Resistance to selective serotonin reuptake inhibitors: role of serotonergic mechanisms

Thesis

Resistance to selective serotonin reuptake inhibitors: role of serotonergic mechanisms

Eleonora Calcagno

Mario Negri Institute for Pharmacological Research, Milan, Italy

in collaboration with the Open University, London, UK

Thesis submitted for the degree of

Doctor of Philosophy

September 2009

Submission date: 30 March 2009
Date of award: 21 Sept. 2009

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ABSTRACT

SSRIs are the mainstay in the treatment of depression. However, approximately 30% of depressed patients do not respond to antidepressants and the causes of resistance to these drugs are largely unknown.

Thus, it is necessary to develop experimental models to investigate the neurobiological changes underlying the response to SSRIs.

The approach used in this project was the inter-strain comparison of mice, such as DBA/2 and C57BL/6, differing in serotonin (5-HT) synthesis, due to a spontaneous mutation of tryptophan hydroxylase-2, the rate-limiting enzyme in brain 5-HT synthesis.

The genotype-dependent deficit of 5-HT was associated with no response to acute and chronic citalopram and paroxetine in the forced swimming test (FST), an experimental procedure to assess the antidepressant potential of drugs, and with lower basal and citalopram-induced rise of extracellular 5-HT.

The administration of the 5-HT precursor, tryptophan, or the blockade 5-HT_{1A} autoreceptors and 5-HT_{2C} receptor-mediated inhibitory feedback reinstated the antidepressant-like effect of citalopram.

These findings suggest that boosting 5-HT neurotransmission might be a useful strategy to restore the antidepressant effect in treatment-resistant depressed patients.

Further studies suggested that GABAergic inhibition is involved in the mechanism by which 5-HT_{2C} receptor inactivation augments the effects of SSRIs whereas the ability of tryptophan to restore the antidepressant-like

effect of SSRIs may involve non-serotonergic mechanisms.

Finally, chronic citalopram induced opposite changes in brain-derived neurotrophic factor (BDNF), a neurotrophin linked to the long-term action of antidepressants, in the nucleus accumbens of DBA/2 and C57BL/6 mice. This suggests a possible role of this protein in the lack of response to SSRIs.

Overall, these studies provide new insight into the role of 5-HT and BDNF in the response to antidepressant drugs and remark on the inter-strain comparison of mice with a spontaneous tryptophan hydroxylase-2 mutation as a useful tool for understanding the mechanism underlying the response to SSRIs and testing new potential therapeutic strategies.

To whom has been able to wait...

Preface

The following body of work was performed at the Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy, during the years 2005-2009, under the direction of Dr. R.W. Invernizzi.

Declaration

This thesis has not been submitted in whole or in part for a degree or diploma or other qualification at any other University. The experimental work described in this thesis was performed by myself and includes work done in collaboration with Dr. L. Cervo and Dr. M. Gobbi.

Publications

Some of the experimental findings have been published:

Calcagno E, Guzzetti S, Canetta A, Fracasso C, Caccia S, Cervo L, Invernizzi RW. Enhancement of cortical extracellular 5-HT by 5-HT1A and 5-HT2C receptor blockade restores the antidepressant-like effect of citalopram in non-responder mice. Int J Neuropsychopharmacol. 2009 Jan 5:1-11. [Epub ahead of print]

Guzzetti S, Calcagno E, Canetta A, Sacchetti G, Fracasso C, Caccia S, Cervo L, Invernizzi RW. Strain differences in paroxetine-induced reduction of immobility time in the forced swimming test in mice: role of serotonin. Eur J Pharmacol. 2008 Oct 10;594(1-3):117-24.

Calcagno E, Canetta A, Guzzetti S, Cervo L, Invernizzi RW. Strain differences in basal and post-citalopram extracellular 5-HT in the mouse medial prefrontal cortex and dorsal hippocampus: relation with tryptophan hydroxylase-2 activity. J Neurochem. 2007 Nov;103(3):1111-20.

Acknowledgements

First I am grateful to Dr. R.W. Invernizzi, the director of this study, for giving me the opportunity to attend the Open University PhD program and for his support and suggestions during these years. I am especially grateful to my supervisor, Dr. M. Rattray, whose suggestions have contributed greatly to the work presented in this dissertation.

My special thanks also go to Sara Guzzetti, Alessandro Canetta, Giusi Sacchetti, Luigi Cervo, Marco Gobbi and Mara Canovi who had helped me in several capacities during the execution of the experiments presented in this dissertation.

I am also grateful to the "Mario Negri" Institute, including Prof. Silvio Garattini, for making available all the facilities necessary for this work.

My greatest thanks go to Mrs. E. Mancini for a fellowship supporting my work and to Ing. P. De Sanctis who founded part of these studies.

A particular thanks to my colleagues for their friendship and support during these years in the lab!

INDEX

LIST OF ABBREVIATIONS

5-HIAA 5-hydroxyindole acetic acid

5-HT serotonin

5-HTP 5-hydroxytryptophan

5-HTT serotonin transporter

AAAD aromatic amino acid decarboxylase

AC adenylate cyclase

aCSF artificial cerebrospinal fluid

ACTH adrenocorticotropin

ANOVA analysis of variance

BDNF brain derived neurotrophic factor

BST brainstem

CMS chronic mild stress paradigm

CRF corticotropin-releasing factor

CSF cerebrospinal fluid

CX rest of the cortex

DH dorsal hippocampus

DMSO dimethylsufoxide

DOPA dihydroxyphenylalanine

DR dorsal raphé nuclei

DSM-IV-TR American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders

ECT Electroconvulsive therapy

Ed extraction fraction

FCX frontal cortex

FRL Flinders Resistant Line

FST forced swim test

FSL Flinders Sensitive Line

GABA γ-Aminobutyric acid

GAD glutamic acid decarboxylase

GLU glutamate

HIPP hippocampus

HPA hypothalamic-pituitary-adrenal axis

ICD-10 World Health Organization's International Statistical Classification of Diseases and Related Health Problems

i.p. intraperitoneal

LH learned helplessness

mAb monoclonal antibody

MAO monoamine oxidase

MAO-A monoamine oxidase A

MAOIs monoamine oxidase inhibitors

mBDNF mature BDNF

MDD Major depressive disorder

mPFC medial prefrontal cortex

MR median raphé nuclei

NA noradrenaline

NAc nucleus accumbens

NET noradrenaline transporter

NRIs noradrenaline reuptake inhibitors

NSD-1015 m-hydroxybenzylhydrazine

OB olfactory bulbectomy

p75NTR p75 low-affinity neurotrophin receptor

pAb polyclonal antibody

pCPA p-chlorophenylalanine

proBDNF pro-form of BDNF

s.c. subcutaneous

SNP single nucleotide polymorphism

SNRIs serotonin/noradrenaline reuptake inhibitors

SSRIs selective serotonin reuptake inhibitors

STAR*D Sequenced Treatment Alternatives to Relieve Depression

TCAs tricyclic and tetracyclic antidepressants

TPH tryptophan hydroxylase

TRD Treatment-resistant depression

TrkB tropomyosin-related kinase B receptor

Trp tryptophan

TST tail suspension test

TTX tetrodotoxin

VNTR variable number tandem repeat

VTA ventral tegmental area

WKY Wistar-Kyoto

LIST OF FIGURES

page

LIST OF TABLES

Chapter 1

GENERAL INTRODUCTION

1.1 Major depressive disorder

Major depressive disorder (MDD) is one of the most disabling diseases in the world and is characterized by a pervasive low mood, loss of interest in usual activities and diminished ability to experience pleasure (Wong and Licinio, 2001; Wong and Licinio, 2004; Belmaker and Agam, 2008; Schechter et al., 2005). It is both biologically and genetically a heterogeneous disorder, with symptoms manifested at the psychological, behavioural and physiological level (Fava and Kendler, 2000; Kaplan and Sadock, 2000). Moreover MDD is associated with significant potential morbidity and mortality (Ebmeier et al., 2006; Kalia, 2005), contributing as it does to suicide, medical illness, disruption in interpersonal relationships, substance abuse, and lost work time (Belmaker and Agam, 2008; Nemeroff, 2007; Licinio and Wong, 2005; Murray and Lopez, 1997).

The course varies widely: it can be a once-in-a-lifetime event or have multiple recurrences; it can appear either gradually or suddenly; and can either last for a few months or be a life-long disorder (Mueller and Leon, 1996).

Depressive disorders come in different forms and the diagnosis can be arbitrary, based on the patient's self-reported experiences and observed behaviour. There are several criteria lists and diagnostic tools that are used to identify and evaluate depression (Fava and Kendler, 2000; Nemeroff, 2007). The most widely used criteria for diagnosing depressive conditions are found in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, the current version being DSM-IV-TR

(American Psychiatric Association., 1994) and the World Health Organization's International Statistical Classification of Diseases and Related Health Problems, currently the ICD-10 (World Health Organization, 1992; Ebmeier et al., 2006; see ***Box 1.1***). The latter system is typically used in European countries, while the DSM criteria are used in the USA and many other non-European nations. Other evaluation scales commonly used include Hamilton Depression Rating Scale designed by psychiatrist Max Hamilton in 1960 (Hamilton, 1960) and the Montgomery-Åsberg Depression Rating Scale (Montgomery and Asberg, 1979).

According to the DSM-IV-TR, major depressive disorder is characterized by one or more major depressive episodes without a history of manic, mixed, or hypomanic episodes. A major depressive episode is characterized by at least 2 weeks during which there is a new onset or clear worsening of either depressed mood or loss of interest or pleasure in nearly all activities. Four additional symptoms must also be present including changes in appetite, weight, sleep, and psychomotor activity; decreased energy; feelings of worthlessness or guilt; difficulty thinking, concentrating, or making decisions; or recurrent thoughts of death or suicidal ideation, plans, or attempts. The episode must be accompanied by distress or impairment in social, occupational, or other important areas of functioning.

Box 1.1 ICD-10 criteria for depression:

Depressive episode

In typical mild, moderate, or severe depressive episodes, the patient suffers from lowering of mood, reduction of energy, and decrease in activity. Capacity for enjoyment, interest, and concentration is reduced, and marked tiredness after even minimum effort is common. Sleep is usually disturbed and appetite diminished. Self-esteem and self-confidence are almost always reduced and, even in the mild form, some ideas of guilt or worthlessness are often present. The lowered mood varies little from day to day, is unresponsive to circumstances and may be accompanied by so-called "somatic" symptoms, such as loss of interest and pleasurable feelings, waking in the morning several hours before the usual time, depression worst in the morning, marked psychomotor retardation, agitation, loss of appetite, weight loss, and loss of libido. Depending upon the number and severity of the symptoms, a depressive episode may be specified as mild, moderate or severe.

Mild depressive episode

Two or three of the above symptoms are usually present. The patient is usually distressed by these but will probably be able to continue with most activities.

Moderate depressive episode

Four or more of the above symptoms are usually present and the patient is likely to have great difficulty in continuing with ordinary activities.

Severe depressive episode without psychotic symptoms

An episode of depression in which several of the above symptoms are marked and distressing, typically loss of self-esteem and ideas of worthlessness or guilt. Suicidal thoughts and acts are common and a number of "somatic" symptoms are usually present.

Severe depressive episode with psychotic symptoms

An episode of depression but with the presence of hallucinations, delusions, psychomotor retardation, or stupor so severe that ordinary social activities are impossible; there may be danger to life from suicide, dehydration, or starvation. The hallucinations and delusions may or may not be mood-congruent.

Modified from: www.who.int/classifications/apps/icd/icd10online/

1.1.1 Symptoms of depression

Not everyone who is depressed experiences every symptom (***Box 1.2***). Some people experience a few symptoms, some many. Severity of symptoms varies with individuals and also varies over time (Shelton, 2007; Schechter et al., 2005; Belmaker and Agam, 2008; Kaplan and Sadock, 2000).

Box 1.2 Symptoms of depression

- *Persistent sad, anxious, or "empty" mood*
- *Feelings of hopelessness, pessimism*
- *Feelings of guilt, worthlessness, helplessness*
- *Loss of interest or pleasure in hobbies and activities that were once enjoyed, including sex*
- *Decreased energy, fatigue, being "slowed down"*
- *Difficulty concentrating, remembering, making decisions, impairments in several cognitive domains, such as memory, learning, attentional set-shifting, psychomotor speed, sustained attention, planning, inhibitory control and problem solving.*
- *Insomnia, early-morning awakening, or oversleeping*
- *Appetite and/or weight loss or overeating and weight gain*
- *Thoughts of death or suicide; suicide attempts*
- *Restlessness, irritability*
- *Persistent physical symptoms that do not respond to treatment, such as headaches, digestive disorders, and chronic pain*

Depression often co-exists with other illnesses that may precede the depressive episode, cause it and/or be a consequence of it. These other co-occurring illnesses need to be diagnosed and treated. They include anxiety disorders such as post-traumatic stress disorder, obsessive-compulsive

disorder, panic disorder, social phobia and generalized anxiety disorder (Gilmer et al., 2005). Alcohol and other substance abuse may also occur with depression. MDD also co-exists with other serious medical illnesses such as heart disease, stroke, cancer, diabetes and Parkinson's disease (Fava and Kendler, 2000).

1.1.2 Epidemiology

Major depressive disorder is one of the most common psychiatric disorders (Murray and Lopez, 1997) and has an incidence of about 4% and a lifetime prevalence estimates in the community range from 12 to 20% in Europe (Paykel et al., 2005). A sixth of people in the community will have major depressive disorder during their lifetime.

It occurs twice as frequently in women as in men, can begin at any age, but has its average age of onset in the mid-20s (Ebmeier et al., 2006; Gilmer et al., 2005). Only between a quarter and half of patients will be in contact with the health services for their depression. In half the cases, the illness is incapacitating, leading to role impairment at work or at home. The risk of premature death is increased, in part because of a greater risk of suicide (Nestler et al., 2002; Ebmeier et al., 2006).

Fifty to 60% of individuals who have had a single major depressive disorder episode can be expected to have a second episode. Relapses often occur 5-10 years after first presentation and residual disability is common.

Epidemiologic studies show that about 30-40% of the risk for depression is genetic (see *section 1.4*). In addition, vulnerability to depression is only partly genetic, with nongenetic factors also being important.

The nongenetic factors implicated in the etiology of depression are gender, stress and emotional trauma, adverse childhood experiences, viral infections (e.g., Borna virus), medical illnesses (including Cushing's disease, hypothyroidism, multiple sclerosis, Huntington's disease, Parkinson's disease, myocardial infarction, stroke, diabetes, cancer, and rheumatoid arthritis) and even stochastic processes during brain development (Kaplan and Sadock, 2000; Fava and Kendler 2000; Belmaker and Agam 2008). Also certain personality traits have been consistently associated with MDD, with the best evidence available for the trait termed "neuroticism". Neuroticism is a stable personality trait that reflects the level of emotional stability versus the predisposition to develop emotional upset under stress (Fava and Kendler 2000).

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, the largest prospective, randomized treatment study to date of outpatients with MDD (Rush et al., 2003) found that chronic depression is associated with older age, ethnicity, unemployment, lack of private medical insurance, lower income and lower educational level (Gilmer et al., 2005).

The PREDICT study (2008) that took place in six European countries estimated the higher prevalence in the UK and Spain for all mental disorders, with men aged 30-50 and women aged 18-30 with the highest prevalence of MDD (King et al., 2008).

A recent WHO report (***Box 1.3***) ranked depression as the fourth medical condition with the greatest disease burden worldwide, measured in Disability-Adjusted Life Years, which express years of life lost to premature death and years lived with a disability of specified severity and duration.

The same report predicted that depression would be the second condition with the greatest disease burden worldwide by 2020.
Moreover it estimates that depression can lead to suicide, a tragic fatality associated with the loss of about 850,000 lives worldwide every year.

Studies also show that MDD contributes to higher mortality and morbidity in the context of other medical illnesses, such as myocardial infarction, and that successful treatment of the depressive episode improves medical and surgical outcomes (Zellweger et al., 2004; Keck, 2006).

Considering that about 21 million people in Europe suffer for depression (Sobocki et al., 2006), the economic cost for this disorder is high, but the cost in human suffering cannot be estimated. Depressive illnesses often interfere with normal functioning and cause pain and suffering not only to those who have a disorder, but also to those who care about them. Serious depression can destroy family life as well as the life of the ill person.

