reduction was further enhanced by treatment with imipramine (Fig. 17). Stress had no effect on the phosphorylation states of Ser^{831}- or Ser^{845}-GluA1. However, treatment with tianeptine, but not imipramine, increased Ser^{831}-GluA1 (Fig. 17). Neither stress nor tianeptine or imipramine had any effects on the phosphorylation states of Ser^{896}- and Ser^{897}-GluN1. Stress had no effect on Tyr$^{490/515}$-TrkA/B at this early timepoint, but tianeptine and imipramine increased the phosphorylation state of TrkA/B in non-stressed animals and tended to cause an increase also in stressed animals (Fig. 17).

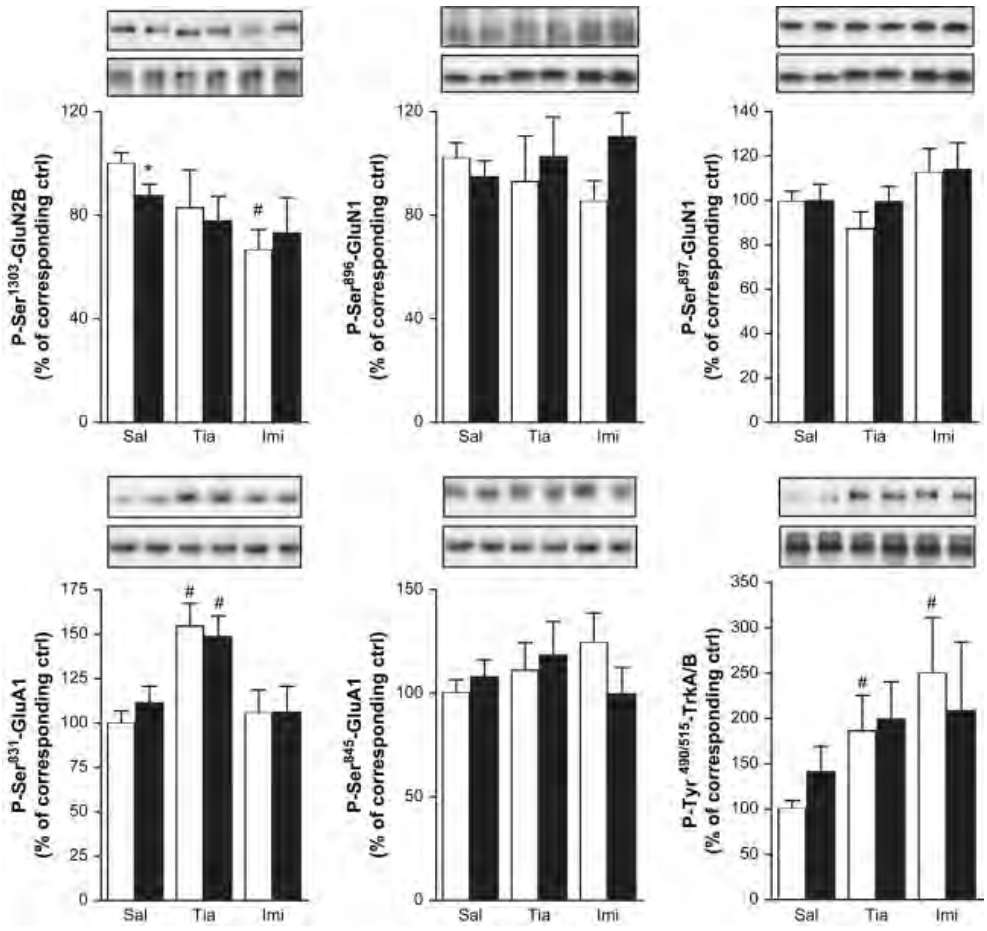


Fig. 17. Immunoblots and histograms showing the effects of tianeptine and imipramine on the phosphorylation state at multiple receptor subunits 30 min after control conditions (white bars) or elevated

platform stress (black bars). Upper immunoblots illustrates the phosphorylated form of the protein, whereas lower immunoblots illustrate the total protein. The number of animals per group is 6. Unpaired, two-tailed, Student's T-test. *p < 0.01, compared with corresponding control group; #p < 0.01, compared with saline treated control or stressed animals.

Treatment with both imipramine and tianeptine increased phosphorylation of $Ser^{217/221}$-MEK and counteracted the stress-induced decrease in phosphorylation at this site (Fig. 18). Both imipramine and tianeptine increased the phosphorylation states at Thr^{202}/Tyr^{204}-p42MAPK and Thr^{183}/Tyr^{185}-p44MAPK. We also studied the effects of imipramine and tianeptine on the stress-induced effects on Thr^{218}/Tyr^{220}-ERK5 and Thr^{180}/Tyr^{182}-p38MAPK. It was found that imipramine, by itself, increased Thr^{218}/Tyr^{220}-ERK5, an effect that was not seen in stressed animals. No effect of tianeptine was found. No significant effects of imipramine or tianeptine on the phosphorylation state of Thr^{180}/Tyr^{182}-p38MAPK were found. Moreover, tianeptine and imipramine had no effects on the phosphorylation states of Thr^{286}-CamKIIb.

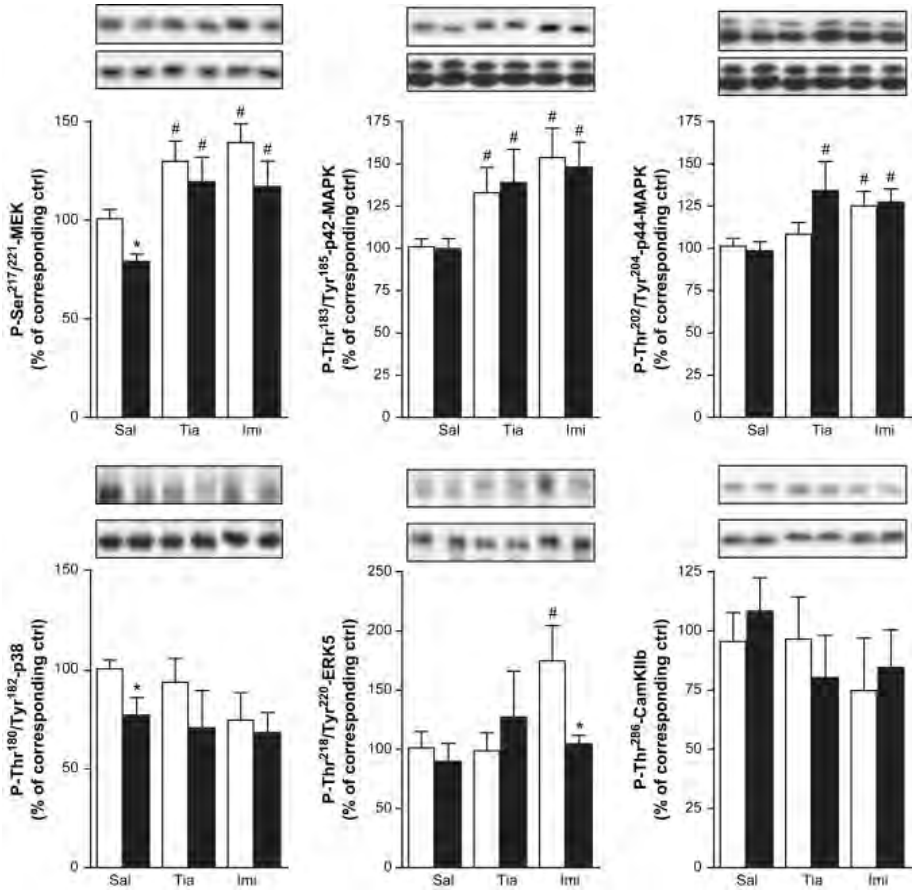


Fig. 18. Immunoblots and histograms showing the effects of tianeptine and imipramine on the

phosphorylation state of intracellular signaling proteins 30 min after control conditions (white bars) or elevated platform stress (black bars). Upper immunoblots illustrates the phosphorylated form of the protein, whereas lower immunoblots illustrate the total protein. The number of animals per group is 6. Unpaired, two-tailed, Student's T-test. *p < 0.05, compared with corresponding control group; #p < 0.05, compared with saline treated control or stressed animals

Tianeptine and imipramine had no effects on the phosphorylation states of Ser^{133}-CREB or Ser^{63}-ATF-1 at the studied timepoint (Fig. 19).