Box 1.3 WHO REPORT 2008

It establishes that:

- *Depression is common, affecting about 121 million people worldwide.*
- *Depression is among the leading causes of disability worldwide.*
- *Depression can be reliably diagnosed and treated in primary care.*
- *Fewer than 25% of those affected have access to effective treatments.*

Depression can be reliably diagnosed in primary care. Antidepressant medications and brief, structured forms of psychotherapy are effective for 60-80 % of those affected and can be delivered in primary care. However, fewer than 25% of those affected (in some countries fewer than 10%) receive such treatments. Barriers to effective care include the lack of resources, lack of trained providers, and the social stigma associated with mental disorders including depression.

Primary care based quality improvement programs for depression have been shown to improve the

- *quality of care,*
- *satisfaction with care*
- *health outcomes,*
- *functioning,*
- *economic productivity,*
- *and household wealth at a reasonable cost*

(www.who.int/mental_health/management/depression)

1.2 Antidepressant treatments

Even if the pathophysiology of depression is still poorly understood and the disorder is often underdiagnosed and undertreated, many effective treatments are available.

Mild depression responds to different forms of psychotherapy. More severe forms of depression respond to a host of antidepressant medications, with a combination of medication and psychotherapy providing optimal treatment. Electroconvulsive therapy (ECT) is one of most effective treatments for depression, but is usually reserved for the more severely ill (Berton and Nestler, 2006).

All available antidepressants are based on serendipitous discoveries made in the 1950s and act via the monoamine neurotransmitters (***Table 1.1***).

Several controlled trials have established the antidepressant efficacy of medications that modulate monoaminergic neurotransmission, primarily the serotonin (5-HT) and noradrenaline (NA) systems. These medications typically act by inhibiting reuptake of NA and/or 5-HT from the synapse, inhibiting monoamine oxidase (MAO), the enzyme that degrades these neurotransmitters, or acting at receptors that modulate monoaminergic transmission. Monoamine neurotransmission modulators include the tricyclic and tetracyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), noradrenaline reuptake inhibitors (NRIs), selective serotonin reuptake inhibitors (SSRIs), serotonin/noradrenaline reuptake inhibitors (SNRIs) and various "atypical" antidepressants with less well-described mechanisms of action (Fava and Kendler, 2000; White et al., 2005;

Schechter et al., 2005; Nemeroff, 2007; Holtzheimer and Nemeroff, 2006; Millan, 2006).

Drug treatments remain the mainstay of antidepressant therapy but clinical therapy also includes physical treatments as ECT and focal brain stimulation (see ***Table 1.1***).

***Table 1.1** Current antidepressant treatments*

ANTIDEPRESSANT DRUGS	
Class	**Example**
TCAs	Amitriptyline Amoxapine Clomipramine Doxepin Imipramine Trimipramine Desipramine Nortriptyline Protriptyline
NRIs	Reboxetine
MAOIs	Isocarboxazid Phenelzine Selegiline Tranylcypromine
SSRIs	Citalopram Escitalopram Fluoxetine Fluvoxamine Paroxetine Sertraline
SNRIs	Duloxetine Venlafaxine
Atypical	Bupropion Buspirone Mirtazapine Nefazodone Trazodone
ANTIDEPRESSANT TREATMENTS	
ECT	
Focal Brain Stimulation	VNS TMS MST DBS

Tricyclic and tetracyclic antidepressants (TCAs); noradrenaline reuptake inhibitors (NRIs); monoamine oxidase inhibitors (MAOIs); selective serotonin reuptake inhibitors (SSRIs); serotonin/noradrenaline reuptake inhibitors (SNRIs); electroconvulsive therapy (ECT); vagus nerve stimulation (VNS); transcranial magnetic stimulation (TMS); magnetic seizure therapy (MST); deep brain stimulation (DBS).

Despite the availability of effective treatment, we are still faced with the dilemma that MDD is widely under-recognized and undertreated, time to response may take several weeks and relapse is common. Furthermore side effects (***Box 1.4***) are still a serious problem even with the newer medications (Berton and Nestler, 2006; Carrasco and Sandner, 2005; Edwards and Anderson, 1999).

Recently there has been a controversy about the real efficacy of antidepressant medications. Over a thousand randomized trials have been conducted with antidepressant drugs and statistically significant benefits have been repeatedly demonstrated. However, two recent meta-analyses bring into question their usefulness. The first meta-analysis used data submitted to Food and Drug Administration (FDA) for the approval of 12 antidepressants (Turner et al., 2008). While only half of these trials had significant effectiveness, published reports almost ubiquitously claimed significant results. Negative trials were left unpublished. A second meta-analysis using also FDA-submitted data examined the relationship between treatment effect and baseline severity of depression (Kirsch et al., 2008). Drug-placebo differences increased with increasing baseline severity and the difference became large enough to be clinically important only in few patients with severe major depression. These data suggest that antidepressants may be less effective than their wide marketing suggests. However Randomized Controlled Clinical Trials, usually lasting 6 weeks, may not adequately reflect the chronic course of the disease and overestimate the placebo effect. In fact, it is important to consider the effectiveness of antidepressant along time and the impact of depression if it is not treated (*see* Blier, 2008). This debate opens the question if antidepressants really

work and the need of investigate on biological, pharmacokinetic and genetic factors implicated in the efficacy of the antidepressant treatment.

Box 1.4 General side effects of depression medication

- *Dry mouth*
- *Urinary retention*
- *Blurred vision*
- *Constipation*
- *Sedation (can interfere with driving or operating machinery)*
- *Sleep disruption*
- *Weight gain*
- *Headache*
- *Nausea*
- *Gastrointestinal disturbance/diarrhoea*
- *Abdominal pain*
- *Inability to achieve an erection*
- *Inability to achieve an orgasm (men and women)*
- *Loss of libido*
- *Agitation*
- *Anxiety*

One key factor in the lack of diagnostic tests for depression is the limited knowledge of the brain regions and neural circuits involved.

The broad range of symptoms of depression suggests that many brain regions might be involved (Berton and Nestler, 2006; Fava and Kendler, 2000). This is supported by human brain imaging studies that have shown changes in blood flow or related measures in several brain areas, including regions of the prefrontal and cingulate cortex, hippocampus, striatum, amygdala and thalamus (Mayberg, 2003; Ressler and Mayberg, 2007).

1.2.1 Resistance to antidepressant treatments

One of the still unsolved problems is that fewer than 50% of all patients with depression show full remission with optimized treatment (Fava and Kendler, 2000). Therefore, there is still a great need for more effective treatments for depression.

Almost half of depressed patients continue to have some residual depressive symptoms despite adequate treatment and up to 20% may show minimal or no response to even the most aggressive interventions (Fava and Kendler, 2000).

However, because no one treatment is universally effective for everyone, many depressed patients do not experience a satisfactory clinical benefit from the treatment received. Some patients respond to one treatment, some to another, and some may require the combination of two or more treatments.

Treatment-resistant depression (TRD) typically refers to the occurrence of an inadequate response following adequate antidepressant therapy (Fava, 2003). Adequate antidepressant therapy is typically considered to consist of one or more trials with antidepressant medications with established efficacy in major depressive disorder and at the effective doses (Quitkin, 1985) and duration (Quitkin et al., 1986). Thase and Rush (1997) first proposed a model of staging the various levels of resistance in TRD.

1) nonresponse (< 25% symptom reduction from baseline); 2) partial response (25% to 49% symptom reduction from baseline); and 3) response without remission (50% or greater symptom reduction from baseline without achieving remission). Using such classification, a meta-analysis of clinical trials found that, among antidepressant-treated depressed patients,

partial response occurs in 12% to 15%, and nonresponse in about 30% (Fava and Davidson, 1996; Stimpson et al., 2002).

STAR*D study was aimed at defining which subsequent treatment strategies, in what order or sequence, and in what combination(s) (***Figure 1.1***) are both acceptable to patients and provide the best clinical results with the least side effects (*www.star-d.org*; Rush et al., 2003). The results obtained suggest potential benefit for using more vigorous treatments in the earlier steps (Warden et al., 2007).

The STAR*D trial enrolled 4,041 outpatients and the first results reported that only about 30% of patients remitted during citalopram treatment. Remission rate at the end of two treatments step (level 2, ***Figure 1.1***) was approximately 50% and there were no differences in remission rate or time to remission among medication switching or augmentation strategies (Warden et al., 2007).

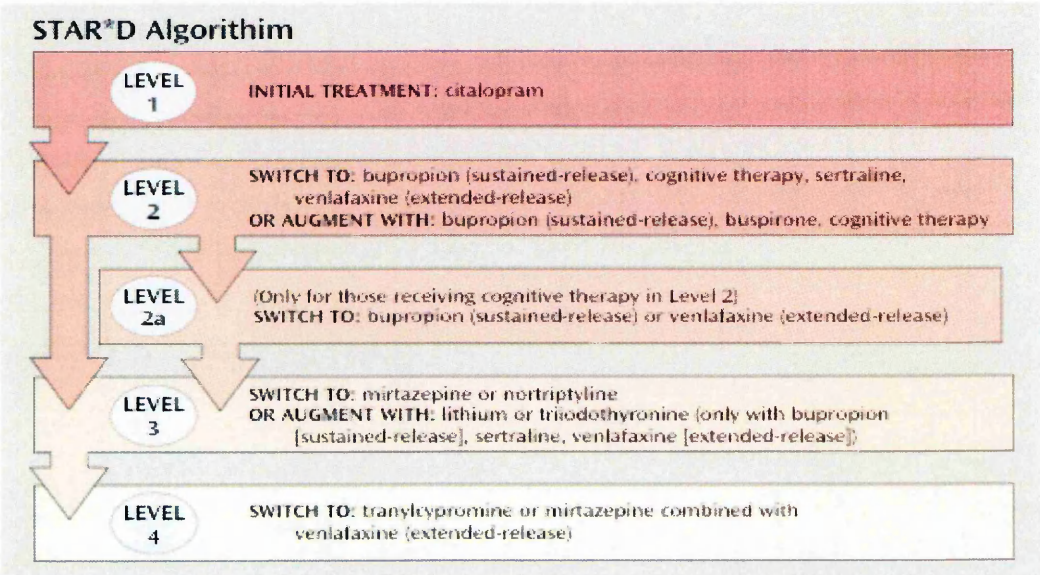


Figure 1.1 STAR*D PROJECT

In the management of TRD it was suggested to increase the dose of antidepressant, switching to a different class of antidepressant drugs, adding psychotherapy or augmenting with lithium (Stimpson et al., 2002; McAllister-Williams, 2006) or more recently, with the β-adrenoceptor/5-HT_{1A-1B} receptor antagonist, pindolol (Artigas et al., 1994; Artigas et al., 1996; *for review see* Nelson, 2000). The lack of clear rationale behind most of these strategies reflects the still scarce knowledge on the factors underlying treatment response. Therefore researche into the neurobiological, neuroanatomical and genetic bases for depression could suggest promising new directions for antidepressant treatment development.

1.3 Dysregulation of the Hippocampus and Hypothalamic-Pituitary-Adrenal Axis (HPA)

A prominent mechanism by which the brain reacts to acute and chronic stress is activation of the hypothalamic-pituitary-adrenal (HPA) axis (***Figure 1.2***). Neurons of the hypothalamus secrete corticotropin-releasing factor (CRF), which stimulates the synthesis and release of adrenocorticotropin (ACTH) from the anterior pituitary. ACTH then stimulates the synthesis and release of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal cortex that exerts profound effects on general metabolism and affects behaviour via direct actions on numerous brain regions (Wong and Licinio 2004; Pariante and Lightman, 2008).

The activity of the HPA axis is controlled by several brain pathways, including the hippocampus and the amygdala. Glucocorticoids, regulating hippocampal and hypothalamic neurons, exert powerful feedback effects on the HPA axis. Levels of glucocorticoids under normal physiological condition enhance hippocampal inhibition of HPA activity and cognitive function of the hippocampus. However, sustained elevations of glucocorticoids, after prolonged and severe stress, may damage hippocampal neurons, reducing dendritic branching and the highly specialized dendritic spines. Stress and the resulting hypercortisolemia also reduce the birth of new neurons in the adult hippocampal dentate gyrus (Nestler et al., 2002).

Hypercortisolemia and abnormal activation of the HPA axis have been observed in approximately half of individuals with depression, and these abnormalities are corrected by antidepressant treatments. Several patients exhibit increased cortisol production, as measured by increases in urinary

free cortisol and decreased ability of the potent synthetic glucocorticoid, dexamethasone, to suppress plasma levels of cortisol, and ACTH (*for review see* Pariante and Lightman, 2008). Animal studies are consistent with human data. Maternal deprivation in rodents (see *section 1.5*) induces abnormalities in HPA axis function, which resemble those seen in some depressed humans. These abnormalities can persist into adulthood and can be corrected by antidepressant treatments (Nestler et al., 2002).

Hovewer, it is still unknown whether HPA axis abnormalities are a primary cause of depression or, instead, secondary to some other initiating cause. Nevertheless, there is growing evidence that glucocorticoid receptor antagonists may be useful in treating some cases of depression (Fava and Kendler, 2000; Nestler et al., 2002).

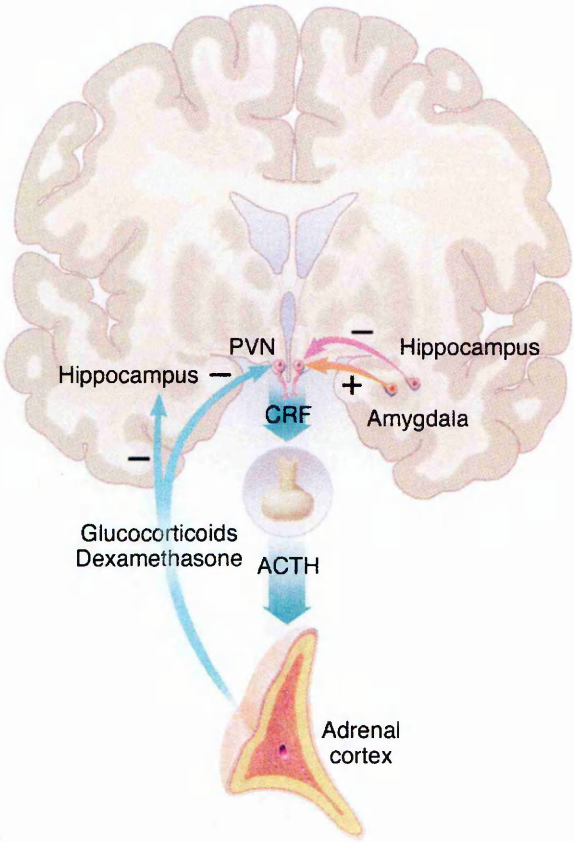


***Figure 1.2** Regulation of the Hypothalamic-Pituitary-Adrenal Axis*

CRF-containing neurons of the paraventricular nucleus (PVN) of the hypothalamus integrate information relevant to stress.
The figure shows excitatory afferents from the amygdala and inhibitory afferents from the hippocampus. CRF is released by these neurons into the hypophyseal portal system and acts on the corticotrophs of the anterior pituitary to release ACTH. ACTH reaches the adrenal cortex via the bloodstream, where it stimulates the release of glucocorticoids. At higher levels, glucocorticoids also impair, and may even damage, the hippocampus, which could initiate and maintain a hypercortisolemic state related to some cases of depression.

1.4 Genetics of depression and antidepressant response

Several lines of evidence suggest that genetic factors partially influence overall risk of illness but also influence the sensitivity of individuals to the "depressogenic" effects of environmental adversity.

Because of the pattern of inheritance in MDD, but also because of heterogeneity of clinical samples, no genes of major effect have been identified (Lesch, 2004). A multitude of genes with small effects are likely to be identified, which will be related to certain aspects of genetic vulnerability to depression and will work alongside or interact with environmental factors. Different points of vulnerability in the brain may predispose to depressive disorder (Shelton, 2007; Wurtman, 2005; Bolonna et al., 2004).

Investigation of gene–environment interactions in humans and nonhuman primates, as well as gene inactivation studies in mice, have further advanced the identification of genes that are essential for the development and plasticity of brain systems related to depression (Lesch, 2004).

Studies between monozygotic and dizygotic twins suggest a heritability of about 37% (Sullivan et al., 2000). Some aspects of the normal personality, such as avoidance of harm, anxiousness, and pessimism, are also partly heritable. Kendler et al. (2005) showed that although depression is due in part to heritable depression-prone personality traits, it is also the result of heritable factors that are independent of personality. Early-onset, severe, and recurrent depression may have a higher heritability than other forms of depression (Kovacs et al., 1997; Kendler et al., 1999).

The efficacy of antidepressant action was associated with several polymorphisms, located on coding genes of proteins thought to be involved in the different mechanisms of action of antidepressant treatments. Most of the published genetic association studies with antidepressant response (Belmaker and Agam, 2008; Levinson, 2006; Serretti et al., 2008; Serretti and Artioli, 2004; Serretti et al., 2005a; Lin and Chen, 2008; Li and He, 2006; Tsai et al., 2003) have focused on polymorphisms in the loci encoding the serotonin transporter (5-HTT), receptors, biosynthetic and metabolic enzymes, some dopamine receptors, and noradrenaline transporter (NET) (summarized in ***Table 1.2***).

Table 1.2 Pharmacogenetic association studies on antidepressant response

Gene	Polymorphism	Results
5-HTT	5-HTTLPR	l allele subjects were more likely to responde to Fluvoxamine and Fluoxetine
	5-HTTLPR	s allele is associated with less favourable and slower response to Paroxetine in caucasian population
	5-HTTLPR	s/s showed better response to SSRIs in asiatic population
TPH-1	A779C A218C	A/A and A/C genotypes are associated with slower response to SSRIs
	A779C A218C	strong association with suicide
5-HT$_{2A}$	T102C	contrasting results in association with various antidepressants
	A1438G	contrasting results in association with various antidepressants
5-HT$_{6}$	C267T	lack of association with antidepressant response
G protein beta3	C825T	T/T is associated with response to various antidepressant treatments
DRD2	S311C	lack of a clear association
DRD4	Variable Number Tandem Repeats	lack of a clear association
MAO	Variable Number Tandem Repeats	no association with various antidepressant treatments
5-HT$_{1A}$	C1019G	C allele is associated with a better response to antidepressants
NET	T182C	T allele is associated with a better response
	G1287A	G allele is associated with a faster response

The most frequently examined candidate gene codes for the serotonin transporter (5-HTT, see *section 1.5.1*).

Ramamoorthy et al. (1993) identified and cloned a single gene encoding the human 5-HTT. Heils et al. (1996) reported a polymorphism in the transcriptional control region of the 5HTT coding sequence. It consists of a 44-bp insertion or deletion, resulting in a long (l) or short (s) variant of this gene. Lesch et al. (1996) called it 5-HTTLPR and found that the basal activity of the long variant in vitro was more than twice that of the short form of the 5HTT gene promoter. It causes reduced uptake of 5-HT into the presynaptic neurones (Lesch and Gutknecht, 2005).

More than twelve different human behavioural traits and several medical disorders are associated with 5-HTT gene variation (Murphy and Lesch, 2008). This contains a regulatory variation that has been associated with anxiety, susceptibility for depression, emotion and social cognition (Canli and Lesch, 2007). Clinical studies show that individuals in an epidemiological sample with one or two copies of the short allele of the 5-HTT promoter polymorphism showed more depressive symptoms, diagnosable depression, and tendency to commit suicide in relation to stressful life events than individuals homozygous for the long allele (Caspi et al., 2003).

In a large, prospective epidemiologic study, Caspi et al. (2003) have demonstrated a causal interaction between the polymorphic variant of the 5HTT, early and late life adversity, and the occurrence of depression. They found that 5-HTTLPR genotype influenced stress reactivity rather than directly causing depression.

The occurrence of stressors conferred increased risk primarily in persons carrying the s allele.

However there is no consensus in the literature (Kendler et al., 2005; Arango et al., 2003) on the association of the 5-HTTLPR genotype with mood disorders. Some authors reported that patients with two alleles of the l-variant generally have increased 5-HT uptake, 5-HTT binding sites and mRNA in brain and platelets (Hanna et al., 1998; Little et al., 1998; Greenberg et al., 1999) and show a better clinical response to SSRIs in studies of mainly white patients but not in Asian population (Serretti et al., 2008; Kim et al., 2000; Kim et al., 2006). Other studies reported no association between 5-HTTLPR genotype and depression (Mann et al., 2000; Minov et al., 2001) and others found an association between the s-allele (Neumeister et al., 2002; Joiner et al., 2003) or the l-allele and depression (Moreno et al., 2002). Therefore the functional link between this polymorphism and altered 5-HT transmission remains unclear.

Pharmacogenomic studies of subjects in the STAR*D trial have included analyses of the 5-HTT in relation to remission and response to citalopram. These findings do not replicate previous studies with smaller sample size; in fact, it failed to detect an association between any of the polymorphisms of 5-HTT and the SSRI response (Kraft et al., 2007). Nevertheless, a recent report (Mrazek et al., 2008) found an association between multiple variations in the gene encoding for 5-HTT with remission after citalopram in white non-Hispanic subjects of the STAR*D study, in particular in patients of European origin. This suggests that ethnicity is an important factor to account for response to antidepressant drugs.

Moreover, in association studies, 5-HTTPR was independently linked to efficacy for a range of treatments, other polymorphisms located on the tryptophan hydroxylase (TPH) gene, 5-HT_{2A} receptor and G-protein beta 3 (Arango et al., 2003; Chotai et al., 2003; Murphy and Lesch, 2008; Lesch and Gutknecht, 2005). Furthermore variants of the gene encoding for 5-HT_{1A} receptor combined to polymorphism of the 5-HTT could be associated to the response to SSRIs (Arias et al., 2005).

Other analysed polymorphisms belong to the synthetic and catabolic pathways of 5-HT.

Tryptophan hydroxylase 1 (TPH-1) is the rate-limiting enzyme of peripheral 5-HT synthesis. Many investigators have examined intronic variants of the TPH-1 gene, but after the discovery of a second isoform of TPH, named TPH-2, responsible for brain 5-HT synthesis (Walther et al., 2003) and localized exclusively in the brain (Zill et al., 2004a), most attention has shifted to this enzyme.

The best-studied TPH-1 variants are two polymorphisms, on position 218 (A218C) and 779 (A779C) of intron 7. These polymorphism were associated with suicidal behaviour and, in several studies, A allele was associated with a poorer response to SSRIs compared to C/C subjects (*for review see* Serretti et al., 2005), even if STAR*D study failed to found an association (*for review see* Lin and Chen, 2008). Nevertheless, it is surprising that a polymorphism located on TPH-1 could influence the antidepressant response. A possible explanation may be that TPH-1 partially contributes to 5-HT synthesis in the human brain. In fact, TPH-1 mRNA is 25% of TPH-2 in the human raphé nuclei and similar or even predominant expression of TPH-

1 is found in hypothalamus, amygdala, cortex, thalamus, hippocampus, and cerebellum (Zill et al., 2007).

Recently, pharmacogenetic studies showed that the functional polymorphism of TPH-2, is linked to increase risk of depression (Van Den Bogaert et al., 2006) and the lack of response to SSRIs (Zhang et al., 2005). The authors identified a single nucleotide polymorphism (SNP), G1463A, in which the highly conserved Arg 441 is replaced with His (R441H) and resulted in about 80% loss of function in 5-HT production when mutant TPH-2 was expressed in PC12 cells. Identification of a loss-of-function mutation in TPH-2 suggested that defect in brain 5-HT synthesis may represent an important risk factor for MDD. Although independent researchers confirmed the existence of the polymorphism G1463A in the same sample analyzed in the study of Zhang et al. (2005), other studies have been unable to find it in different populations of depressed patients (Blakely, 2005; Henningsson et al., 2007) suggesting that this polymorphism is not common in depressed populations. SNP association and haplotype studies have indicated that other polymorphisms in the promoter and surrounding regions of TPH-2 (for example rs1386494; A40237G; rs11178997; A22879G) may be associated with MDD and suicide victims (Haghighi et al., 2008; Zill et al., 2004b; Ke et al., 2006; Van Den Bogaert et al., 2006). A recent study reported an association between polymorphisms in the TPH-2 gene (rs10897346 and rs1487278) and the antidepressant drug response, in particular with SSRIs (Tzvetkov et al., 2008).

A variable number tandem repeat (VNTR) in the regulatory region of the gene for monoamine oxidase A (MAO-A), the principal enzyme for the degradation of monoamines, has been shown to affect the transcriptional activity of the MAO-A gene promoter but no clear association with several antidepressant treatments was found (Serretti and Artioli, 2004; D'Souza and Craig, 2006; Lin and Chen, 2008).

Other possible candidate genes for pharmacogenetic studies were those coding for serotonin receptors. A polymorphism in the promoter region of the $5HT_{1A}$ gene was reported; it is a G to C substitution at position - 1019, and the homozygous G allele was more represented in depressed patients compared to controls (Serretti et al., 2005a). Moreover female patients carrying the C/C genotype showed a better response to SSRIs compared to G carriers (Lin and Chen, 2008).

Three polymorphisms on the gene encoding for $5\text{-}HT_{2A}$ receptor were investigated: two in the promoter region (A-1438G and C-1420T) and a silent substitution in position 102 (T102C). Contrasting results were found in association studies between these polymorphisms and antidepressant response (*for review see* Serretti et al., 2005b; D'Souza and Craig, 2006). Recently the STAR*D study reported to detect a significant association between rs7997012 polymorphism in HTR2A gene and response to SSRIs (Lin and Chen, 2008).

A silent T267C polymorphism within the $5\text{-}HT_6$ receptor gene was investigated by Wu et al. (2001) but results on association with

antidepressant response were contradictory (Wu et al., 2001; Lee et al., 2005).

Also polymorphisms in dopamine receptor genes were analyzed. However, studies testing the S311C and the VNTR polymorphisms respectively in D2 and D4 receptors genes failed to show evidence of associations with antidepressant response (Serretti and Artioli, 2004; Serretti et al., 2005b).

Regarding the noradrenergic pathway, an association was demonstrated for the T allele of the T182C polymorphism in the gene encoding for the NA transporter (NET) with a better antidepressant response. In addition the G allele of the NET G1287A was associated with a faster therapeutic response (Yoshida et al., 2004).

Signal transduction pathways are also possibly involved in the response to treatment with antidepressants. The majority of the studies focused on the Gβ3 subunit (GNB3). T/T genotype of the C825T polymorphism was associated with better response to antidepressant treatments. However no association was found between citalopram outcome and GNB3 gene in the STAR*D reports (*for review see* Lin and Chen, 2008).

1.5 Animal models of depression

The understanding of MDD psychopathology relies on the availability of experimental models potentially mimicking the disease (El Yacoubi et al., 2003).

It is generally thought that the development of better validated, and more appropriate, animal model is a task of major importance for psychiatry. However, giving the wide range of symptoms, both somatic and cognitive, and the disease heterogeneity it is very difficult to develop and validate reliable models of depression. Moreover, two human symptoms, recurring thoughts of death or suicide, and excessive thoughts of guilt, are impossible to model in laboratory animals (Cryan et al., 2002).

McKinney and Bunney (1969) proposed that the minimum requirements for a valid animal model of depression are that:

1) it is "reasonably analogous" to the human disorder in its manifestations or symptoms;

2) there is a behavioural change that can be monitored objectively;

3) the behavioural changes observed should be reversed by the same treatments effective in humans;

4) it should be reproducible between investigators.

These principles provide a valuable guide to modelling depression or endophenotypes of this disorder in the animal (Dalvi and Lucki, 1999; Cryan et al., 2002).

However many criteria have to be considered to evaluate the validity of an animal model in particular the predictive, face and construct validity (*for review see* Willner and Mitchell, 2002; Willner, 1995).

The predictive validity is defined as the extent to which the model correctly identifies antidepressant treatments and how the potency in the model correlates with clinical potency. This concept implies that manipulations known to influence the pathological state should have similar effects in the model. In practice, the predictive validity of animal models of depression is determined largely by their response to antidepressant drugs.

Construct validity defines the theoretical rationale underlying the model. Theories of depression that could be used to evaluate the construct validity of animal models could relate to neurobiological mechanisms, aetiology or psychological mechanisms.

Face validity for an animal model of depression refers to a phenomenological similarity between the model and the disorder representing how well the model resembles the human depressive state. It takes account of the necessity, or not, to use chronic administration to have an antidepressant effect and the specificity of observed features.

These three sets of validation criteria provide a valid support to set up an animal model of depression and ensure that different models are comparable.

Various paradigms have been developed (Cryan and Slattery, 2007) and are used in detecting the antidepressant-like potential of novel compounds in preclinical settings (McArthur and Borsini, 2006). Basically, animal models of depression are mainly based on the concept that an unavoidable stress produces behavioural changes reminiscent of aspects of depression, which are typically reversible with antidepressant drugs. This has some

analogies with the observation that episodes of major depressive disorder are frequently precipitated by exposure to stressors (Kessler, 1997).

Although not initially developed as an animal model of depression, the "learned helplessness" (LH) is based on the fact that following repeated uncontrollable shocks, rodents demonstrate escape deficits that are reversible by antidepressant agents (Cryan et al., 2002; Maier and Watkins, 2005). The exposure to an inescapable electric shock induces an impairment of escape behaviour and leads to debilitating consequences, including a "depressive-like" phenotype.

Many of the more recently developed models, however, are based not on stress exposure and symptom precipitation, but on long-term manipulations that are better considered as modelling a predisposition to depression, rather than a depressive response to a precipitating event.

The chronic mild stress paradigm (CMS, *for review see* Willner, 1997; Willner, 1995; Cryan and Holmes, 2005) was developed with the objective of modelling anhedonia, a core symptom of depression that would be reversed by chronic antidepressant treatments. By chronic exposure to mild stressors such as restraint, wet bedding, constant lighting, food deprivation and novel housing, the uncontrollable stress induces a decrease in sensitivity to reward and long-term behavioural, neurochemical, neuroimmune and neuroendocrine alterations that resemble those observed in depressed patients. The CMS model fulfils the criteria of validity previously described.

The experimental procedures sensitive to antidepressant treatments include also the forced swim test (FST) (also known as Porsolt's test; Porsolt et al., 1977a), which is probably the most widely and most

frequently used paradigm (Dalvi and Lucki, 1999; Cryan et al., 2002; Cryan and Holmes, 2005). The FST is based on the observation that rodents placed in an enclosed (inescapable) cylinder filled with water will initially engage in vigorous escape-orientated movements, but then within minutes will exhibit increasing immobility. Even if it does not fully respond to construct and face validity criteria, the FST responds to effective antidepressant treatments, is easy to perform and, from an ethical point of view, it requires only one (for mice) or two (for rats) exposure to a stressor. A related task is the tail suspension test (TST, Steru et al., 1985; Cryan et al., 2005b), in which mice suspended by the tail also exhibit passive immobility after minutes of futile struggling. Antidepressant drugs, given before either test, induce mice to actively engage in escape-directed behaviours, reducing the immobility time. These tests are also frequently used as phenotypic screens for depression-related behaviours in mutant mice (Cryan and Mombereau, 2004).

Other animal models of depression are used, including social stress, in which animals are exposed to various types of stress (proximity to dominant males, odors of natural predators) and show behavioral abnormalities; early life stress (animals are separated from their mothers at a young age and show some persisting behavioral and HPA axis abnormalities as adults, some of which can be reversed by antidepressant treatments); fear conditioning, in which animals show fear-like responses when exposed to previously neutral cues or context that has been associated with an aversive stimulus; reward-based tests (animals show highly reproducible responses to drugs of abuse in classical conditioning and operant conditioning assays); bilateral olfactory bulbectomy (OB) that

reliably mimics many of the neurochemical and behavioural changes seen in human depression, and the delayed onset of therapeutic action with antidepressants (Cryan and Holmes, 2005; Nestler et al., 2002; Wong and Licinio, 2004; Cryan and Slattery, 2007).

Genetic research on animal models consists primarily of inbred strain and selection studies. Different inbred strains of mice (Cryan et al., 2002; Cryan and Mombereau, 2004; Jacobsen et al., 2008) or rats (Lahmame et al., 1997; Malkesman et al., 2006; El Yacoubi and Vaugeois, 2007) show remarkable differences in measures of depression-related and anxiety-related behaviour (Cryan and Holmes, 2005; Jacobson and Cryan, 2007; Cryan et al., 2005a; Cryan et al., 2002). In particular, mice strains are used to identify genetic loci that contribute to behavioural traits, including fearfulness and emotionality. Comparisons between different strains (El Yacoubi and Vaugeois, 2007) include animals susceptible to learned helplessness (Cryan et al., 2002; Vollmayr and Henn, 2001), high and low FST responders (David et al., 2003; Jacobson and Cryan, 2007) and animals with high or low immobility time in the TST (Cryan et al., 2005b).