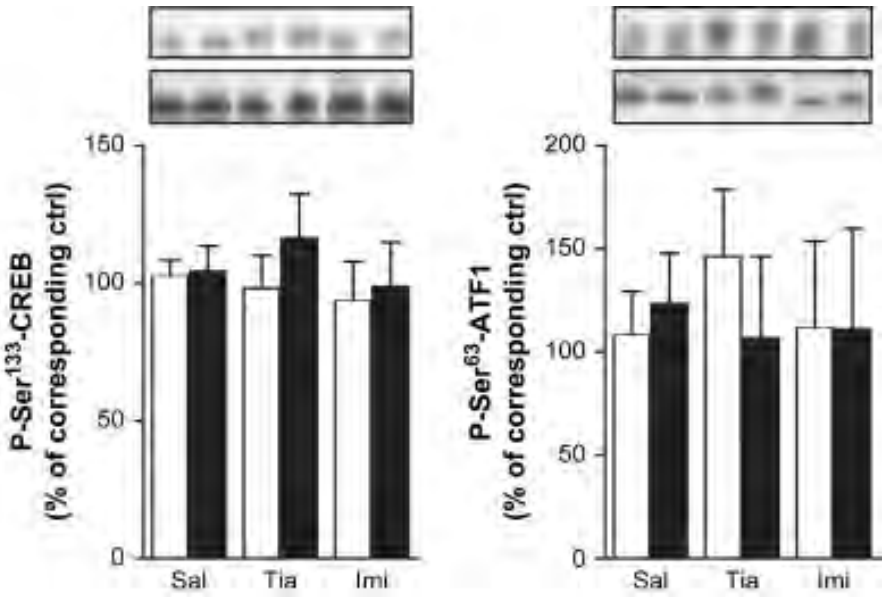


Fig. 19. Immunoblots and histograms showing the effects of tianeptine and imipramine on the phosphorylation state of Ser^{133}-CREB and Ser^{63}-ATF1 30 min after control conditions (white bars) or elevated platform stress (black bars). Upper immunoblots illustrates the phosphorylated form of the protein, whereas lower immunoblots illustrate the total protein. The number of animals per group is 6. Unpaired, two-tailed, Student's T-test. *p < 0.05, compared with corresponding control group; #p < 0.05, compared with saline treated control or stressed animals.

4.3.5 Regulation of BDNF by elevated platform stress

Using ELISA and Western blotting, 30 min after elevated platform stress there was a reduction in BDNF levels throughout frontal cortex (Fig. 20). This effect could be reversed by both tianeptine and imipramine (Fig. 20). No changes in BDNF were found 140 and 180 min after stress.

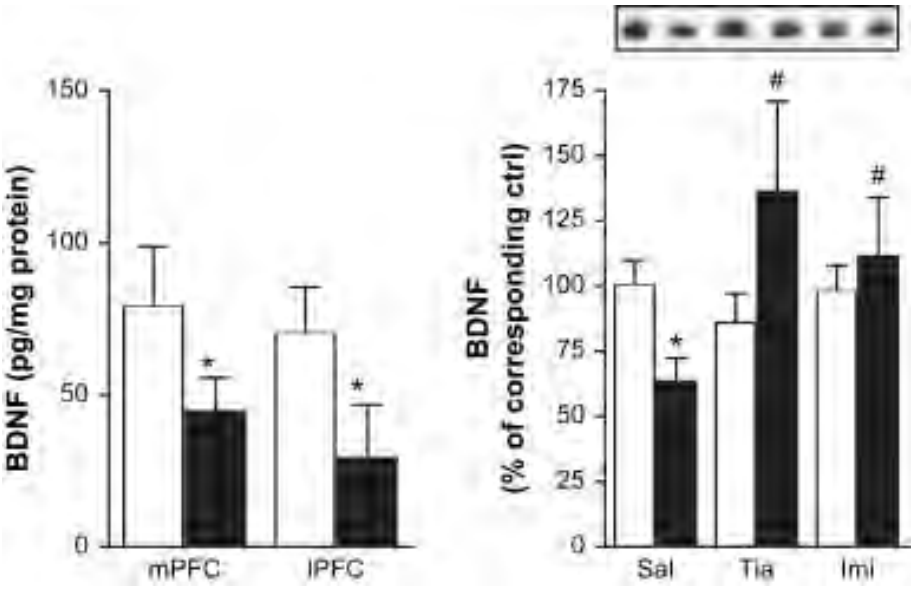


Fig. 20. **Regulation of BDNF by elevated platform stress.** Histogram showing the levels of BDNF 30 min after control conditions (white bars) or elevated platform stress (black bars) using ELISA. Immunoblot and histogram showing the effects of tianeptine and imipramine on the levels of BDNF. The number of animals per group is 6. mPFC, medial prefrontal cortex; lPFC, lateral prefrontal cortex. Unpaired, two-tailed, Student's T-test. *$p < 0.05$, compared with corresponding control group.

4.4 EFFECTS OF MIFEPRISTONE ON PHOSPHO-SER$^{217/221}$- AND TOTAL MEK LEVELS IN THE FRONTAL CORTEX (PAPER IV)

Professor Therese Jay's laboratory carried out electrophysiological experiments were carried in control (i.e. nonstressed rats injected with vehicle) and stressed rats (injected with vehicle). As previously reported, exposure to acute elevated platform stress significantly and robustly impaired LTP in the prefrontal cortex for the whole 120 min of recording after HFS. Acute injection of the selective GR antagonist mifepristone (20 mg/kg, i.p.) at the end of stress prevented impairment of LTP compared with stressed vehicle-treated animals and led to normal LTP that was not distinguishable from LTP in the vehicle-treated group.

The exposure of elevated platform stress caused a significant reduction in the phosphorylation state of Ser$^{217/221}$-MEK when compared with normal littermates (Fig. 21). This reduction was not accompanied by any changes in the total level of MEK.

There was no change in phosphorylation of any of the other proteins measured (GluA1; NR1, NR2B receptors, CamKII, CREB) (data not shown). Treatment with mifepristone (20 mg/kg; i.p) counteracted the inhibitory action of stress on the levels of phospho-MEK (Fig. 21).

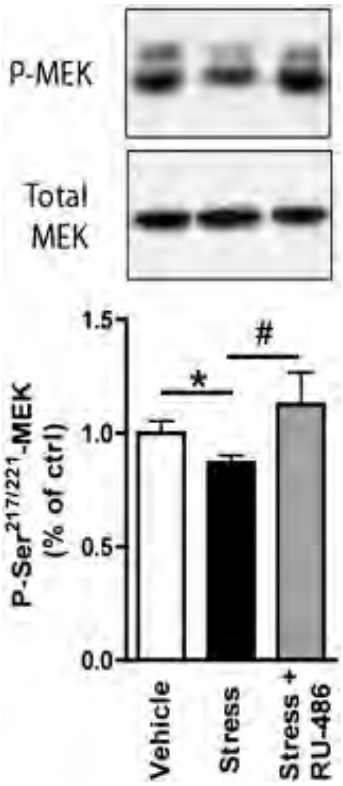


Fig. 21. Mifepristone reverses stress-induced decreases of phospho-Ser$^{217/221}$-MEK levels in frontal cortex. Immunoblots and histogram illustrating that stress reduces phospho-Ser$^{217/221}$-MEK levels without affecting total MEK levels. An acute injection of mifepristone (20 mg/kg) reverses the stress-mediated reduction in phospho-MEK. Values are mean±SEM from 9–10 animals per group. #p<0.05 vs mifepristone-treated stressed rats. *p<0.05 vs nonstressed normal rats.

4.5 BIOCHEMICAL EVIDENCE FOR ANTIDEPRESSANT-LIKE EFFECTS OF 5-HT6 RECEPTOR STIMULATION (PAPER V)

4.5.1 The 5-HT$_6$ receptor antagonist, SB271046, reverses biochemical antidepressant-like effects of Fluoxetine

In agreement with our previous study (Svenningsson et al., 2002), fluoxetine (20 mg/kg) increased the levels of phospho-Ser^{845}-GluA1 in the frontal cortex (Fig. 22) and striatum (data not shown). Treatment with SB271046 (10 mg/kg) alone had no effect on phospho-Ser^{845}-GluA1 in these regions but significantly counteracted fluoxetine-induced phospho-Ser^{845}-GluA1 in the frontal cortex (Fig. 22).

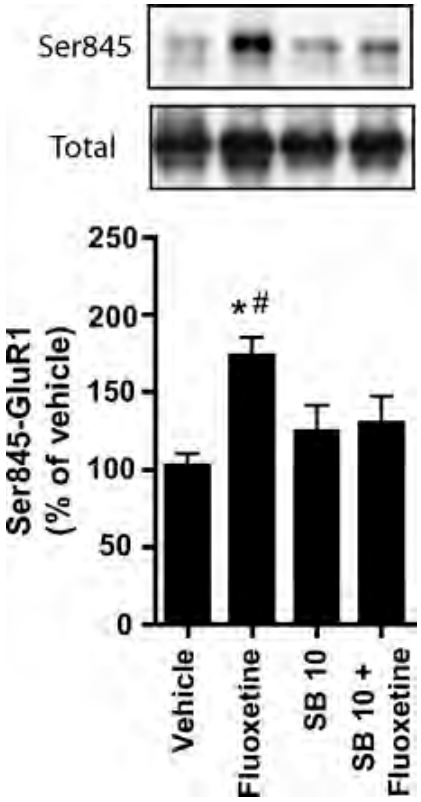


Fig. 22. Regulation by fluoxetine and SB271046 of phospho-Ser^{845}-GluR1 in the frontal cortex in intact mice. Top, Immunoblots showing the levels of phospho-Ser^{845}-GluR1 and total GluR1 in the frontal cortex 30 min after intraperitoneal administration of saline, fluoxetine (20mg/kg), SB271046 (10 mg/kg), or SB271046 (10 mg/kg) together with fluoxetine (20 mg/kg) in mice. Bottom, The histogram shows the quantification of phospho-Ser^{845}-GluR1 in the frontal cortex after the indicated treatments. Data represent means ±SEM for five to six mice per group. *p<0.05 compared with saline-treated mice; #p<0.05 compared with SB271046 (10mg/kg) plus fluoxetine-cotreated mice; one-way ANOVA followed by Newman–Keuls test for pairwise comparisons.

4.5.2 Effects of SB271046 on antidepressant-like effects of Fluoxetine in the tail suspension test

It is well established that acute treatment with various antidepressant drugs reduces immobility in the tail suspension test. In agreement with the biochemical data, SB271046 (1, 5, or 10 mg/kg) had no effect in the tail suspension test when administered alone. When SB271046 (5 or 10 mg/kg) was given in conjunction with an antidepressant-like dose of fluoxetine (20 mg/kg), however, there was a partial reversal of its anti-immobility effect in this test (Fig. 23). It can be concluded from these studies that specific biochemical and behavioral actions of fluoxetine that are associated with its antidepressant effects may involve activation of 5-HT$_6$ receptors.

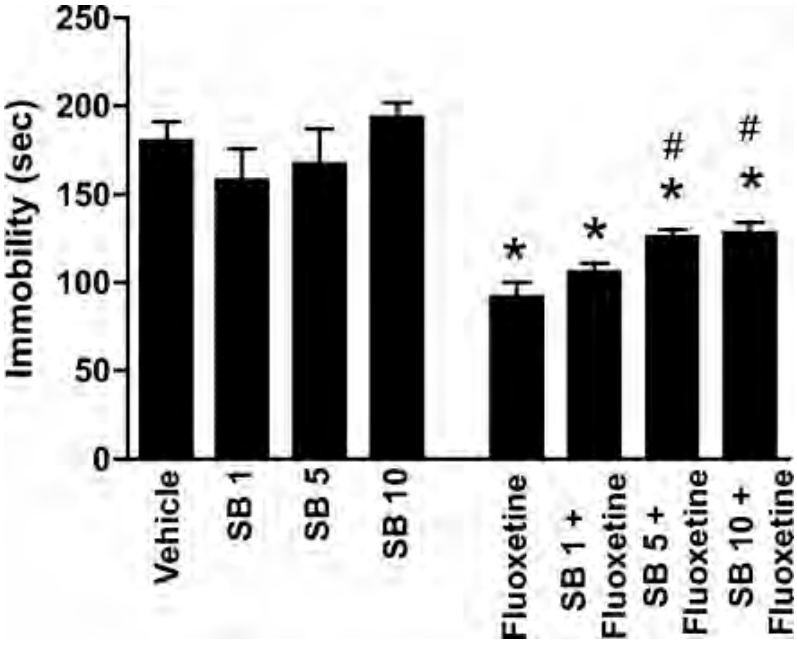


Fig. 23. Effects of SB271046 on antidepressant-like effects of fluoxetine in the tail suspension test. Saline, fluoxetine (20 mg/kg), SB271046 (1,5, or 10 mg/kg), or SB271046 (1,5, or 10mg/kg) combined with fluoxetine (20 mg/kg), 30 min before the tail-suspension test trial. The trial was conducted for a period of 5 min, during which the duration of immobility was recorded. Data represent means ±SEM for eight mice per group. *$p<0.05$ compared with saline; #$p<0.05$ compared with fluoxetine; one-way ANOVA followed by Duncan's test.

4.5.3 Antidepressant effects of the 5-HT_6 receptor agonist EMDT

We next assessed the ability of the 5-HT_6 receptor agonist EMDT to mimic some of the antidepressant-like biochemical and behavioral effects of fluoxetine. First, we measured its ability to regulate the phosphorylation state of two PKA phosphosubstrates, Thr^{34}-DARPP-32 and Ser^{845}-GluA1, in striatal slices. EMDT increased the phosphorylation states of Thr^{34}-DARPP-32 and Ser^{845}-GluA1 in a dose-dependent manner (Fig. 24).

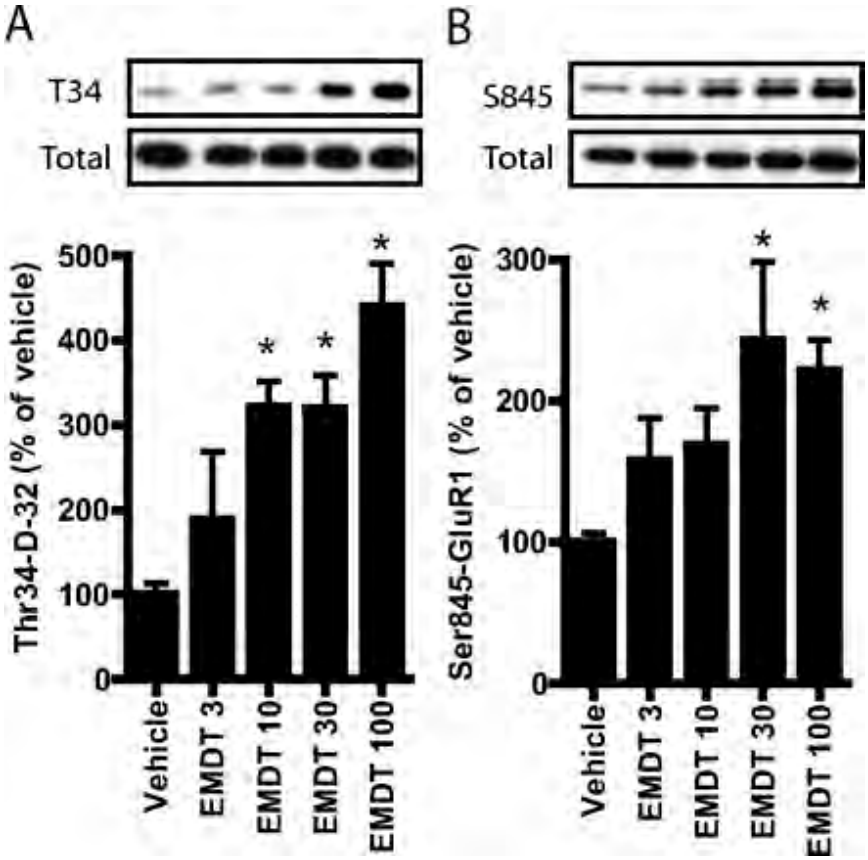


Fig. 24. Regulation by EMDT of the phosphorylation states of DARPP-32 and GluR1 in slices of neostriatum. Dose-response experiments of in vitro regulation by EMDTof phosphorylation of Thr^{34}-DARPP-32 (**A**) and Ser845-GluR1 (**B**) in striatal slices. Slices were incubated with EMDT (3, 10, 30, and 100μM) for 5 min. Data represent means±SEM (n=6 –10). *p<0.05 compared with vehicle; one-way ANOVA followed by Newman–Keuls test.

To determine whether the effect of EMDT on phospho-Thr^{34}-DARPP-32 and phospho-Ser^{845}-GluA1 involved D1 receptor activation, we compared the effect of EMDT (100 μM) on these phosphosubstrates in wild-type and D1 receptor knock-out mice. As shown in Figure 25, EMDT significantly increased phospho-Thr^{34}-DARPP-32 and phospho-Ser^{845}-GluA1 not only in slices from wild-type mice but also in slices from D1 receptor knockout mice. It can be concluded that the stimulatory effect of EMDT on phospho-Thr^{34}-DARPP-32 and phospho-Ser^{845}-GluA1 is independent of D1 receptor activation.