The Flinders Sensitive Line (FSL) rat is the result of selective breeding for sensitivity to the hypothermic effect of cholinergic agonists (*for review see* Overstreet et al., 1995; Overstreet et al., 2005). This line of rats exhibits behavioural features characteristic of depression, and responds to chronic, but not acute, antidepressant treatments. They also show greater immobility in the FST compared to the control Flinders Resistant Line (FRL). This model, therefore, shows some features of depression, but there are

also some oddities in contrast to those observed in depressed patients, for example there is a reduced HPA axis activity.

As shown in ***Table 1.3***, genetic models have been developed based on an underlying alteration in the function of a selective neurotransmitter system. So far, about 80 different mutant lines have been reported to have phenotypes interpreted as abnormal "depression-related" or "anxiety-related" behaviour (Cryan et al., 2002; Cryan and Holmes, 2005). Molecular techniques such as gene knockout partially support the monoamine theory of depression (see *section 1.5*).

Some examples of knockout mice, such as those with targeted deletion of the 5-HT_{1A} receptor and the NET, are expected to show antidepressant-related phenotypes given the large body of evidence implicating these proteins in antidepressant action (Ramboz et al., 1998; Perona et al., 2008).

Moreover, several findings in mice with a targeted inactivation of the 5-HTT gene emphasize the relevance of adaptive 5-HT uptake function and 5-HT homeostasis in the developing human brain as well as molecular processes underlying anxiety and depressive- related traits. (Lesch, 2004; Murphy and Lesch, 2008; Perona et al., 2008; Kim et al., 2005).

Both genetic and behavioural strategies could be used as complementary and together may yield further information about the disease (Gould and Gottesman, 2006).

Table 1.3 Some genetically altered mice with depressive or antidepressive phenotype

Gene	Results
5-HTT knockout	Antidepressant-like effects in the FST and TST
5-HT_{1A} receptor knockout mouse	Antidepressant-like effects in the FST and TST
5-HT_{1B} receptor knockout mouse	Increase sensivity to the effects of SSRIs in the TST
	Blockade of antidepressant-like effects of various antidepressants in the FST
Dopamine-β-hydroxylase knockout	Blockade of antidepressant-like effects of various antidepressants in the FST
α_{2A}-adrenoceptor knockout	Depressive-like effects and blockade of the antidepressant-like effect of imipramine in the FST
α_{2C}-adrenoceptor knockout	Antidepressant-like effects in the FST
α_{2C}-adrenoceptor overexpressing	Depressive-like effects in the FST
NET knockout	Antidepressant-like effects in the FST and TST
MAO-A knockout	Antidepressant-like effects in the FST
MAO-B knockout	Antidepressant-like effects in the FST
Mu opioid receptor knockout	Antidepressant-like effects in the FST
Delta opioid knockout	Depressive-like effects in the FST
$G_2\alpha$ G-protein knockout	Blockade of antidepressant-like effects of desipramine and reboxetine in the FST
Glucocorticoid receptor impaired transgenic	Antidepressant-like effects in the FST
Glutamic acid decarboxylase knockout	Antidepressant-like effects in the FST
Adenosin A_{2A} receptor knockout	Antidepressant-like effects in the FST and TST
DARPP-32 knockout	Reduced sensivity to Fluoxetine in the FST
CREB mutant mouse	Antidepressant-like effects in the FST and TST

Modified from Cryan et al., 2002.

The causes of insensitivity to antidepressant treatments remain poorly understood and no model of drug-resistant depression is available. Although non-responder strains to antidepressant drugs are identified in rodents behavioural tests (Jacobson and Cryan, 2007; Lucki et al., 2001; Yalcin et al., 2008), attempts to correlate the response to drugs to neurochemical mechanisms are scarce or gave inconsistent results.

The Wistar-Kyoto (WKY) rat strain has been proposed as an interesting model of resistance to antidepressant treatment.

WKY strain, compared to many other strains of rats, demonstrated hormonal, behavioural and psychological alteration emulating those found in depressive patients and exhibited depressive-like behaviour in several behavioural paradigms. Several studies have cited significant differences between WKY rats and other strains in behavioural response to antidepressants. Although some authors reported that desipramine reduce immobility time in the FST (Tejani-Butt et al., 2003; López-Rubalcava and Lucki, 2000) other studies have found a reduced responsiveness to most antidepressant drugs (Lahmame et al., 1997; Lahmame and Armario, 1996). Different sensitivity of WKY rats may be dependent by the antidepressant drug class used and this may provide information on the substrate involved in the response.

The demonstration of strain differences, also combining genetic and behavioural strategies in the response to antidepressant drugs, could provide new models for the detection of genes that influence the clinical effects of antidepressants.

Recently, it has been suggested that differences in 5-HT levels between some mouse strains may be driven by a functional polymorphism in the TPH-2 gene. The gene encoding mouse TPH-2 shows a SNP (C1473G), in the same region of those found in human TPH-2, that results in the substitution of Pro^{447} with Arg^{447}, with a different allelic distribution in different strains of mice (Zhang et al., 2004). DBA/2J and BALB/C mice, homozygous for the allele 1473G, have a lower 5-HT synthesis rate than C57BL/6J and 129/Sv mice, homozygous for the allele 1473C (Zhang et al., 2004; Cervo et al., 2005).

Recent studies in our department shown that citalopram dose-dependently reduces immobility time in C57BL/6J and 129/Sv but had no effect on DBA/2J and BALB/C mice (Cervo et al., 2005).

The data supported the hypothesis that this TPH-2 polymorphism, influencing the serotonergic system, reduced sensitivity to citalopram in the FST.

1.6 Relevance of serotonergic system in depression

The development of new treatments for depression depends on our understanding of the pathophysiology of the disease and of the mechanisms by which drugs relieve symptoms of depression.

Depressive illness was first recognized as a biochemical phenomenon in the mid sixties of the last century (Schildkraut, 1965; Kaplan and Sadock, 2000). Since then, the monoamine theory of depression became widely accepted. It simply states that mental depression is due to deficiency of brain monoaminergic activity and restoring the normal function of 5-HT- and NA-associated signalling pathway has been the target of antidepressants.

The idea that monoamines are involved in the aetiology of depression came initially from three main lines of evidence. Firstly, drugs such as reserpine that cause depletion of brain monoamines can induce symptoms of depression; secondly, some depressed patients have reduced levels of monoaminergic metabolites in cerebrospinal fluid (CSF); and finally, antidepressant drugs immediately attenuate the mechanisms by which 5-HT and NA are inactivated (*for review see* Blier, 2003).

The earliest clinical report of the relationship between brain monoamines and depression was published by Freis (1954). He reported on 5 hypertensive patients who developed mental depression after treatment with high doses of reserpine. Since then, the pharmacological discoveries that depletion of brain catecholamines and serotonin by reserpine-like drugs, increased availability of NA and/or 5-HT by MAOIs and tricyclic drugs

have provided ample support of the earlier clinical observations (Blier and de Montigny, 1994; Blier, 2003).

5-HT and NA can be depleted experimentally in humans by oral treatments (*for review see* Ruhé et al., 2007). A drink containing all amino acids except tryptophan (Trp) stimulates the liver to synthesize proteins and rapidly depletes the plasma (and therefore the brain) of Trp (Delgado et al., 1990). Trp is rate-limiting for serotonin synthesis in the brain (see *section 1.5.1*). Such oral Trp depletion does not induce depression in healthy subjects but will cause a relapse of depression in patients who have been successfully treated with a SSRI (Jans et al., 2007; Hood et al., 2005; Bell et al., 2005; Neumeister, 2003; Van der Does, 2001; Delgado et al., 1999; Delgado et al., 1991). Similarly, {alpha}-methyl paratyrosine inhibits tyrosine hydroxylase, the rate-limiting step in catecholamine synthesis. Treatment with {alpha}-methyl paratyrosine does not induce depression in normal subjects but will induce a relapse in patients who have been treated successfully with a NRI (Ruhé et al., 2007; Delgado et al., 1993). These findings suggest that NA and 5-HT have critical roles in the mechanisms of these treatments of depression but that additional neurochemical factors are necessary to cause depression.

Although evidence exists for the participation of both 5-HT and NA neurotransmission in the aetiology of depression and the mechanism of action of the antidepressants, more recently, the focus has shifted to the 5-HT system. This is principally because of the great success of the SSRIs as antidepressants.

In particular, 5-HT appears to play a major role and is a major pharmacological target for the action of antidepressant drugs (Graeff et al., 1996; Jans et al., 2007; Blier and de Montigny, 1998; Blier and de Montigny, 1994). Its role is supported by the fact that SSRIs, which are the most widely prescribed antidepressant drugs today, enhanced extracellular level of 5-HT in animal models.

It is widely accepted that diminished serotonergic function is involved in the onset and course of depression (Jans et al., 2007). Serotonergic system has been implicated in the pathophysiology of affective disorders (Coppen and Wood, 1982), and drugs that increase serotonergic activity generally exert antidepressant effects on patients (Blier and de Montigny, 1994; Carrasco and Sandner, 2005). Loading depressed patients with the 5-HT precursor Trp or 5-hydroxytryptophan (5-HTP), with or without standard antidepressant treatments, has been found to be beneficial in the treatment of depression (Shaw et al., 2002).

Despite an intense effort to correlate 5-HT deficiency with depression, the findings of most studies have been inconclusive.

Dysfunction at various levels of the 5-HT system may be present in at least some depressed patients (Jans et al., 2007). In particular, 5-HT and/or its metabolites are found to be reduced in urine and CSF of patients with affective illness, the 5-HT content in brains of suicide victims was found to be low as compared with controls. In addition, there was some evidence that there was decrease in the 5-HT metabolite, 5-hydroxyindole acetic acid (5-HIAA), in the suicide group (Owens and Nemeroff, 1994).

Post-mortem studies have shown both an increase in the density of serotonin 5-HT_2 receptor binding sites, and a decreased number of

serotonin 5-HTT binding sites in brain tissue of depressed patients and suicide victims (Owens and Nemeroff, 1994), as well as an increase in the serotonin 5-HT_{1A} autoreceptors in the midbrain dorsal raphé of suicide victims with MDD (Stockmeier et al., 1998). Post-mortem studies show that the levels of p11, a protein that enhances the efficiency of 5-HT_{1B} receptor signalling, are decreased in the brains of patients with depression (Svenningsson et al., 2006; Belmaker and Agam, 2008). Decreased serotonergic activity in MDD is further supported by the results of recent imaging studies that have evidenced widespread reductions in serotonin 5-HT_{1A} autoreceptor and a reduction in the density of brain 5-HTT binding sites (Malison et al., 1998; Sargent et al., 2000).

Although 5-HT deficiency alone cannot explain the pathophysiology of mood disorders, the interaction of low 5-HT levels in the brain with other neurotransmitter systems in the CNS has been considered to be important in the aetiology of depression and other mood disorders (Kalia, 2005).

Several factors, such as genetic factors, gender, stress and drug use or manipulation such as 5-HT challenges or acute tryptophan depletion, may disrupt or modify the serotonergic system. This "serotonergic vulnerability" can predispose to develop disorders related to 5-HT, indicating that the development of depression is associated with the presence of a pre-existent abnormality in the functioning of the 5-HT system (Jans et al., 2007; Gaspar et al., 2003).

1.6.1 Serotonin system

5-HT-containing neuronal cell bodies are restricted to discrete clusters or groups of cells located along the midline of the brainstem (Adell et al., 2002; Siegal et al., 1999; Azmitia and Segal, 1978; Michelsen et al., 2007). Their axons, however, innervate nearly every area of the CNS (***Figure 1.3***).

Most 5-HT neurones are found in the raphé nuclei, on the midline of the rhomboencephalon, with a smaller number in the reticular formation. Dahlstrom and Fuxe (1964) described nine groups of 5-HT-containing cell bodies designated B1 through B9 and corresponded for the most part with the raphé nuclei.

They cluster as two main groups: the caudal division (B1–B5, corresponding to the raphé pallidus, magnus, obscurus and pontis), and the rostral division (B6–B9, corresponding to the dorsal and median raphé nuclei, respectively DR and MR; Lidov and Molliver, 1982; Wallace and Lauder, 1983).

The total number of serotonergic neurons is small — around 20,000 neurons in the rat (Jacobs and Azmitia, 1992) — compared with the total number of neurons in the CNS — about 10^{10}, but serotonergic neurons provide a relatively dense innervation to all the brain areas and the spinal cord, by way of an extensive and diffuse collateralization of their axons.

The largest group of serotonergic cells is B7, which is continuous with a smaller group of serotonergic cells, B6. Groups B6 and B7 are considered together as the DR, with B6 being its caudal extension. Another prominent serotonergic cells group is B8, which corresponds to the MR, also termed the nucleus central superior. Group B9 forms a lateral extension of the MR. Ascending serotonergic projections innervating the cerebral cortex and

other regions of the forebrain arise primarily from the DR, MR and B9 cell group (Dahlström and Fuxe, 1964; Waselus et al., 2006; Törk, 1990; Jacobs and Azmitia, 1992).

The two main ascending serotonergic pathways from the midbrain raphé nuclei to the forebrain are the dorsal periventricular path and the ventral tegmental radiations. Both pathways converge in the caudal hypothalamus, where they join the medial forebrain bundle.

Ascending projections from the raphé nuclei to forebrain structures are organized in a topographical manner. The DR and MR nuclei give rise to distinct projections to forebrain regions. The MR projects heavily to hippocampus, septum and hypothalamus, whereas the striatum is innervated predominantly by the DR. They both send overlapping neuronal projections to the neocortex. Within the DR and MR, cells are organized in particular zones that send axons to specific areas of brain. For example, the frontal cortex receives heavy innervation from the rostral and lateral subregions of the DR. The other raphé nuclei, B1 to B4, are situated more caudally in the midpons to caudal medulla and contain a smaller number of serotonergic cells. These cell body groups give rise to serotonergic axons that project within the brainstem and to the spinal cord. The spinal cord receives a strong serotonergic innervation.

Afferent connections to the raphé nuclei include those between the DR and MR, B9, B1 and B3 (Jacobs and Azmitia, 1992; Molliver, 1987; Törk, 1990). Such innervation may have considerable physiological and pharmacological importance as 5-HT released in the vicinity of serotonergic cell bodies regulates the firing of serotonergic neurons through the activation of somatodendritic autoreceptors (mainly 5-HT_{1A}). The raphé nuclei also

receive input from other cell body groups in the brainstem, such as the substantia nigra and ventral tegmental area (VTA; dopamine), superior vestibular nucleus (acetylcholine), locus ceruleus (NA) and nucleus prepositus hypoglossi and nucleus of the solitary tract (adrenaline). Other afferents include neurons from the hypothalamus, thalamus and limbic forebrain structures.

In most of the raphé nuclei, the majority of neurons are not serotonergic. For example, the DR contains the largest number of serotonergic neurons; however, only 40 to 50% of the cell bodies in the DR are serotonergic.

Serotonergic axon terminals appear to exhibit morphological differences related to the raphé nucleus of origin (Molliver, 1987; Rattray et al., 1999). Serotonergic axons from the MR look relatively coarse with large spherical varicosities. By contrast, axons from the DR are very fine and typically have small, pleomorphic varicosities. DR axons appear to be more vulnerable to certain neurotoxic amphetamine derivatives, such as d-fenfluramine, 3,4-methylenedioxymethamphetamine (MDMA) or parachloroamphetamine (PCA). MR axons appear to be more resistant to the neurotoxic effects of these drugs. Blockade of the 5-HTT prevents the neurotoxic effects of these amphetamine derivatives, indicating that activity of this transporter is critical for the neurotoxic effects of these drugs.