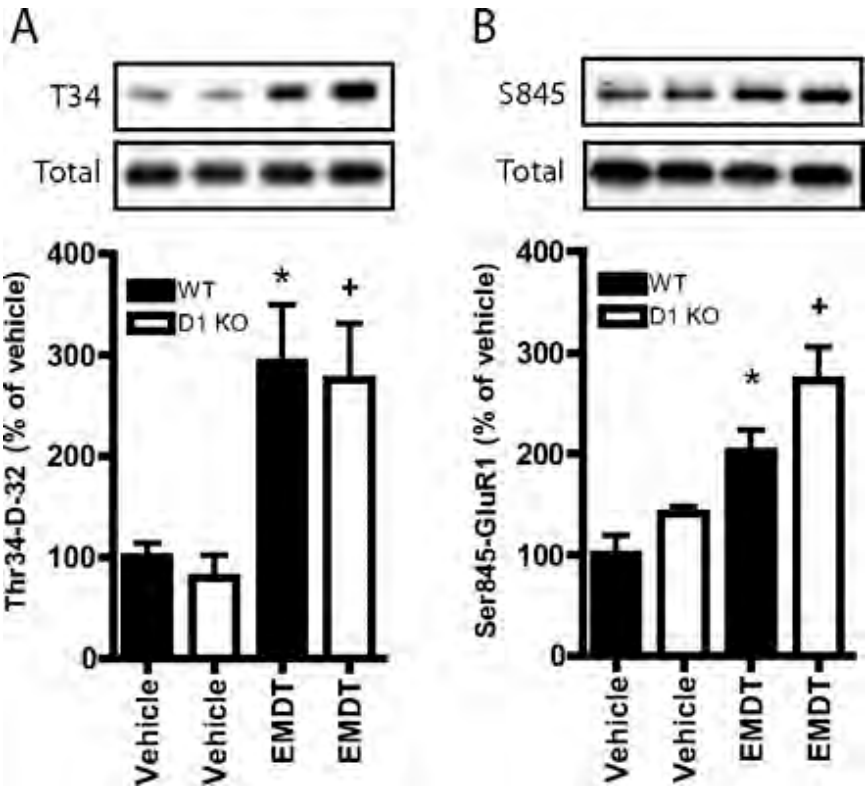


Fig. 25. Comparison of the regulation by EMDT of the phosphorylation states of DARPP-32 and GluR1 in striatal slices from wild-type (WT) and D1 receptor knock-out (D1 KO) mice. In vitro regulation of Thr^{34}-DARPP-32 (A) and Ser^{845}-GluR1 (B) phosphorylation by EMDT (100μM) in slices of neostriatum from wild-type and D1 knock-out mice. The amounts of phospho-Thr^{34}-DARPP-32 and phospho-Ser^{845}-GluR1 in extracts of slices were quantified by densitometry. Data represent means ±SEM (n=6–12). *p<0.05 compared with wild-type control; p<0.05 compared with D1 knock-out control; unpaired two-tailed Student's t test.

We next examined the effect of systemic administration of EMDT on the PKA sites, phospho-Thr^{34}-DARPP-32 and phospho-Ser^{845}-GluA1. It was found that 5 mg/kg of EMDT increased the phosphorylation states of both Thr^{34}-DARPP-32 and Ser^{845}-GluA1 in striatal extracts (Fig. 26). No significant alterations of phospho-Thr^{75}-DARPP-32 or phospho-Ser^{831}-GluA1 were found in the same extracts. Treatment with EMDT also increased phospho-Ser^{845}-GluA1, but not phospho-Ser^{831}-GluA1, in the frontal cortex (Fig. 26). These data indicate that the effects of EMDT on phosphorylation of PKA phosphosubstrates in brain slices can be reproduced by its systemic administration to intact animals.

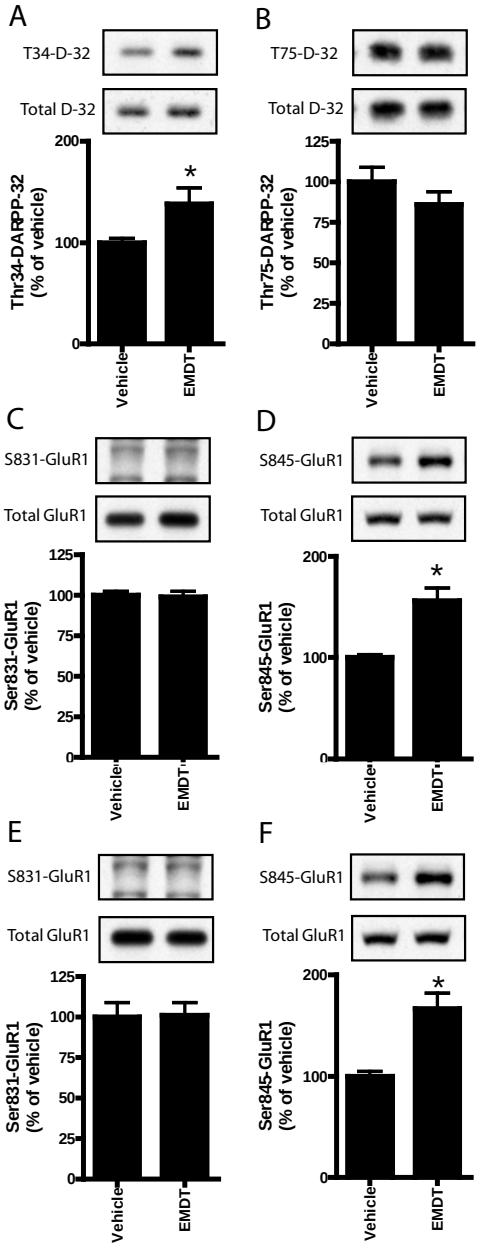


Fig. 26. Regulation by EMDT of the phosphorylation states of DARPP-32 and GluR1 in striatal and cortical extracts from intact mice. Regulation of Thr^{34}- and Thr^{75}-DARPP-32 and Ser^{831}- and Ser^{845}-GluR1 phosphorylation in vivo in the striatum (A–D) and frontal cortex (E, F) by EMDT. Mice were injected intraperitoneally with saline or EMDT (5 mg/kg). Fifteen minutes later, mice were killed by focused microwave irradiation. Data represent means±SEM for 5–10 mice per group. *$p<0.05$ compared with saline-treated mice; one-way ANOVA followed by Newman–Keuls test.

5 GENERAL DISCUSSION

During the past decades, inhibitors of monoamine reuptake have been the standard phamocological treatment of major depression, but a significant proportion of patients do not receive clinically meaningful relief from these agents. Among a number of strategies to overcome these shortcomings some of these approaches circumvent the aminergic synapse, whilst others remain grounded in monoaminergic theories of depression and the identification of more specific, receptor subtype-based, treatments. The work presented in this thesis has added information related to both these approaches on developing novel antidepressants.

Tianeptine has challenged the monoaminergic hypothesis of depression, as well as the proposed monoaminergic mechanisms whereby the action of most known antidepressants is explained. Since it is a serotonin reuptake enhancer, or at least does not increase monoamine levels, events beyond the monoaminergic regulation are probably relevant to its clinical antidepressant efficacy. Previous work has shown that the neurobiological properties of tianeptine involve a critical ability to restore normal neuroplasticity in circumscribed limbic brain regions and to reverse stress-induced impairments. In accordance with the findings, we have here provided novel evidence that tianeptine is likely to potentiate synaptic glutamate transmission by increasing AMPA receptor phosphorylation (Fig 27). The effects of tianeptine were more pronounced on Ser831-GluA1, than Ser845-GluA1 where fluoxetine and imipramine only regulate Ser845-GluA1, suggesting that fluoxetine, imipramine and tianeptine increase AMPA receptor phosphorylation via different primary mechanisms (Fig 27). As phosphorylation of GluA1 subunits is a way of potentiating AMPA receptor function, these data add further evidence that potentiation of AMPA receptor function may be implicated in antidepressant actions. Along this line, there are several reports

showing that allosteric modulators that act at extracellular domains to potentiate AMPA receptors possess antidepressant properties at least in animal models (Alt et al., 2006). Tianeptine was also found to potentiate the BDNF/MEK/MAPK signaling cascade (Fig 27) which is associated with enhanced synaptic plasticity.

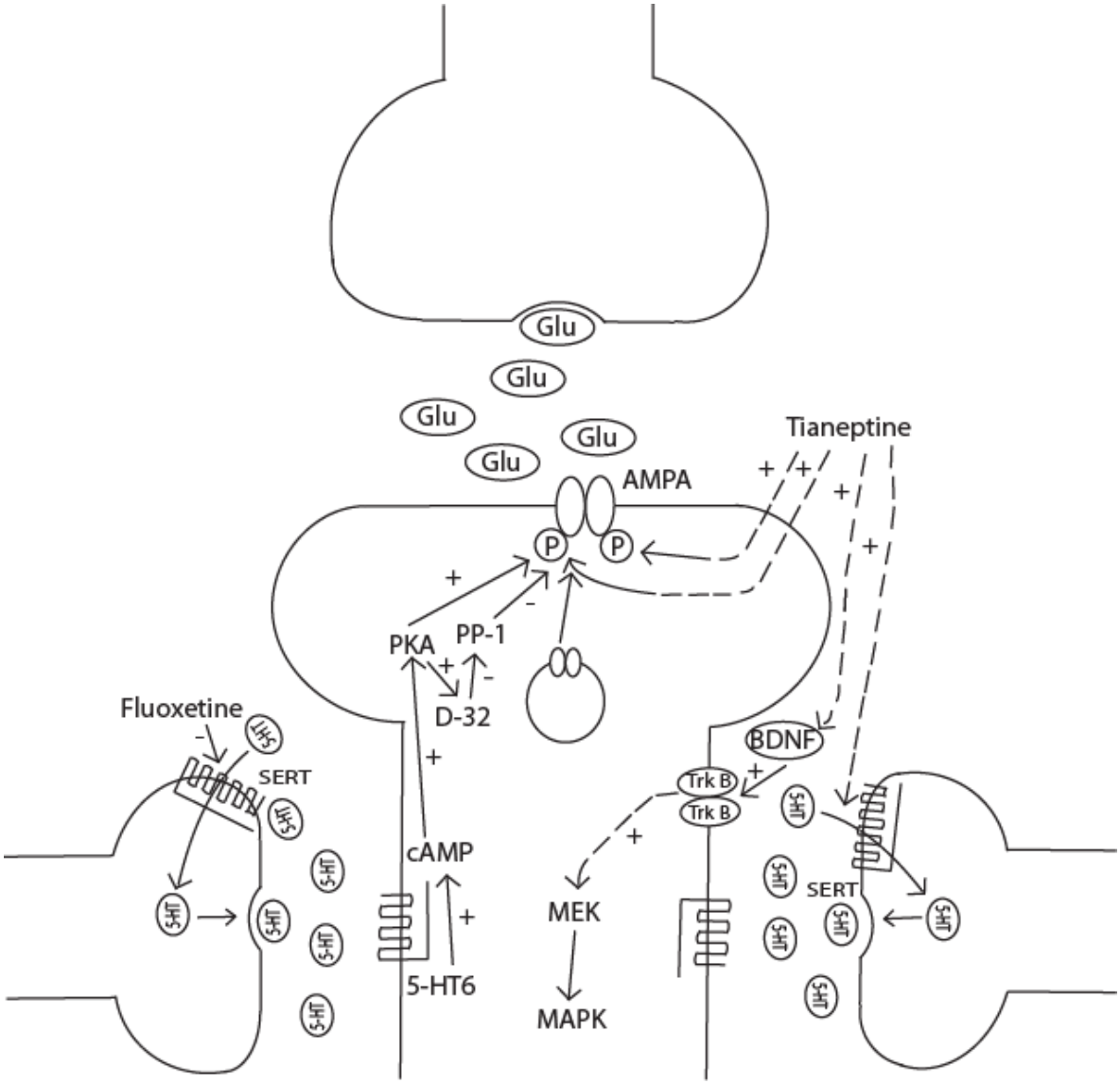


Fig. 27. Schematic summary of data obtained in the current thesis. Tianeptine and fluoxetine have different effects on serotonin reuptake. However, tianeptine and fluoxetine both increase AMPA receptor phosphorylation on GluA1 subunits but via different primary mechanisms**.** Tianeptine causes a robust increase of GluA1 phosphorylation at both Ser831- and Ser845-GluA1, whereas fluoxetine only affects the latter site proably by increased PKA activity and an amplification by means of activation of DARPP-32/PP-1 resulting is less dephosphorylation of Ser845-GluA1. In addition of potentiating synaptic glutamate transmission by increasing AMPA receptor phosphorylation, tianeptine also potentiates the BDNF/MEK/MAPK signaling cascade.

Interestingly, the stress model developed by Professor Therese Jay engages brain circuitries which are targeted antidepressant actions by deep brain stimulation (Mayberg et al., 2005) indicating a high relevance. Using this model tianeptine reversed stress-induced impairments of this BDNF/MEK/MAPK signalling cascade. Altogether, these studies have provided strong biochemical evidence that tianeptine enhances neuronal plasticity processes which may underlie its therapeutic actions. These data reinforce the idea of developing novel antidepressants based on their ability to enhance neuronal plasticity in relevant brain circuitries.

Another line of reseach in the depression field is to find more specific drug targets derived from currently used compounds. Fluoxetine exerts its primary antidepressant action by inihibiting reuptake of serotonin from the synaptic cleft and thereby stimulating multiple 5-HT receptors. The distribution of 5-HT6 receptors in the mouse brain is uniform throughout the cortex and striatum. Here we describe for the first time that blockade of 5HT6 receptors counteracts biochemical and behavioural effects of fluoxetine. Furthermore, stimulation of 5-HT6 receptors causes antidepressant-like behavioral and biochemical effects and provide support to the idea that 5-HT6 receptors may contribute to serotonergic modulation of clinically relevant psychopharmacological processes. Interestingly, 5HT6 receptors also stimulate AMPA receptor phosphorylation (Fig 27), further reinforcing a role of glutamate neurotransmission and neuroplasticity in antidepressant-like actions.

6 CONCLUSIONS

From the data presented in this thesis some conclusions can be drawn:

1. Treatment with tianeptine increases GluA1 phosphorylation in frontal cortex and hippocampus in brain slices and in intact animals indicative of enhanced synaptic plasticity.
2. There is altered behavioural and biochemical responsivity towards tianeptine in dual-phosphomutant GluA1 mice showing the role of these subunits in mediating antidepressant-like actions of tianeptine.
3. Tianeptine potentiates the BDNF/MEK/MAPK signaling cascade in frontal cortex.
4. Acute elevated platform stress downregulates a BDNF/MEK/MAPK signaling cascade which can be counteracted by the antidepressants tianeptine and imipramine.
5. Acute stress significantly reduces MEK phosphorylation in frontal cortex and this effect can be counteracted by the GR antagonist mifepristone.
6. Blockade of the 5-HT_6 receptor with the antagonist SB271046 counteracts the stimulatory actions of fluoxetine on cortical phospho-Ser^{845}-GluA1 and reduces the antidepressant-like action of fluoxetine in the tail suspension test.
7. 5-HT_6 receptor agonist EMDT mimics antidepressant-like biochemical effects of fluoxetine.

A general conclusion of this thesis is that agents which enhance neuronal plasticity by stimulating AMPA receptor phosphorylation or BDNF/MEK/MAPK signaling, in relevant brain regions, including prefrontal cortex and hippocampus, may exert antidepressant actions.

7 ACKNOWLEDGEMENTS

Many people have contributed directly or indirectly to the present thesis work. I would especially like to express my sincere gratitude to all those who helped and encouraged me during my PhD study, special thanks to:

Per Svenningsson, my supervisor, for accepting me as a PhD student in his laboratory, for introducing me to the research on depression with your vast knowledge and for all your support.

Alexei Morozov, my cosupervisor, for accepting me as a Ph.D student.

Karima Chergui, for letting me use the lab to do slice experiments.

Former and present members of our research group, **Benita Sjögren, Martin Egeland**, **Therese Eriksson**, **Carly Kiselycznyk, Alexandra Madeira, Martin Paucar Arce, Ebba Gregorsson Lundius, Karl Bjork, Nicoletta Schintu, Alexandra Alvarsson, Anna Kindlundh Högberg, Riccardlo Piona, Serena Balasso, Elenora Tandoi,** and **Mashid Hassan Pour**, for all your help and all the good time both inside and outside the lab!

The former and present head of the department of Physiology and Pharmacology, **Professor Bertil Fredholm** and **Associate Professor Stefan Eriksson,** for providing good working conditions at the department.

All the professors and researchers in Pharmacology, particularly **Professor Göran Engberg, Professor Magnus Ingelman-Sundberg, Professor Torgny Svensson, Professor Jon Lundberg, Docent Jan Kehr, Docent Kent Jardemark,** for the nice atmosphere and environment for science.

The administrative and technical staff at FYFA, especially **Eva Gipperth, Ulla Wester, Monica Pace-Sjöberg, Camilla Fors Holmberg, Inger Johansson, Hasse Svensson, Micke Elm, Peter Wolf, Eva Näsström, Renee Andersson,** for all practical help. The people in the animal department, especially **Per-Arne Åberg, Benny Gustafsson,** for taking excellent care of my mice and rats.