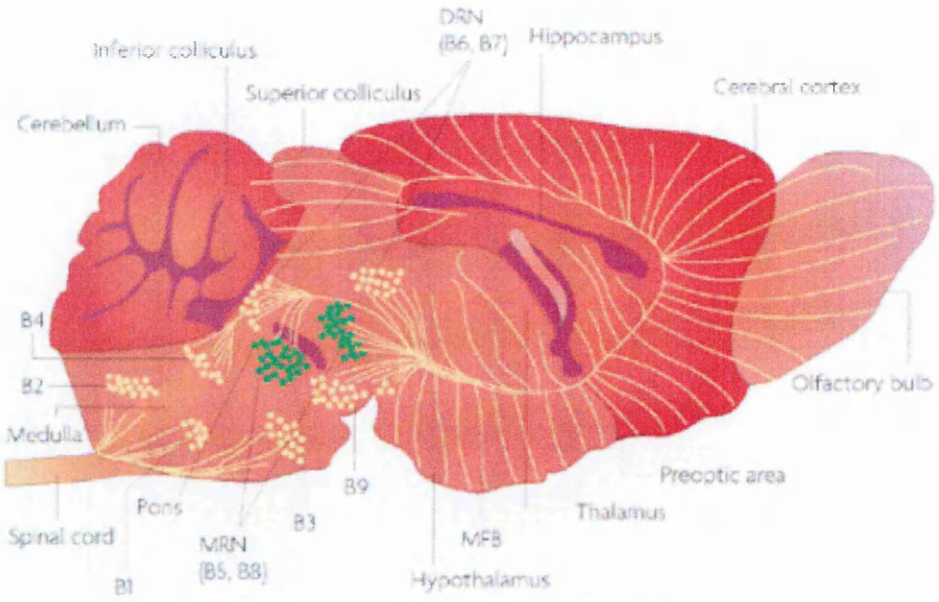


***Figure 1.3** Central serotonergic system*

Although high amounts of 5-HT are present in the periphery, cerebral 5-HT has to be synthesised by the brain itself, as it cannot cross the blood brain barrier under normal conditions (Sharma et al., 1990). Plasma Trp, the precursor of 5-HT, is mainly derived from diet and it is actively transported into the brain. Competition exists for this active transport between several aminoacids like aromatic amino acids (tyrosine and phenylalanine), branched chain amino acids (leucine, isoleucine and valine), and others (methionine and histidine). As a consequence, the amount of Trp available in the brain not only depends on the plasma concentration of Trp, but also on other amino acids (Daniel et al., 1976).

5-HT is synthesized from Trp, in a two-step process (***Figure 1.4***) involving the hydroxylation of the essential amino acid Trp in position 5 of the aromatic ring to obtain 5-HTP. The enzyme responsible in the brain for this

limiting step is TPH-2. The second step is the conversion of 5-HTP into 5-HT by the aromatic amino acid decarboxylase (AAAD).

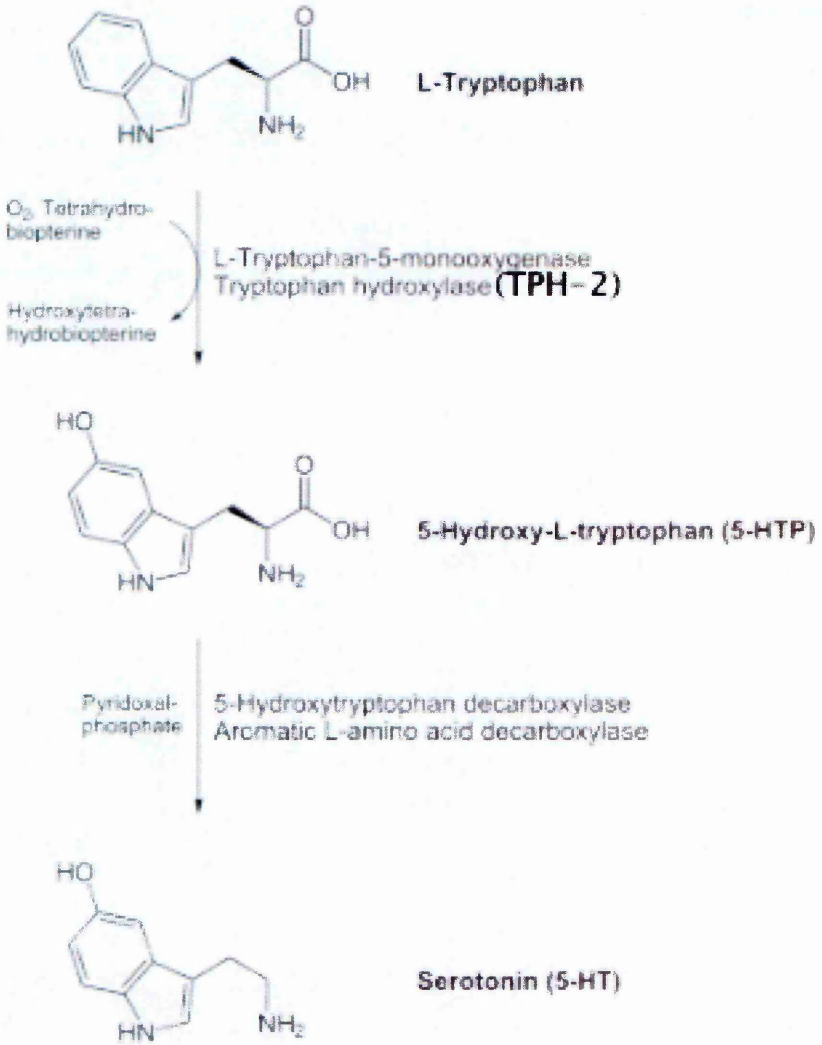


Figure 1.4 Brain 5-HT synthesis
Modified from http://en.wikipedia.org/wiki?title=Serotonin

5-HT is stored in vesicles in the presynaptic neuron and released into the synaptic cleft upon neuronal membrane depolarization, thus affecting both presynaptic and postsynaptic neurons (***Figure 1.5***). Reuptake of 5-HT by serotonin transporter (5-HTT) is a key point in serotonergic neurotransmission because it is the main way by which released 5-HT is cleared from the synapses.

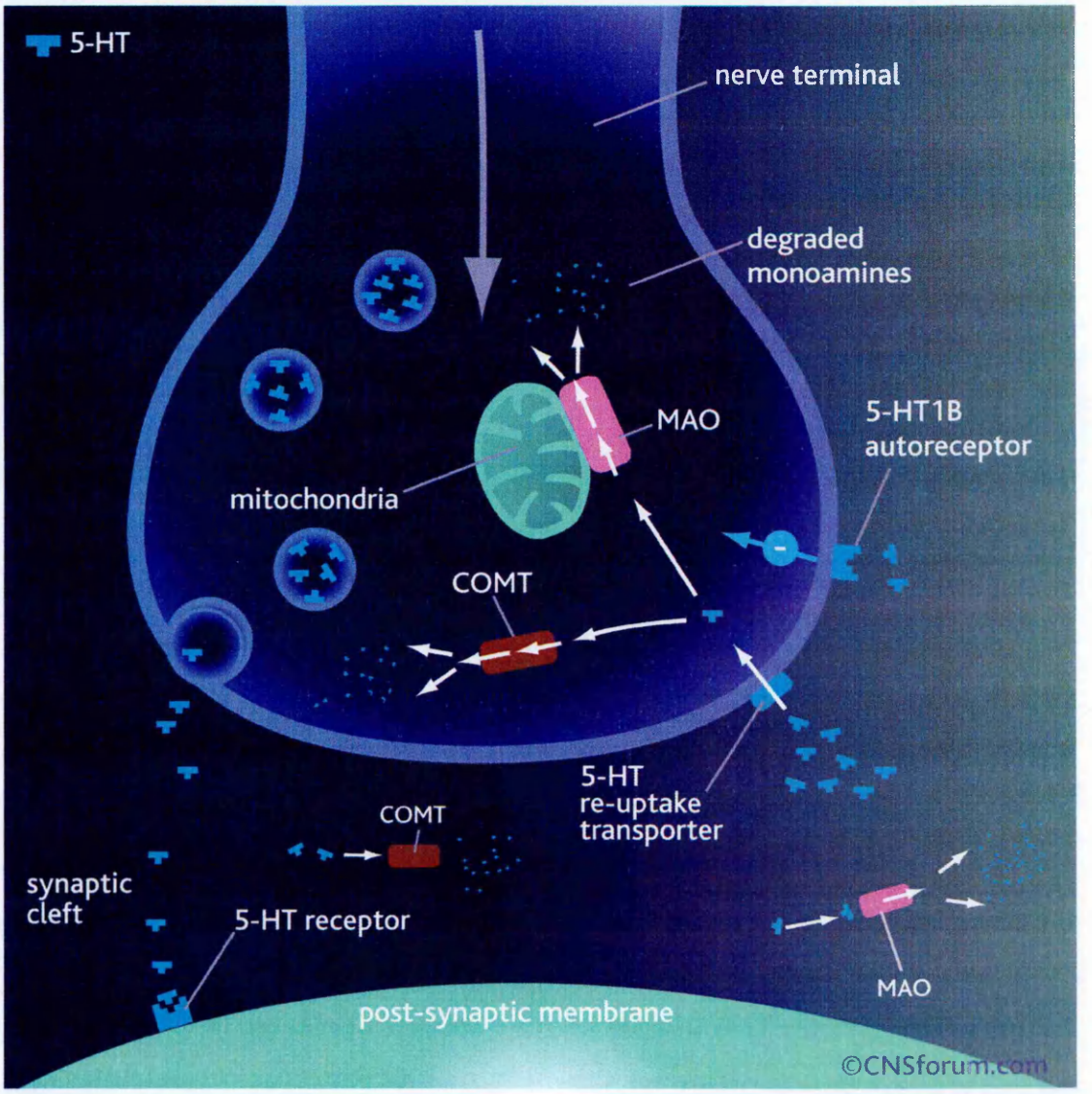


Figure 1.5 5-HT neurotransmission. *(www.cnsforum.com/imagebank)*

Cessation of the synaptic action of the neurotransmitters occurs also by means of the feedback control of release through the presynaptic 5-HT_{1A} and 5-HT_{1B} regulatory autoreceptors and MAO-A that indirectly regulates vesicular content.

In the CNS, catabolism of serotonin mainly occurs by MAO-A to form 5-hydroxyindoleacetaldehyde. Oxidation of this intermediate by aldehyde dehydrogenase forms 5-HIAA, which is the predominant metabolite of serotonin in the brain (***Figure 1.6***). In the pineal gland, 5-HT is converted into melatonin by two additional enzymatic steps.

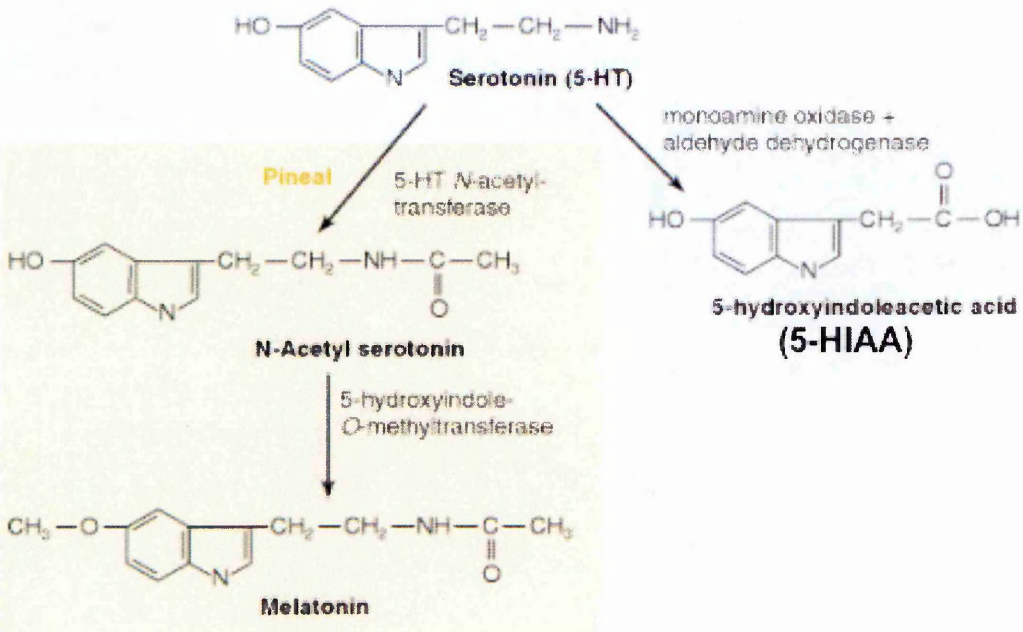


Figure 1.6 ***Catabolism of 5-HT***
Modified from www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=bnchm.figgrp.955

Serotonergic pathways have both inhibitory and facilitatory functions in the brain.

Due to the rapid developments in the field of molecular biology, the number of serotonin receptors that have been identified has expanded significantly in the last decades.

At least 14 distinct mammalian 5-HT receptor subtypes have been identified (Stahl, 1998; Barnes and Sharp, 1999; Elhwuegi, 2004; Jans et al., 2007). Based on structural and functional properties, seven types of serotonin receptors have been classified until now (***Table 1.4***). However, a more complex picture may emerge due to evidence that some receptor subtypes (5-HT_{2C}, 5-HT_3, 5-HT_4 and 5-HT_7) can occur as multiple isoforms due to gene splicing, post-transcriptional RNA editing and polymorphic variants (Stam et al., 1997; Gerald et al., 1995; Canton et al., 1996; Werner et al., 1994).

Furthermore 5-HT receptors are categorized into four groups according to their second messenger coupling pathways: the 5-HT_1 receptors, which are coupled to Gi proteins (5-HT_{1A}, 5-HT_{1B-C} and 5-HT_{1D-F}); the 5-HT_2 receptors, which are coupled to Gq proteins (5-HT_{2A-C}); the 5-HT_4, 5-HT_6 and 5-HT_7 receptors, which are coupled to Gs proteins; and the 5-HT_5 receptors (5-HT_{5A} and 5-HT_{5B}), which resemble the previous group but whose signal transduction cascade is not entirely clear. 5-HT_{3A} and 5-HT_{3B} are ionotropic receptors.

Table 1.4 Overview of 5-HT receptors

5-HT receptor subtypes	AGONISTS	ANTAGONISTS	SIGNALLING	CNS LOCALIZATION and FUNCTIONS
5-HT_{1A}	8OH-DPAT, ipsapirone	methiothepin, pindolol, spiperone, WAY 100635	decreasing cellular levels of cAMP	Hippocampus, amygdala, septum, cortex, hypothalamus, raphé nuclei Neuronal inhibition, behavioural effects (sleep, feeding, thermoregulation, aggression, anxiety)
5-HT_{1B}	Sumatriptan, Zolmitripan	metergoline, methiothepin, risperidone, GR 55562	decreasing cellular levels of cAMP	Striatum, nucleus accumbens, tuberculum olfactorium, cortex, hypothalamus, hippocampus, thalamus, dorsal raphé and cerebellum Presynaptic inhibition, behavioural effects
5-HT_{1D}	Sumatriptan, Zolmitripan,	GR 127935, BRL 15572	decreasing cellular levels of cAMP	Substantia nigra, basal ganglia, superior colliculus, entorhinal cortex, dorsal raphè, cerebellum Locomotion, anxiety
5-HT_{1E}	BRL 54443		decreasing cellular levels of cAMP	Cortex, claustrum, caudate putamen Unknown
5-HT_{1F}	LY334370, (BRL 54443)		decreasing cellular levels of cAMP	Cerebral cortex, striatum, hippocampus, olfactory bulb Migraine
5-HT_{2A}	DOI, DOB	Ketanserin, MDL 100,907	increasing cellular levels of inositol trisphosphate (IP3) and diacylglycerol (DAG)	Claustrum, cerebral cortex, olfactory tubercle, striatum, nucleus accumbens Neuronal excitation, behavioural effects, learning, anxiety
5-HT_{2B}	CP809101 BW723C86	RS127445, SB204741, SB206553	increasing cellular levels of inositol trisphosphate (IP3) and diacylglycerol (DAG)	Amygdala, septum, hypothalamus and cerebellum Anxiety
5-HT_{2C}	mCPP, Ro60-0175, WAY 161503	SB206553, RS102221, SB242084	increasing cellular levels of inositol trisphosphate (IP3) and diacylglycerol (DAG)	Choroid plexus, globus pallidus, cerebral cortex, hypothalamus, septum, substantia nigra Depression, anxiety
5-HT_{3}	2-methyl-5-HT, SR 57227A	Granisetron, Ondansetron	ligand-gated Na+ and K+ cation channel	Hippocampus, cortex, amygdala, nucleus accumbens Neuronal excitation, anxiety, emesis, cognition
5-HT_{4}	BIMU 8, RS 67506	GR113808, SB204070	increasing cellular levels of cAMP	Hippocampus, striatum, olfactory tubercle, substantia nigra Neuronal excitation, learning, memory, anxiety, cognition
5-HT_{5A}	5-CT	SB699551	inhibiting adenylate cyclase activity	Hippocampus, hypothalamus, cerebral cortex, thalamus, pons, striatum, raphé and medulla Locomotion, sleep
5-HT_{5B}			inhibiting adenylate cyclase activity	No or low level in the brain Unknown
5-HT_{6}	Carboxamidotryptamine	Ro 04-6790	increasing cellular levels of cAMP	Olfactory tubercle, cerebral cortex , nucleus accumbens, striatum, hippocampus, cerebellum, caudate nucleus Anxiety, cognition, memory
5-HT_{7}	Carboxamidotryptamine	SB258719	increasing cellular levels of cAMP	Cerebral cortex, septum, thalamus, hypothalamus, amygdala, superior colliculus, raphé nuclei and hippocampus Circadian rhythms, sleep, depression

The 5-HT_1 receptor family is subdivided to at least five receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}). These receptors are linked to inhibition of adenylate cyclase (AC) activity through Gi protein, thus inhibiting formation of the second messenger c-AMP, or to regulation of K^+ or Ca^{2+} channels.