To the committee at my half time seminar: **Professor Jens D. Mikkelsen, Professor Sven Ove Ögren, and Professor Ernst Brodin,** for giving me constructive feedback on my work.

All my current and former colleagues in Department of Physiology and Pharmacology,. Special thanks to **Stina Johansson, Olga Björklund Karovic, Eva Lindgren, Karin Lindström Törnqvist, Liyue Huang, Yingqing Wang, Ying Dou, Shijin Zhang, Xiaojing Hu, Qing Xu, Na Guan, Jinling Huang,** thank you all for the chatting about everything during lunch time or outside of KI.

Jinjing Pei and **Huixin Wang,** for the happy time of Christmas and New Year that we spent together.

Guojun Cheng, Jianjing Zheng, Tian Cheng, for all the good food and the time we spent together, especially the trip to Norway.

My Chinese friends in Sweden: **Min Wan, Ming Liu, Qiaolin Deng, Zhe Jin, Xin Wang, Xiaofeng Zheng, Xiaowei Zheng, Jiangning Yang, Yan Li, Bing Zhang, Mingmei Shang, Ying Sun, Liqun He, Bin Zhao, Jinfeng Shen, Zhuochun Peng, Qing Cheng, Jikui Guan, Guanqun Meng, Linlin Zhang, Hong Jiang, Mengmeng Wu, Yang Shi, Kaiyu Liu,** for being wonderful companions, for the food and for all the moments we enjoyed together, as well as for all the help from you. The cute babies: **Lele, Ruirui, Chuchu, Tongtong,** for the happiness you bring.

Lei, Yumeng, Huajun, Hailiang, Dongfang, Qiu, Yougen, Qing, Xin, Wenkan, Tiantian, Yongjie, Xiyan, Kaichao, Hongchao, for the sweat and laugh, for sharing the No.1 hobby in my life.

My uncle and aunt: **Kejun Li** and **Shujing Zhang**, for endless support, love, advice and encouragement.

Jinsong and **Ronghua,** my parents-in-law, for your understanding, love and support. **Xiaowei and Di,** my brother and sister-in-law, **Ziling**, my cute niece, for the nice time we spend together.

Huan, my dad, I know you're always there looking at me, encouging me go ahead bravely. **Yan,** my mum, for giving without taking all the time, thank you and I love you.

My beloved wife**, Xiaoqun,** for the love and support! My lovely son, **Ian,** for giving me a big smile! My world is always beautiful with you two!

8. REFERENCES

Agid Y., Buzsaki G., Diamond D.M., Frackowiak R., Giedd J., Girault J.A., Grace A., Lambert J.J., Manji H., Mayberg H., Popoli M., Prochiantz A., Richter-Levin G., Somogyi P., Spedding M., Svenningsson P.,Weinberger D. (2007) How can drug discovery for psychiatric disorders be improved? Nat. Rev. Drug Discov. 6, 189–201.

Aihara M., Ida I., Yuuki N., Oshima A., Kumano H., Takahashi K., Fukuda M., Oriuchi N., Endo K., Matsuda H., Mikuni M. (2007) HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions. Psychiatry Res. 155, 245–256.

Alt A., Nisenbaum E.S., Bleakman D., Witkin J.M. (2006) A role for AMPA receptors in mood disorders. Biochem. Pharmacol. 71, 1273–1288.

Atmaca M., Kuloglu M., Tezcan E., Buyukbayram A. (2003) Switching to tianeptine in patients with antidepressant-induced sexual dysfunction. Hum. Psychopharmacol. 18, 277-280.

Bai F., Bergeron M., Nelson D.L. (2003) Chronic AMPA receptor potentiator (LY451646) treatment increases cell proliferation in adult rat hippocampus. Neuropharmacology 44, 1013–1021.

Barbui C., Hotopf M., Freemantle N., Boynton J., Churchill R., Eccles M.P., Geddes J.R., Hardy R., Lewis G., Mason J.M. (2007) WITHDRAWN: Treatment

discontinuation with selective serotonin reuptake inhibitors (SSRIs) versus tricyclic antidepressants (TCAs). Cochrane Database Syst Rev. 18, CD002791.

Barria A., Muller D., Derkach V., Griffith L.C., Soderling T.R. (1997) Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science 276, 2042–2045.

Belanoff, J.K., Kalehzan, M., Sund, B., Fleming Ficek, S.K., Schatzberg, A.F. (2001) Cortisol activity and cognitive changes in psychotic major depression. Am. J. Psychiatry 158, 1612–1616.

Belmaker R.H. and Agam G. (2008) Major depressive disorder. N. Engl. J. Med. 358, 55-68.

Bergink V., van Megen H.J., Westenberg H.G. (2004) Glutamate and anxiety. Eur. Neuropsychopharmacol. 14, 175-183.

Berton, O. and Nestler, E.J. (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat. Rev. Neurosci., 7, 137–151.

Bonierbale M., Lancon C., Tignol J. (2003) The ELIXIR study: evaluation of sexual dysfunction in 4557 depressed patients in France. Curr. Med. Res. Opin. 19, 114-124.

Brion S., Audrain S., De Bodinat C. (1996) Major depressive episodes in patients over 70 years of age. Evaluation of the efficiency and acceptability of tianeptine and mianserin. Presse. Med. 25, 461-468.

Brown G.W. (1998) Genetic and population perspectives on life events and depression. Soc Psychiatry Psychiatr Epidemiol. 33, 363-72.

Caspi A. and Moffitt T.E. (2006). Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat. Rev. Neurosci. 7, 583–590.

Caspi A., Sugden K., Moffitt T.E., Taylor A., Craig I.W., Harrington H., McClay J., Mill J., Martin J., Braithwaite A., Poulton R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389.

Cerqueira J.J., Mailliet F., Almeida O.F., Jay T.M., Sousa N. (2007) The prefrontal cortex as a key target of the maladaptive response to stress. J. Neurosci. 27, 2781–2787.

Chamba G., Lemoine P., Flachaire E., Ferry N., Quincy C., Sassard J., Ferber C., Mocaer E., Kamoun A., Renaud B. (1991) Increased serotonin platelet uptake after tianeptine administration in depressed patients. Biol. Psychiatry 30, 609-617.

Chen B., Dowlatshahi D., MacQueen G.M., Wang J.-F., Young L.T. (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol. Psychiatry 50, 260–265.

Collingridge G.L., Olsen R.W., Peters J., Spedding M. (2009) A nomenclature for ligand-gated ion channels. Neuropharmacology 56, 2-5.

Curzon G., Kennett G.A., Sarna G.S., Whitton P.S. (1992) The effects of tianeptine and other antidepressants on a rat model of depression. Br. J. Psychiatry Suppl. 15, 51-55.

Czeh B., Michaelis T., Watanabe T., Frahm J., de Biurrun G., van Kampen M., Bartolomucci A., Fuchs E. (2001) Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc. Natl. Acad Sci. U. S. A. 98, 12796-12801.

Diorio D., Viau V., Meaney M.J. (1993) The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J. Neurosci. 13, 3839–3847.

Drevets W.C., Bogers W., Raichle M.E. (2002) Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. Eur. Neuropsychopharmacol. 12, 527–544.

Du J., Suzuki K., Wei Y., Wang Y., Blumenthal R., Chen Z., Falke C. Zarate C.A. Jr., Manji H.K. (2007) The Anticonvulsants lamotrigine, riluzole, and valproate differentially regulate AMPA receptor membrane localization: relationship to clinical effects in mood disorders. Neuropsychopharmacology 32, 793–802.

Duman R.S., Heninger G.R., Nestler E.J. (1997) A molecular and cellular theory of depression. Arch. Gen. Psychiatry 54, 597-606.

Duman C.H., Schlesinger L., Kodama M., Russell D.S., Duman R.S. (2007) A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. Biol. Psychiatry 61, 661–670.

Duman R.S., Malberg J., Thome J. (1999) Neural plasticity to stress and antidepressant treatment. Biol. Psychiatry 46, 1181-1191.

Dwivedi Y., Rizavi H.S., Conley R.R., Tamminga C.A., Pandey G.N. (2003) Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects, Arch. Gen. Psychiatry 60, 804–815.

Eisch A.J., Bolanos C.A., Wit J. de, Simonak R.D., Pudiak C.M., Barrot M. (2003) Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: A role in depression. Biol. Psychiatry 54, 994–1005.

Fattaccini C.M., Bolanos-Jimenez F., Gozlan H., Hamon M. (1990) Tianeptine stimulates uptake of 5-hydroxytryptamine in vivo in the rat brain. Neuropharmacology 29, 1-8.