Activation of 5-HT_{1A} receptors leads to the opening of an inwardly rectifying K^+ conductance, which leads to hyperpolarization and neuronal inhibition (Aghajanian and Lakoski, 1984). Overall, the 5-HT_{1A} receptor is regarded as an inhibitory receptor. It is located in the raphé nuclei, but is also highly abundant in terminal areas such as prefrontal cortex and hippocampus (Hensler et al., 1991). It is well known that somatodendritic 5-HT_{1A} autoreceptors in the DR causes inhibition of the firing rate of 5-HT neurons and therefore inhibit 5-HT release at the nerve terminals (Sprouse and Aghajanian, 1986). They also regulate 5-HT release from the forebrain projection areas (*for review see* Piñeyro and Blier, 1999).

5-HT release from serotonergic neurons is also under the control of terminal 5-HT_{1B} and 5-HT_{1D} autoreceptors (Starkey and Skingle, 1994; Adell et al., 2001). These receptors can modify 5-HT release with minor or no effects on 5-HT neuron firing activity (Crespi et al., 1990; Adell et al., 2001).

5-HT_{1E} receptors are located in cortex, claustrum and caudate putamen and little is known about their physiological role (Barnes and Sharp, 1999), whereas 5-HT_{1F} receptors have been found in cortex, hippocampus and DR and agonists might have a role in treatment migraine (Barnes and Sharp, 1999).

There are three subtypes of 5-HT_2 receptors, the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. These receptors have in common that their signalling pathway activates phosphoinisitide metabolism, which mobilises Ca^{2+} and activates protein kinase C. Centrally, the 5-HT_{2A} receptor is mainly found in cortex, claustrum and basal ganglia (Hoyer et al., 1994). In man, 5-HT_{2B} receptors are mostly located peripherally, but presence of 5-HT_{2B} receptors has been observed in amygdala, septum, hypothalamus and cerebellum. In contrast to 5-HT_{2B} receptors, 5-HT_{2C} receptors are mainly found in the central nervous system. High levels of 5-HT_{2C} receptors have been observed in the choroid plexus, cerebral cortex, hippocampus, striatum, and substantia nigra of rats as well as humans (Barnes and Sharp, 1999; Hoyer et al., 1994; Di Giovanni et al., 2006).

Several lines of evidence indicate an important role of 5-HT_1 and 5-HT_2 subtypes in depression (Middlemiss et al., 2002, see *section 1.5* and *Chapter 4*).

The 5-HT_3 receptor is the only ligand gated ion channel in the serotonin receptor family. It enhances depolarisation by increasing permeability to cations. The receptor is highly abundant in the gastric system, whereas in the CNS it is most abundant in the area postrema, nucleus tractus solitarius, substantia gelatinosa and nuclei of the lower brain stem.

Centrally acting 5-HT_3 antagonists have behaved as anxiolytics and antiemetics (Hoyer et al., 1994).

The 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 subgroups have been identified by molecular cloning and characterized biochemically, but a clear role of these receptors in depression is not yet known.

5-HT_4 subtypes receptors are positively coupled to AC. It has been found centrally in the nigrostriatal and mesolimbic systems (Barnes and Sharp, 1999) as well as peripherally.

The 5-HT_5 receptor is probably the least understood receptor of the 5-HT class. Whereas molecular biological research has provided evidence for 5-HT_{5A} and 5-HT_{5B} subtypes, their pharmacology is largely unknown. The presence of the 5-HT_{5A} receptor has been observed in hippocampus, hypothalamus, cerebral cortex, thalamus, pons, striatum, raphé and medulla. No 5-HT_{5A} receptors have been observed in peripheral tissue (Barnes and Sharp, 1999).

Conversely, 5-HT_{5B} receptors have been observed in peripheral tissue like heart, kidney and lungs, though no or low levels of the receptors were found in brain tissue (Barnes and Sharp, 1999).

5-HT_6 subtype receptors appear to be largely confined to the CNS, though some have been observed in peripheral structures like stomach and adrenal gland as well. High levels of 5-HT_6 mRNA have been observed in the caudate nucleus, olfactory tubercle, nucleus accumbens and hippocampus. Their activation has been shown to enhance cAMP formation. 5-HT_6 knockout mice tended to show enhanced anxiety in behavioural paradigms (Barnes and Sharp, 1999) but their relevance in depression is not clear. Recent evidence shows that activation of 5-HT_6 receptors initiates a cascade

of intracellular events that may be involved in the antidepressant-like effects of SSRIs (Svenningsson et al., 2007).

The 5-HT_7 receptor is the most recently described 5-HT receptor but it has already gained a lot of interest (Bonaventure et al., 2007, Guscott et al., 2005). This receptor is highly abundant in the raphé nuclei, thalamus, hypothalamus and hippocampus (Barnes and Sharp, 1999). The observation that 5-HT_7 receptors were downregulated upon chronic treatment with various antidepressants might indicate relevance of these receptors in depression (Mullins et al., 1999).

1.6.2 Revised monoaminergic hypothesis

Emerging evidence indicates that the monoamine hypothesis of 5-HT and NA modulation fails to explain the whole mechanism of antidepressants and in particular the discrepancy between the acute effect of antidepressant drugs and the delay in the onset of their therapeutic action.

First, some drugs as cocaine and amphetamine, that can increase brain monoaminergic activity, are not effective as antidepressants (Belmaker and Agam, 2008). Second, not all depressed patients respond equally to the same antidepressant treatments. Third, and most importantly, the pharmacological and biochemical effects of the antidepressant drugs occur within minutes, they do not produce their effects for at least 14 days after initiation of treatment. This lag time suggests that antidepressants act via a delayed postsynaptic receptor-mediated event (Malberg and Blendy, 2005; Blier, 2003).

Monoamines produce their effect by inducing complex biochemical changes in postsynaptic neurons by interacting with signalling proteins (G proteins) inside the postsynaptic cell membrane.

The modified amine theory has suggested that the acute increase in the levels of the monoamines at the synapse may be only an early step in a cascade of events that ultimately results in antidepressant activity (Blier and de Montigny, 1998; Piñeyro and Blier, 1999). This acute increase in the amount of the monoamine at the synapse has been found to induce desensitization of the 5-HT_{1A} and α2-adrenoceptors, located on the soma and/or dentrites, respectively of serotonergic and noradrenergic neurones, or functioning as heteroreceptors controlling neurotransmitter release in

several brain regions (*for review see* Blier, 2003). The desensitization of these receptors would result in higher central monoaminergic activity that coincides with the appearance of the therapeutic response (Stahl, 1998).

More recently, molecular events downstream of antidepressants action on the monoamines have been elucidated, generating new theories about the pathophysiology of depression and the action of antidepressant treatments. These hypotheses put forward that antidepressants activate second messenger systems and subsequently transcription factors such as cAMP response element-binding protein (CREB), neurotrophic pathways and increase hippocampal neurogenesis (*for review see* Krishnan and Nestler, 2008). Recent preclinical and clinical studies demonstrate structural alterations occurring in response to stress and in depressed patients that can be reversed by antidepressant treatments (*for review see* Duman, 2002). Therefore neural plasticity and neurogenesis may play a significant role in the etiology and treatment of depression and the neurotrophic pathway, and in particular the brain derived neurotrophic factor (BDNF), seems to be involved.

1.7 Neurotrophic hypothesis and BDNF

The neurotrophic hypothesis can be considered as complementary to the monoamines hypothesis of depression (Duman and Monteggia, 2006; Duman, 1998; Duman, 2002; Pittenger and Duman, 2008; Malberg and Blendy, 2005). It postulates that a loss of BDNF plays a major role in the pathophysiology of depression, and its restoration may represent a critical mechanism underlying antidepressant efficacy. This theory comes from converging lines of data (Pittenger and Duman, 2008). First, antidepressant drugs require at least 2 weeks administration to see clinical efficacy (Nestler et al., 2002). This time lag may represent a necessity for long-term adaptations in the signalling pathways, such as neurotrophic pathways, before seeing a therapeutic effect. Secondly, it was hypothesized that depression can arise from the failure of the CNS to exhibit the appropriate synaptic plasticity in response to stress, which may be offset or reversed by neurotrophic support induced by antidepressant treatments (Duman et al., 2001). Finally, it has been recently hypothesized that neurogenesis, or the birth and survival of new neurons, is involved in antidepressant action (Malberg and Blendy, 2005). BDNF is a neurotrophic peptide, critical for axonal growth, neuronal survival, and synaptic plasticity, and its levels are affected by stress and cortisol (Duman, 2004a; Duman, 1998; Smith et al., 1995; Angelucci et al., 2005; Sapolsky, 1996).

The neurotrophic hypothesis of depression was originally based on findings in rodents that acute or chronic stress decreases expression of BDNF mRNA in the hippocampus and that several classes of antidepressants produce the

opposite effect and prevent the actions of stress (Duman et al., 2001; Duman, 2004b).

Chronic antidepressant drugs and electroconvulsive therapy up-regulate BDNF and other neurotrophic and growth factors (Duman et al., 2000; Balu et al., 2008); BDNF protein acutely administered directly into the lateral ventricles or into the dentate gyrus has antidepressant-like effects in the FST and LH paradigm (Siuciak et al., 1996; Shirayama et al., 2002). The hippocampus seems to play a major role in the antidepressant-like effects of BDNF while infusion of this neurotrophin into the VTA produces pro-depressive effect (Eisch et al., 2003).

Post-mortem studies reported reduced BDNF levels in the hippocampus of patients with depression who had committed suicide (Karege et al., 2005; Dwivedi et al., 2003). Clinically, increased hippocampal BDNF levels have been observed in patients treated with antidepressants, along with decreased serum BDNF levels in untreated depressed subjects (Chen et al., 2001; Groves, 2007).

One study showed that the hippocampus was smaller than normal in patients with depression who carried a polymorphism giving rise to a BDNF variant (V66M) allele (Frodl et al., 2007). Several studies suggest an association between polymorphisms in BDNF and depression and between the M allele and response to antidepressant treatments, but subsequent reports only partially confirmed these results (Lin and Chen, 2008; Duncan et al., 2008; Levinson, 2006).

Overall these studies suggest that BDNF is an important link between stress, neurogenesis and hippocampal atrophy in depression. However, a genetic association of the BDNF V66M polymorphism with depression has

not been replicated in most studies, and BDNF may be related not only to depression but also to multiple psychiatric disorders (Angelucci et al., 2005; Balu et al., 2008). Furthermore both BDNF-knockout mice and Knock-in mice that homozygously express M-66 BDNF have behaviours unrelated to depression (Lyons et al., 1999; Chen et al., 2006; Krishnan et al., 2007).

Castrén (2005) has proposed that antidepressant treatments may increase synaptic sprouting and allow the brain to use input from the environment more effectively to recover from depression. Moreover Adachi et al. (2008) recently suggest that the loss of hippocampal BDNF per se is not sufficient to mediate depression but it is involved in mediating the therapeutic effect of antidepressants. Further studies are needed to clarify the role of BDNF in the neuroplasticity and neurogenesis mechanisms that contribute to the pathophysiology of depression and the action of antidepressant drugs.

AIM of the thesis

Despite several decades of research, the changes that antidepressant drugs induce in the brain that underlie their therapeutic action remain unclear.

The fundamental goal of the work described in this thesis is to provide a better understanding of the role played by the neurochemical factors involved in a new animal model predictive of the antidepressant effect in resistant subjects, and in particular to evaluate the role of serotonergic mechanisms.

The preceding pages have set out the general background of this work.

Recent results obtained in our department (Cervo et al., 2005) suggested that the impairment of 5-HT synthesis might be involved in the lack of response to SSRIs in mice carrying the mutant isoform of TPH-2 (***Figure 1.7***).

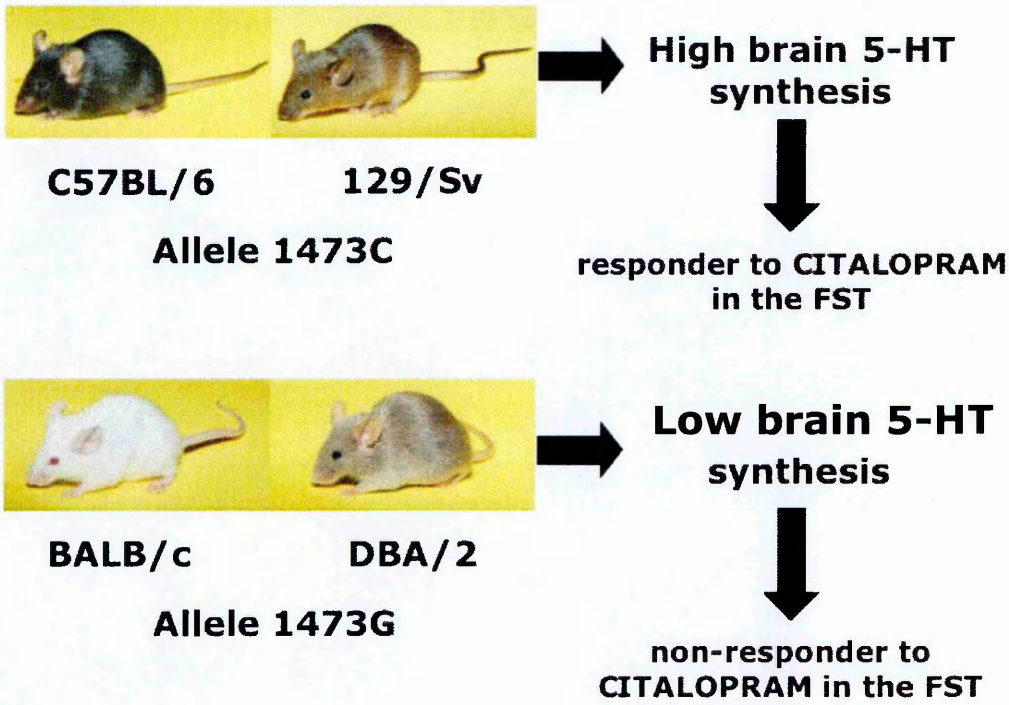


***Figure 1.7** Strain differences in single nucleotide polymorphism of TPH-2*

Tryptophan-hydroxylase-2, the rate-limiting enzyme in the synthesis of brain serotonin, shows single nucleotide polymorphism (C1473G). Inbred mice carrying the 1473G allele (DBA/2 and BALB/c) show a reduced rate of serotonin synthesis compared to C57BL/6 and 129/Sv strains, homozygous for the C-allele. Using the forced swimming test (FST), it has been shown that the genotype-dependent impairment of serotonin synthesis determines the failure of DBA/2 and BALB/c mice to respond to citalopram (Cervo et al., 2005).

The results acquired suggest that inter-strain comparisons of mice carrying allelic variants of TPH-2 may serve as a good model for a clearer understanding of the mechanisms underlying the response to SSRIs.

The experimental methods have concentrated on neurochemical measurement of monoamine averflow using *in vivo* microdialysis (see *Chapter 2*). The combination of this methodological approach with a behavioural model, the FST, is well suited to delve into the role of 5-HT. In

particular it provides information on alteration in brain neurotransmission and allows manipulating it, mainly acting on pre- and post-synaptic 5-HT mechanisms.

The work has four main parts:

1) In order to test the role of serotonergic mechanism involved in the lack of response to SSRIs in the FST, I evaluated strain differences in 5-HT synthesis and synaptic availability of 5-HT under basal conditions and in response to acute administration of SSRIs.

2) In the second target of the project, I tested pharmacological strategies aimed at improving the effect of SSRIs in mice not responding to the SSRI alone, verifying whether intervention aimed at enhancing the effect on extracellular 5-HT restored the antidepressant-like effect in the FST.

3) To enquire into the role of serotonergic mechanism, I examined the inhibitory control of GABA on 5-HT neurons in the dorsal raphé and the existence of a negative-feedback loop involving reciprocal connections between GABAergic interneurons and 5-HT neurones.

4) It is well known that antidepressant drugs produce their effects about two weeks after initiation of treatment. This lag time suggests that adaptive changes are required for their effects. Therefore the last goal of the project was to evaluate the role of BDNF, a neurotrophin that has been linked to the long-term action of antidepressant drugs and can be involved in regulating neural structure and plasticity in the brain.

Chapter 2

GENERAL METHODS

This chapter contains a description of the general methods and techniques used in this thesis to examine the role of serotonergic mechanisms underlying the response to SSRIs.

The specific procedures and methods that are unique to, or deviate from, the general methods are described in the appropriate experimental chapters.

The methodological approach has concentrated primarily on examining extracellular 5-HT in several regions of the mouse brain using the *in vivo* microdialysis technique. In order to define the role of 5-HT in the antidepressant response, changes in 5-HT release induced by pharmacological manipulation have been associated to the effect of the same treatment in the FST.

Although it is problematic to extrapolate the findings in animals to humans, as discussed in *Chapter 1*, the FST does respond to some 90% of clinically active antidepressant treatments.

All behavioural experiments were done in collaboration with Dr. L. Cervo and the Laboratory of Experimental Psychopharmacology. I contributed to the planning and execution of the experiments whereas the assessment of behaviour was done by Dr. L. Cervo and co-workers of the Laboratory of Experimental Psychopharmacology.

2.1 Animals

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D. L. n. 116,G. U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G. U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

The animals were picked up from the quarantine 3 days after their arrival and housed at constant room temperature (21±1°C) and relative humidity (60±5%) under a regular light/dark schedule (light 07 h 00 min - 19 h 00 min). Food (Teklad global 18% protein rodent diet, Harlan, Italy) and filtered tap water were freely available.

Male C57BL/6J, C57BL/6N, DBA/2J, DBA/2N and BALB/C mice (Charles River Laboratories; Calco, Italy), 6-8 weeks old, were housed in perpex standard cages.

After the microdialysis probe implantation, the animals are housed singly.

Animals are not habituated to handling before the experiment.

Each animal was used only once.

2.2 Intracerebral microdialysis

Since its introduction in the 1980's by Ungerstedt and co-workers, in vivo microdialysis has been an important research tool in neuropharmacology (Zetterström et al., 1983; Benveniste, 1989). It is a well-established technique for *in vivo* monitoring of extracellular neurotransmitters in awake, freely moving animals. The basic principle is the positioning of probe made with a membrane that allows free diffusion of water and low molecular weight solutes between the brain interstitial space and a solution lacking the substance of interest continuously flowing into the probe lumen. The membrane acts as a mechanical barrier to turbulence in the fluid flow reducing the mechanical stimulation of the tissue. Furthermore it acts as a filter against the large molecules and proteins present in the extracellular fluid.

As shown in ***Figure 2.1***, the key element in the microdialysis technique is the dialysis probe. It is perfused with an isotonic and isoionic solution (artificial cerebrospinal fluid, aCSF, see composition in *paragraph 2.2.2*) at a constant flow of 1-2 µL/min with a microinfusion pump.

Although simple in principle, microdialysis is an invasive technique because it attempts to monitor the release of neurotransmitters from nerve terminals by inserting into the brain a probe several orders of magnitude larger than the biological structure under study (Di Chiara, 1990). The injury caused by the insertion of the probe is limited to a short period of time. After 24 h animals exhibited only slight alteration in glucose metabolism and blood-brain barrier permeability (Benveniste, 1989).

Typically after an initial period during which neurotransmitter levels are very high, they decrease and remain stable for several hours or days (Westerink, 1995). The development of reactive gliosis, occurring few days after probe implantation, limits the use of microdialysis probe. Therefore, for longer experiments, it is better to use a guide cannula in which inserts the probe 24 h before the experiment.

An important aspect to consider is that after release neurotransmitters can be involved into processes of uptake and metabolism, which can limit the amount of neurotransmitters recovered in the dialysate. Concentrations obtained by microdialysis sampling both *in vitro* and *in vivo* are substantially lower than those in the sampled fluid, necessitating probe calibration to determine levels released. The ratio of the concentration in the samples collected from the probe to the concentration outside the probe is termed the "relative recovery" (Chaurasia, 1999; Chen et al., 2002). The concentration ratio depends on the flow rate through the probe, the area of the dialysis membrane, the temperature, the molecular weight of the solute and the tortuosity of the surrounding fluid (Benveniste, 1989; Chen et al., 2002). The factor most affecting the probe recovery is the flow rate. When the flow is near to zero, the solutions outside and inside the membrane approach the equilibrium. For monoamines, the amount of neurotransmitter reaching the probes can be determined by the balance between the release of the neurotransmitter and the mechanisms of uptake. To evaluate the contribution of these factors, Lonnroth et al. (1987) developed a method to estimate recovery *in vivo* evaluating the extracellular concentration of the

analyte at the steady state. This method is referred to as "zero-net-flux" and it is discussed in detail in the *Chapter 3*.

Another question is whether the dialysate content of a neurotransmitter is directly related to neurotransmission. This can be evaluated examining the calcium dependency, omitting the calcium ions from the perfusion fluid, and the blockade of fast sodium-channels, infusing the sodium-channel blocker, tetrodotoxin (TTX). Several studies confirmed the neuronal origin of 5-HT measured in dialysate (Di Chiara, 1990; Bortolozzi and Artigas, 2003). More difficult to interpret is the origin of extracellular γ-aminobutyric acid (GABA) and glutamate (GLU) recorded by microdialysis (discussed in *Chapter 5*).

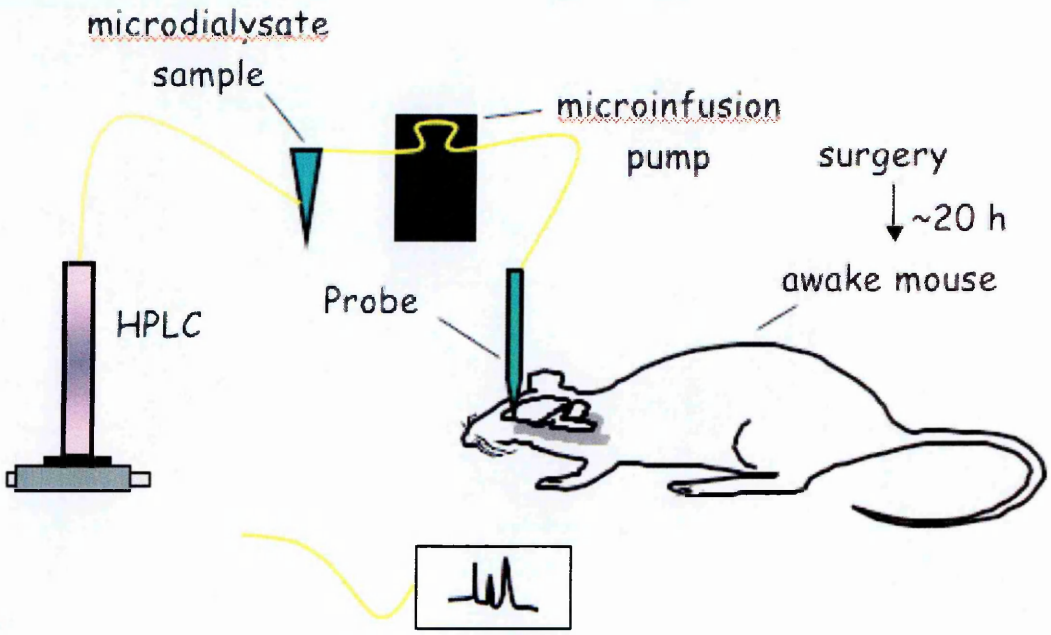


***Figure 2.1** Schematic drawing of the main steps in a microdialysis experiment*

A feature of the microdialysis technique is the possibility of measuring neurotransmitters release in freely moving behaving animals and manipulating the extracellular fluid compartment changing the composition of the perfusing fluid or with drugs administration.

2.2.1 Preparation of the dialysis probe

Concentric dialysis probes (***Figure 2.2***) were prepared essentially as previously described (Robinson and Whishaw, 1988). A tungsten wire (200 µm outer diameter; TW5-3, Clark Electromedical Instr., UK) was insered into a 10 mm long piece of hollow fiber dialysis tubing made of Cuprophan (216 µm outer diameter, 3000 Da cutoff; Sorin Biomedica, Italy). This is the appropriate membrane to measure 5-HT since delayed recovery of 5-HT in the dialysate has been observed with membrane such as AN-69 (Tao and Hjorth, 1992; Ceglia et al., 2004).

The tungsten wire was threaded into a 26 gauge stainless steel cannula and the fiber was stuck into the distal end of the stainless steel tubing with epoxy glue.

When the junction was dry (at least 2 hours), the dialysis membrane was trimmed to the desired length (plus 0.5 mm), the tip sealed (0.5 mm epoxy tip) and then left to dry overnight. The exposed membrane was 2 mm long for the medial prefrontal cortex (mPFC) and 1 mm for the dorsal hippocampus (DH) and the dorsal raphé (DR). The tungsten tube was unthreaded and replaced with a fused silica capillary tube.

A polythene tubing (Portex Ltd., Hythe, UK) with an inside diameter of 0.40 mm (which serves as inlet), was pierced with a 30 gauge needle. The

other end of the silica tube was threaded into this small hole and the tubing was connected to the cannula.

Then the silica tube was threaded in a polythene tubing (inside diameter 0.28 mm), which serves as outlet. The junction between the inlet, the outlet tubing and the cannula was sealed with epoxy glue.

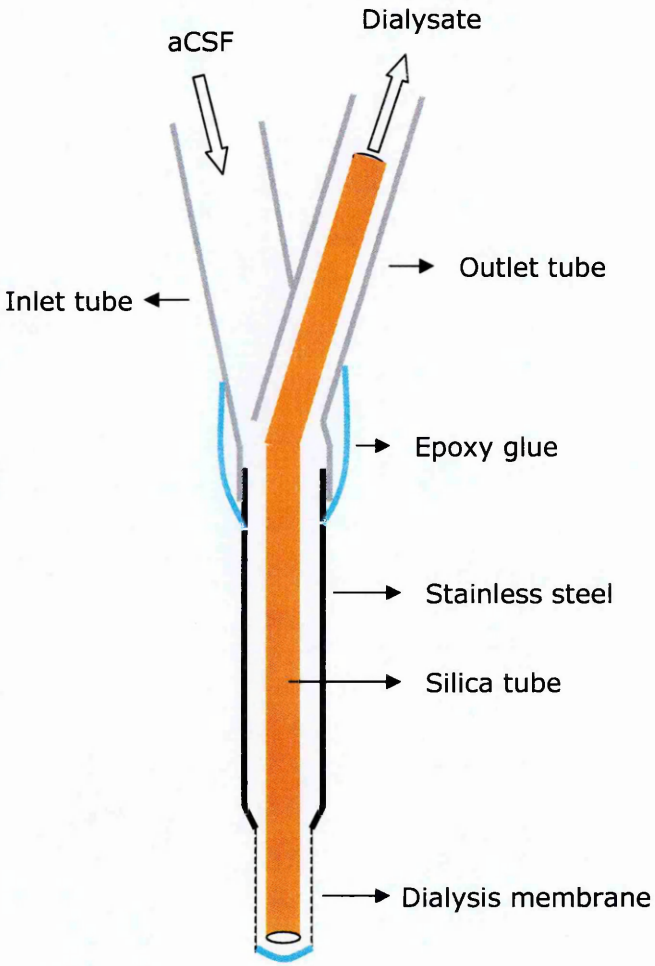


***Figure 2.2** Microdialysis probe*

2.2.2 Surgery

Mice were anesthetized with 3 mL/kg Equithesin intraperitoneally (composition: 1.2 g sodium pentobarbital; 5.3 g chloral hydrate; 2.7 g $MgSO_4$; 49.5 mL propylene glycol; 12.5 mL ethanol and 58 mL distilled water; maintained at +4° C in a dark bottle for 3 weeks) and secured in a stereotaxic frame (model 900, David Kopf, CA) with the incisor bar set at 0 mm.

The skin was shaved, disinfected with Bialcool (Teleflex Medical srl, Italy) and cut with a sterile scalpel to expose the skull. A hole (about 0.6 mm diameter) was drilled to allow the implantation of the probes into the brain parenchyma.

A dialysis probe, while being perfused with artificial cerebrospinal fluid (aCSF, composition in mM: NaCl 145, $CaCl_2$ 1.26, KCl 3, $MgCl_2$ 1, Na_2HPO_4 1.4, pH 7.4 with 0.6 M NaH_2PO_4) at 1 µL/min, was lowered slowly into the mPFC, the DH or DR at the stereotaxic coordinates from the bregma and dura surface taken from the stereotaxic atlas for mouse (Franklin and Paxinos, 1997). The microdialysis probe was secured to the skull with two stainless steel screws and dental cement (Paladur, New Galetti e Rossi, Milan, Italy).

The stereotaxic coordinates (in mm) were AP +2.1, L ±0.3 and V −2.5 for the mPFC and AP -2.3, L ±1.5 and V −2.0 for the DH from bregma and dura surface. For the DR they were AP -4.4 and L ±1.2 mm and V −4.2 at a 20° angle to the dorsal-ventral plane (***Figure 2.3***).

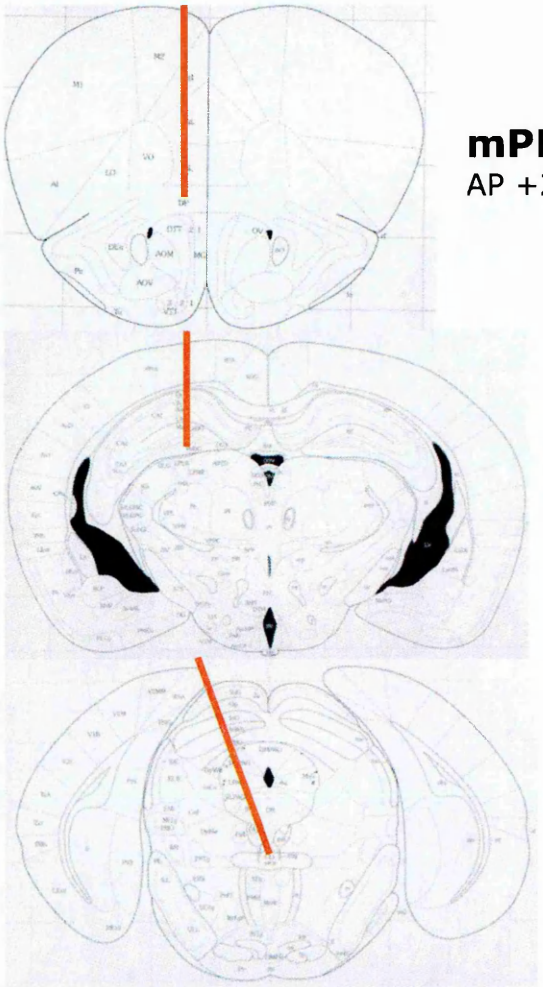


***Figure 2.3** Schematic representation of the positioning of microdialysis probse into the mPFC, DH and DR.*

Stereotaxic coordinates in mm are given for each brain regions according to Franklin and Paxinos (1997).

2.2.3 Sample collection and pharmacological treatment

Twenty hours after implantation, each animal was placed in a Perspex cage and the inlet cannula connected by polythene tubing (about 50-60 cm; Portex Ltd., Hythe, UK) to a 2.5 mL syringe (Icogamma plus, Novico spa, Ascoli Piceno, Italy) with a 26 gauge needle, mounted on a CMA/100

microinjection pump (CMA Microdialysis, Stockholm, Sweden) containing aCSF and perfused at a constant flow-rate of 1 µL/min. To allow the animal to move freely in the cage, inlet and outlet tubing were connected to a two-channel liquid swivel (Instech Laboratories, The Netherlands) mounted above the cage. After 1 hour washout, perfusate was collected every 20 min with a microsampler (Univentor, Zeitun, Malta). One µL antioxidant mixture (acetic acid 0.1 M, Na_2EDTA 0.27 mM, L-Cysteine 3.3 mM, ascorbic acid 0.5mM, pH 3.2) was added to the cortical and hippocampal samples that were stored at -20°C until analysis of 5-HT. DR samples were splitted into 3 aliquots: 10 µL (for 5-HT determination) were added with 1 µL of antioxidant mixture and 2 aliquots of 5 µL each, respectively for the analysis of GABA and GLU (*see Chapter 5*).