Fienberg A.A., Hiroi N., Mermelstein P.G., Song W., Snyder G.L., Nishi A., Cheramy A., O'Callaghan J.P., Miller D.B., Cole D.G., Corbett R., Haile C.N., Cooper D.C., Onn S.P., Grace A.A., Ouimet C.C., White F.J., Hyman S.E., Surmeier D.J., Girault J., Nestler E.J., Greengard P. (1998) DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science 281, 838-842.

Fleischhacker W.W., Hinterhuber H., Bauer H., Pflug B., Berner P., Simhandl C., Wolf R., Gerlach W., Jaklitsch H., Sastre-y-Hernández M. (1992) A multicenter double-blind study of three different doses of the new cAMP-phosphodiesterase inhibitor rolipram in patients with major depressive disorder. Neuropsychobiology 26, 59-64.

Fujimaki K., Morinobu S., Duman R.S. (2000) Administration of a cAMP phosphodiesterase 4 inhibitor enhances antidepressant-induction of BDNF mRNA in rat hippocampus. Neuropsychopharmacology 22, 42-51.

Guelfi J.D., Pichot P., Dreyfus J.F. (1989) Efficacy of tianeptine in anxious-depressed patients: results of a controlled multicenter trial versus amitriptyline. Neuropsychobiology 22, 41-48.

Hirschfeld R.M. (1999) Efficacy of SSRIs and newer antidepressants in severe depression: comparison with TCAs. J Clin Psychiatry. 60, 326-35. Review.

Holsboer, F. and Barden, N. (1996) Antidepressants and hypothalamic-pituitary-adrenocortical regulation. Endocr. Rev. 17, 187–205.

Huang E. and Reichardt L.F. (2003) Trk receptors: Roles in neuronal signal transduction. Annu. Rev. Biochem. 72, 609–642.

Ikenouchi-Sugita A., Yoshimura R., Nakamura J. (2007) Review of pharmacological efficacies and side effects of antidepressants. Nippon Rinsho. 65, 1633-7.

Invernizzi G., Aguglia E., Bertolino A., Casacchia M., Ciani N., Marchesi G.F, Nardini M., Rapisarda V. (1994) The efficacy and safety of tianeptine in the treatment of depressive disorder: results of a controlled double-blind multicentre study vs. amitriptyline. Neuropsychobiology 30, 85-93.

Jay T.M., Rocher C., Hotte M., Naudon L., Gurden H., Spedding M. (2004) Plasticity at hippocampal to prefrontal cortex synapses is impaired by loss of dopamine and stress: importance for psychiatric diseases. Neurotox. Res. 6, 233–244.

Karege F., Perret H., Bondolfi G., Schwald M., Bertschy G., Aubrey J.M. (2002) Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res. 109, 143–148.

Kasper S. and McEwen B.S. (2008) Neurobiological and clinical effects of the antidepressant tianeptine. CNS Drugs 22, 15-26.

Kasper S. and Olie J.P. (2002) A meta-analysis of randomized controlled trials of tianeptine versus SSRI in the short-term treatment of depression. Eur Psychiatry 17 Suppl 3, 331-340.

Kato G. and Weitsch A.F. (1988) Neurochemical profile of tianeptine, a new antidepressant drug. Clin. Neuropharmacol. 11 Suppl 2, S43-S50.

Kelly J.P. and Leonard B.E. (1994) The effect of tianeptine and sertraline in three animal models of depression. Neuropharmacology 33: 1011-1016.

Kendler K.S., Karkowski L.M., Prescott C.A. (1999) Causal relationship between stressful life events and the onset of major depression. Am. J. Psychiatry. 156, 837-41.

Kessler R.C. (1997) The effects of stressful life events on depression. Annu. Rev. Psychol. 48, 191-214.

Kim J.J. and Diamond D.M. (2002) The stressed hippocampus, synaptic plasticity and lost memories. Nat. Rev. Neurosci. 3, 453–462.

Lee H.K., Takamiya K., Han J.S., Man H., Kim C.H., Rumbaugh G., Yu S., Ding L., He C., Petralia R.S., Wenthold R.J., Gallagher M., Huganir R.L. (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112, 631-643.

Lee S.Y., Kang J.S., Song G.Y., Myung C.S. (2006) Stress induces the expression of heterotrimeric G protein beta subunits and the phosphorylation of PKB/Akt and ERK1/2 in rat brain. Neurosci. Res. 56, 180–192.

Lepine J.P., Altamura C., Ansseau M., Gutierrez J.L., Bitter I., Lader M., Waintraub L. (2001) Tianeptine and paroxetine in major depressive disorder, with a special focus on the anxious component in depression: an international, 6-week double-blind study dagger. Hum. Psychopharmacol. 16, 219-227.

Loo H. and Deniker P. (1988) Position of tianeptine among antidepressive chemotherapies. Clin. Neuropharmacol . 11 Suppl 2, S97-102.

Lowy M.T., Wittenberg L., Yamamoto B.K. (1995) Effect of acute stress on hippocampal glutamate levels and spectrin proteolysis in young and aged rats. J. Neurochem . 65, 268-274.

MacQueen G.M., Campbell S., McEwen B.S., Macdonald K., Amano S., Joffe R.T., Nahmias C., Young L.T. (2003) Course of illness, hippocampal function, and hippocampal volume in major depression. Proc. Natl. Acad. Sci. U. S. A. 100, 1387-1392.

Magarinos A.M., Deslandes A., McEwen B.S. (1999) Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. Eur. J. Pharmacol. 371, 113-122.

Malagie I., Deslandes A., Gardier A.M. (2000) Effects of acute and chronic tianeptine administration on serotonin outflow in rats: comparison with paroxetine by using in vivo microdialysis. Eur. J. Pharmacol. 403, 55-65.

Man H.Y., Sekine-Aizawa Y., Huganir R.L. (2007) Regulation of {alpha}-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking through PKA phosphorylation of the Glu receptor 1 subunit. Proc Natl Acad Sci U SA. 104, 3579-84.

Manji, H.K., Drevets, W.C., Charney, D.S. (2001) The cellular neurobiology of depression. Nat. Med. 7, 541–547.

Manji H.K. and Duman R.S. (2001) Impairments of neuroplasticity and cellular resilience in severe mood disorders: implications for the development of novel therapeutics. Sychopharmacol. Bull 35, 5-49.

Manev H, Uz T. (2006) Clock genes as a link between addiction and obesity. Eur. J. Hum. Genet. 14, 5.

Mathé A.A., Husum H., El Khoury A., Jiménez-Vasquez P., Gruber S.H., Wörtwein G., Nikisch G., Baumann P., Agren H., Andersson W., Södergren A., Angelucci F. (2007) Search for biological correlates of depression and mechanisms of action of antidepressant treatment modalities. Do neuropeptides play a role? Physiol Behav. 92, 226-31.

Mayberg H.S., Liotti M., Brannan S.K., McGinnis S., Mahurin R.K., Jerabek P.A., Silva J.A., Tekell J.L., Martin C.C., Lancaster J.L., Fox P.T. (1999) Reciprocal limbic-cortical function and negativemood: converging PET findings in depression and normal sadness. Am. J. Psychiatry 156, 675–682.

Mayberg H.S., Lozano A.M., Voon V., McNeely H.E., Seminowicz D., Hamani C., Schwalb J.M., Kennedy S.H. (2005) Deep brain stimulation for treatment-resistant depression. Neuron 45, 651–660.

Mazzucchelli C. and Brambilla R. (2000) Ras-related and MAPK signaling in neuronal plasticity and memory formation, Cell Mol. Life Sci. 57, 604–611.

McEwen B.S. (2005) Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. Metabolism 54, 20–23.

McEwen B.S. and Olie J.P. (2005) Neurobiology of mood, anxiety, and emotions as revealed by studies of a unique antidepressant: tianeptine. Mol. Psychiatry 10, 525-537.

McEwen B.S., Magarinos A.M., Reagan L.P. (2002) Structural plasticity and tianeptine: cellular and molecular targets. Eur. Psychiatry 17 Suppl 3: 318-330.

Meller E., Shen C., Nikolao T.A., Jensen C., Tsimberg Y., Chen J., Gruen R.J. (2003) Region-specific effects of acute and repeated restraint stress on the phosphorylation of mitogen-activated protein kinase. Brain Res. 979, 57–64.

Mennini T., Mocaer E., Garattini S. (1987) Tianeptine, a selective enhancer of serotonin uptake in rat brain. Naunyn Schmiedebergs Arch Pharmacol. 336, 478-482.

Mizoguchi K., Ishige A., Aburada M., Tabira T. (2003) Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. Neuroscience 119, 887–897.

Nemeroff, C.B. (1996) The corticotropin-releasing factor (CRF) hypothesis of depression: new findings and new directions. Mol. Psychiatry 1, 336–342.

Nibuya M., Morinobu S., Duman R.S. (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments, J Neurosci. 15, 7539–7547.

Novotny V. and Faltus F. (2003) First signs of improvement with tianeptine in the treatment of depression: an analysis of a double-blind study versus fluoxetine. Eur. Neuropsychopharmacol. 13, S230.

Pariante, C.M. (2004) Glucocorticoid receptor function biNin vitrob/iN in patients with major depression. Stress 7, 209–219.

Paul I.A. and Skolnick P. (2003) Glutamate and depression: clinical and preclinical studies. Ann. N. Y. Acad. Sci. 1003, 250-272.

Pine D.S., Cohen P., Johnson J.G., Brook J.S. (2002) Adolescent life events as predictors of adult depression. J. Affect. Disord. 68, 49-57.

Pineyro G., Deveault L., de Montigny C., Blier P. (1995) Effect of prolonged administration of tianeptine on 5-HT neurotransmission: an electrophysiological study in the rat hippocampus and dorsal raphe. Naunyn Schmiedebergs Arch Pharmacol. 351, 119-125.

Pittenger C., Duman R.S. (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 33, 88-109.

Preskorn S.H., Dorey R.C., Jerkovich G.S. (1988) Therapeutic drug monitoring of tricyclic antidepressants. Clin Chem. 34, 822-8.

Qi H, Mailliet F, Spedding M, Rocher C, Zhang X, Delagrange P, McEwen B, Jay TM, Svenningsson P. (2009) Antidepressants reverse the attenuation of the neurotrophic EK/MAPK cascade in frontal cortex by elevated platform stress; reversal of effects on LTP is associated with GluA1 phosphorylation. Neuropharmacology 56, 37-46.

Rapaport M.H. (2007) Dietary restrictions and drug interactions with monoamine oxidase inhibitors: the state of the art. J Clin Psychiatry. 68 Suppl 8, 42-6.

Ridout F. and Hindmarch I. (2001) Effects of tianeptine and mianserin on car driving skills. Psychopharmacology 154, 356-361.

Roberson E.D., English J.D., Adams J.P., Selcher J.C., Kondratick C., Sweatt J.D. (1999) The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. J. Neurosci. 19, 4337–4348.

Roche K.W., O'Brien R.J., Mammen A.L, Bernhardt J., Huganir R.L. (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 16, 1179-1188.

Rocher C., Spedding M., Munoz C., Jay T.M. (2004) Acute stress-induced changes in ippocampal/prefrontal circuits in rats: effects of antidepressants. Cereb. Cortex 14, 224–229.

Rodrigues S.M., Schafe G.E., LeDoux J.E. (2004) Molecular mechanisms underlying emotional learning and memory in the lateral amygdala, Neuron 44, 75–91.

Russo-Neustadt A., Beard R.C., Huang Y.M., Cotman C.W. (2000) Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus, Neuroscience 101, 305–312.

Ryan B., Musazzi L., Mallei A., Tardito D., Gruber S.H., El Khoury A., Anwyl R., Racagni G., Mathé A.A., Rowan M.J., Popoli M. (2009) Remodelling by early-life stress of NMDA receptor-dependent synaptic plasticity in a gene-environment rat model of depression. Int. J. Neuropsychopharmacol. 12, 553-559.

Sanacora G., Rothman D.L., Mason G., Krystal J.H. (2003) Clinical studies implementing glutamate neurotransmission in mood disorders. Ann. N. Y. Acad. Sci. 1003, 292-308.

Santarelli L., Saxe M., Gross C., Surget A., Battaglia F., Dulawa S., Weisstaub N., Lee J., Duman R., Arancio O., Belzung C., Hen R. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805–809.

Schildkraut J.J. (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry. 122, 509-22.

Sheline Y.I. (2003) Neuroimaging studies of mood disorder effects on the brain. Biol. Psychiatry 54, 338-352.

Shen C.P., Tsimberg Y., Salvadore C., Meller E. (2004) Activation of Erk and JNK APK pathways by acute swim stress in rat brain regions. BMC Neurosci. 5, 36–48.

Shirayama Y., Chen A.C.-H., Nakagawa S., Russell D.S., Duman R.S. (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J. Neurosci. 22, 3251–3261.

Siuciak J.A., Lewis D.R., Wiegand S.J., Lindsay R. (1997) Antidepressant-like effect of brain derived neurotrophic factor (BDNF). Pharmacol. Biochem. Behav. 56, 131–137.

Skolnick P., Legutko B., Li X., Bymaster F.P. (2001) Current perspectives on the development of non-biogenic amine-based antidepressants. Pharmacol. Res. 43, 411-423.

Smith M.A., Makino S., Kvetnansky R., Post R.M. (1995) Stress alters the express of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J. Neurosci. 15, 1768–1777.

Stahl S.M. and Felker A. (2008) Monoamine oxidase inhibitors: a modern guide to an unrequited class of antidepressants. CNS Spectr. 13, 855-70.

Stahl S.M., Grady M.M., Moret C., Briley M. (2005) SNRIs: their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants. CNS Spectr. 10, 732-47.

Stimmel, G.L., Dopheide J.A., Stahl S.M. (1997) Mirtazapine: an antidepressant with noradrenergic and specific serotonergic effects. Pharmacotherapy (American College of Clinical Pharmacy).17, 10–21.

Sullivan R.M. and Gratton A. (2002) Prefrontal cortical regulation of hypothalamic-pituitary-adrenal function in the rat and implications for psychopathology: side matters. Psychoneuroendocrinology 27, 99–114.

Svenningsson P, Bateup H, Qi H, Takamiya K, Huganir RL, Spedding M, Roth BL, McEwen BS, Greengard P. (2007) Involvement of AMPA receptor phosphorylation in antidepressant actions with special reference to tianeptine. Eur. J. Neurosci. 26: 3509-3517.

Svenningsson P., Tzavara E.T., Witkin J.M., Fienberg A.A., Nomikos G.G., Greengard P. (2002) Involvement of striatal and extrastriatal DARPP-32 in biochemical and behavioral effects offluoxetine (Pro zac). Proc. Natl. Acad. Sci. U. S. A. 99, 3182–3187.

Sweatt J.D. (2001) The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. J. Neurochem. 76, 1–10.

Szadoczky E. and Furedi J. (2002) Efficacy and acceptability of tianeptine and sertraline in the acute treatment phase of depression. Encephale 28, 343-349.

Thiebot M.H., Martin P., Puech A.J. (1992) Animal behavioural studies in the evaluation of antidepressant drugs. Br. J. Psychiatry Suppl. 15, 44-50.

Wagstaff A.J., Ormrod D., Spencer C.M. (2001) Tianeptine: a review of its use in depressive disorders. CNS Drugs 15: 231-259.

Watanabe Y., Gould E., Daniels D.C., Cameron H., McEwen B.S. (1992) Tianeptine attenuates stress-induced morphological changes in the hippocampus. Eur. J. Pharmacol. 222, 157-162.

Watanabe Y., Sakai R.R., McEwen B.S., Mendelson S. (1993) Stress and antidepressant effects on hippocampal and cortical 5-HT1A and 5-HT2 receptors and transport sites for serotonin. Brain Res. 615, 87-94.

Wilde M.I. and Benfield P. (1995) Tianeptine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depression and coexisting anxiety and depression. Drugs 49, 411-439.

Xu L., Holscher C., Anwyl R., Rowan M.J. (1998) Glucocorticoid receptor and protein/RNA synthesis-dependent mechanisms underlie the control of synaptic plasticity by stress. Proc. Natl. Acad. Sci. U. S. A. 95, 3204–3208.

Xu M., Moratalla R., Gold L.H., Hiroi N., Koob G.F., Graybiel A.M., Tonegawa S. (1994) Dopamine D1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell 79, 729-742.

Yang C.H., Huang C.C., Hsu K.S. (2004) Behavioral stress modifies hippocampal synaptic plasticity through corticosterone-induced sustained extracellular signal-

regulated kinase/mitogen-activated protein kinase activation. J. Neurosci. 24, 11029–11034.

Zarate C.A. Jr., Du J., Quiroz J., Gray N.A., Denicoff K.D., Singh J., Charney D.S., Manji H.K. (2003) Regulation of cellular plasticity cascades in the pathophysiology and treatment of mood disorders: role of the glutamatergic system. Ann. N. Y. Acad. Sci. 1003, 273-291.