After four basal samples the animal received the pharmacological treatment.

2.2.4 5-HT assay

Automated injection of samples were performed using a refrigerated Midas autosampler (Spark-Holland, Emmen, The Netherland) set at 4°C.

5-HT in microdialysis samples (10 µL for the DR and 20 µL for the mPFC and DH) was assayed by HPLC with electrochemical detection as described elsewhere (Invernizzi et al., 1992). Briefly, 5-HT was separated by a reverse phase column (Supelcosil LC18-DB 3 µm, 150 x 4.6 mm; Supelchem, Milan, Italy) and a mobile phase consisting of citric acid 9 mM, sodium acetate 48 mM, Na_2EDTA 0.1 mM, 100 µL/L triethylamine and 40 mL/L acetonitrile, pumped at 1 mL/min. 5-HT was measured by a Coulochem II electrochemical detector equipped with a 5011 analytical cell

(ESA Inc., Chelmsford, MA) at the following potentials: E1 +50 mV, E2 +180 mV. 5-HT was read as the second electrode output signal. Detection limit was 1.1 fmol 5-HT on column (signal-to-noise ratio 2).

2.2.5 Collection of data and calculation

The peak of the neurotransmitter was recognized by the retention time compared with the retention time of the standard. The concentration of the neurotransmitter in the sample, not corrected for the *in vitro* recovery, was expressed in fmol (5-HT) or pmol/20µL (for GABA and GLU, see detailed methods in *Chapter 5*) and was automatically calculated by a data system (Azur, Datalys, Saint Martin d'Heres, France). Briefly, at least three different standard were freshly prepared daily by dilution from stock solutions. Peak height of the compounds of interest, expressed in mV was plotted against the respective concentrations to obtain the calibration curve (***Figure 2.4***). The amount of 5-HT, GABA and GLU in dialysate samples was automatically calculated by interpolating of the height of the corresponding peak into the calibration curve. Corrections for sample dilution were done as necessary.

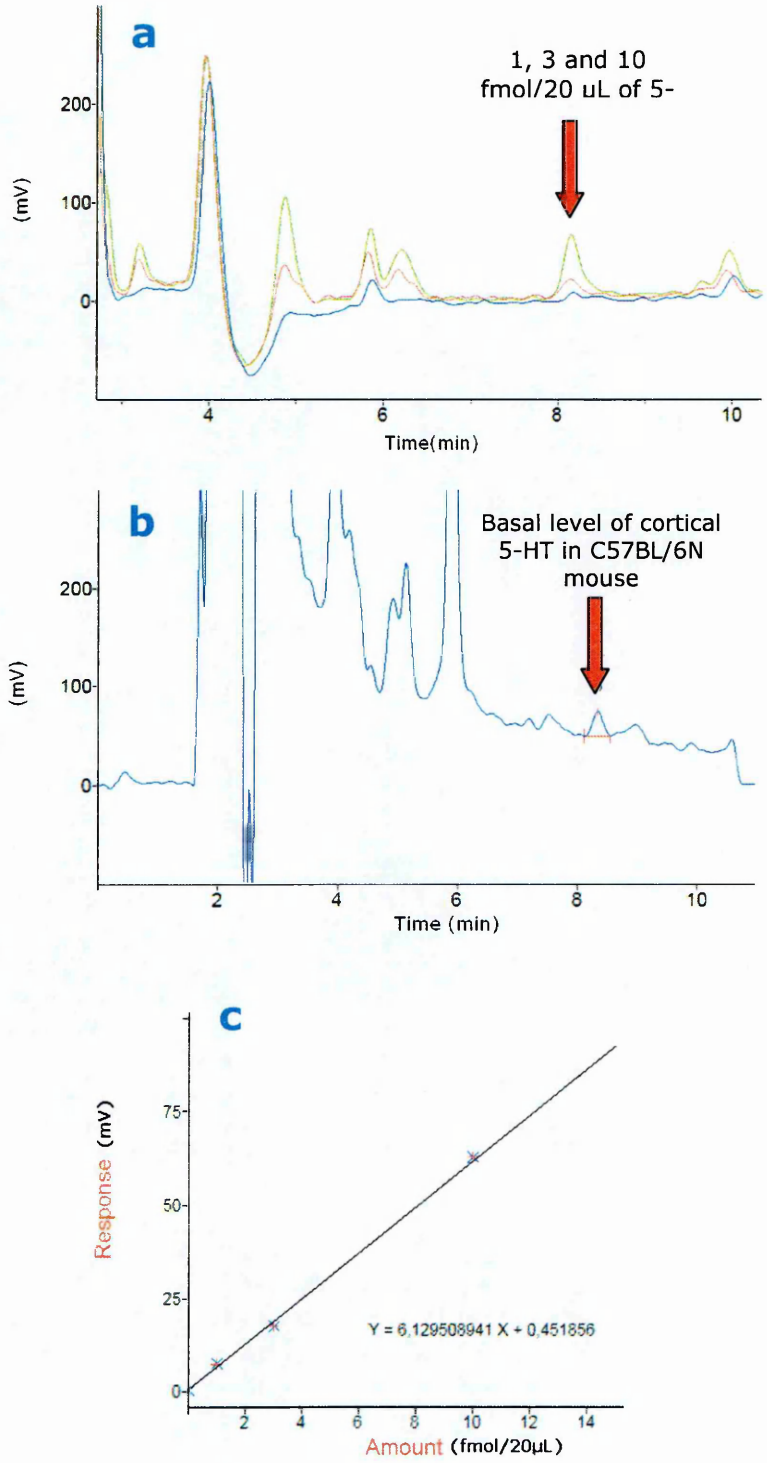


Figure 2.4 ***a)*** Chromatogram of 5-HT standard (1,3 and 10 fmol/20 µL).

b) Chromatogram of mPFC sample (basal; 20 µL) from a C57BL/6N mouse.

c) example of calibration curve for 5-HT.

2.2.6 Histology

At the end of each experiment, animals were killed by decapitation. The brain was removed, placed in 4% paraformaldehyde for 3 days at +4° C, then transferred in sucrose 20% in PBS (100 mL: 800 mg NaCl, 20 mg KCl, 144 mg Na_2HPO_4, 24 mg KH_2PO_4, buffered to pH 7.4) for one day and then frozen at –45° C in n-pentane.

Correct probe placement was verified by visual inspection of the probe track on Nissl-stained coronal sections (30 µm) of each animal. Examples of probe placement in mPFC, DH and DR are shown in ***Figure 2.5***. Animals were included in the results only if no gross alterations of tissue surrounding the probe (as haemorrhage, necrosis or edema) were seen and the probes were correctly positioned in the target regions (±0.3 mm AP; ±0.2 mm L and ±0.2 mm V of the given coordinates).

The Nissl's staining was performed as follows: in successive passages, the slides were dipped in ultra pure water for 1', in ethanol 70% for 5', in 95% for 5', in 100% for 5', in xylene for 5'. All the steps were repeated in the reverse order. Then, the slides were dipped in a 0.5% cresyl-violet solution (prepared in methanol 25%) for 2-3' and, in successive steps in water for ten washings, in ethanol 70% for ten washings, in 95% for 3', in a solution of 95% with 3% acetic acid for 1', in 100% for 3', in xylene for 5'. Finally, slides were covered with the micro cover glass.

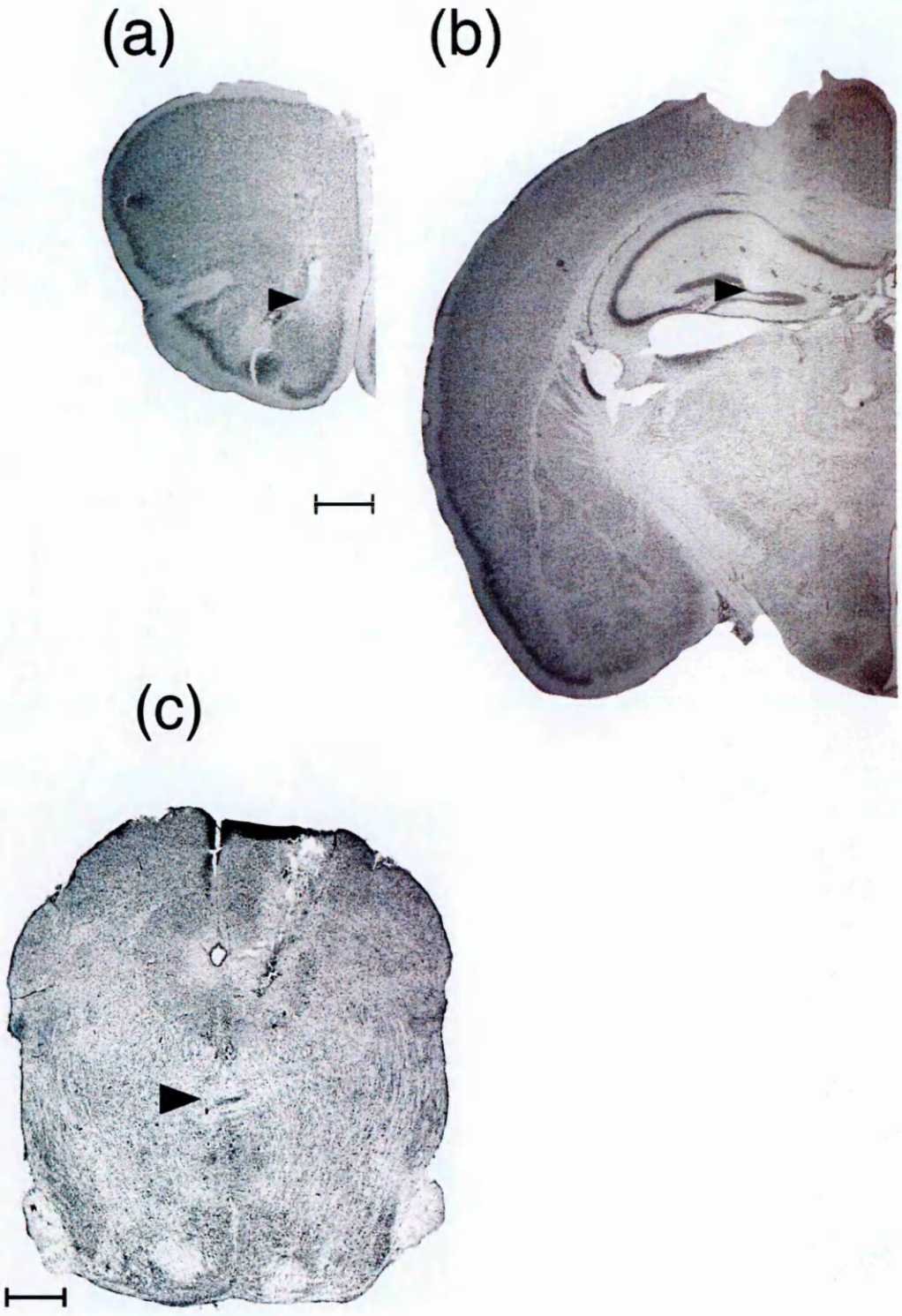


Figure 2.5 Representative positioning of the probe in the mPFC (***a***), DH (***b***) and DR (***c***) of the mouse. Arrowheads indicate the tip of the probe. Scale bar = 0.5 mm

2.3 Behavioural test

The forced swimming test (FST) was designed by Porsolt (1977a) as a primary screening method for antidepressants in rodents. The widespread use of this test is due to its low-cost, but it is also fast, simple to perform and reliable across laboratories. As discussed previously, even if the construct and face validity of the FST are minimal, it has a strong predictive validity.

In fact it has a great sensitivity with all the antidepressant classes and all the mechanisms of action of treatments could be determined, but clinical correlations should be considered very carefully.

For example, one major drawback of the FST is that acute drug treatments are effective in mice and do not correspond to the clinical time course of their action.

In spite of these limitations the FST is a suitable test to detect antidepressant potential of drugs and it is an important tool for looking into the mechanism of action of antidepressants and their relationship with the role played by 5-HT (Cryan et al., 2005a).

In 1977, Porsolt tested a large range of antidepressants and showed a reduction of immobility of mice and rats with all of them. Other clinical therapies such as electroconvulsive shock or sleep deprivation were also effective (Porsolt et al., 1978; Porsolt et al., 1977b).

Porsolt et al. (1978) described the immobility as a behavioural despair reflecting depressive mood and the FST was considered originally as a model of depression. Nevertheless this passive behaviour could also be

considered as aversion to maintain effort in an inescapable situation perceiving it as a successful coping strategy (Lucki et al., 2001). The behavioural abnormalities observed in the FST are situation-specific. Therefore, the FST cannot be considered as a depressive-like state model but can evaluate a pre-existing condition measured as immobility time that is reduced by a variety of antidepressant treatments.

In rats, if antidepressants are given between two exposures to FST, the animals will actively persist engaging in escape behaviour. For reason not yet clear, in mice one exposure is sufficient to generate immobility counteract by antidepressant treatments.

There have been many modifications of the FST both for rats and mice, but improvements of the test are often poorly validated (*for review see* Petit-Demouliere et al., 2005). Many parameters have been assessed in order to increase the sensitivity, specificity and reliability of detection of antidepressant activity. These procedural modifications include:

Depth of water, in fact, the tails should not touch the bottom of the cylinder or the behaviour of the animals would be altered. It has been observed that increasing depth of water decreased the time spent immobile.

To adjust the depth of the water allows distinguishing specific behavioural components of active behaviour in rats: climbing (upward-directed movements), swimming (horizontal movements) and immobility. The modified FST reveals that catecholaminergic agents decrease immobility increasing the climbing behaviour whereas serotonergic drugs such as SSRIs decrease immobility increasing swimming behaviour (Cryan et al., 2005a; Cryan et al., 2002).

Cylinder diameter, that provides a way to distinguish the antidepressant drugs from psychostimulants such as amphetamine and caffeine, anticholinergics, and antihistaminics, which gave a false positive response in 10 cm diameter cylinders in mouse FST.

Also the water temperature is important: a higher temperature (35°C) resulted in shorter immobility time after 10 min of forced swimming (Arai et al., 2000).

Other important parameters that contribute to the behavioural performance of rodents are circadian rhythm, environment of the laboratory, food restriction, gender, age, housing of animals/isolation of animals, observer and strains.

Strain is one of the most important parameters to deal with (Lucki et al., 2001). In fact genetic factors may contribute to the behavioural performance of mice in models of depression and drug sensitivity in the FST is genotype-dependent (David et al., 2003).

Immobility observed in the swim test seems not to be related to behaviour in the tests used in anxiety models but it is important to add an additional test such as the actimeter test, to distinguish between psychostimulant doses in order to avoid false positive results. In fact psychostimulant drugs could reduce immobility without having an antidepressant effect (Porsolt et al., 1977b; David et al., 2003).

2.3.1 Forced swimming test (FST)

The forced swimming test employed was essentially similar to that described elsewhere (Porsolt et al., 1977a; Cervo et al., 2005). Male C57BL/6J, C57BL/6N, DBA/2J, DBA/2N and BALB/C mice (Charles River Laboratories; Calco, Italy; see *section 2.1*), 6-8 weeks old, were dropped individually into a clear Plexiglas cylinders (height: 25 cm, diameter: 10 cm) containing 15 cm water, maintained at 25 ± 1°C, and their behaviour was videotaped for 6 min (***Figure 2.6***). After 2 min of habituation, the total period of immobility (t imm) was timed by two observers unaware of the treatment that mice had received. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water (Porsolt et al., 1978).

All experiments were carried out within 9:00 and 13:00 a.m.

Figure 2.6 The forced swimming test *(modified from* Cryan et al., 2002*)*

2.3.2 Measurement of locomotor activity in mice

Separate groups of mice were used to assess whether treatments reducing immobility in the FST affected locomotor activity. Mice receiving the same treatment of those in the FST, were placed individually in an open field arena made of grey plastic (40 x 40 cm) with the floor divided into 25 equal squares. Spontaneous locomotor activity was videotaped for 6 min and quantified later by counting the number of squares crossed in the last 4-min period, corresponding to the behavioural observation time in the FST.

2.4 Analysis of the data and Statistics

In the microdialysis experiments, the last of three consecutive stable samples was considered the basal value. 5-HT, GABA and GLU outputs were considered stable if three consecutive basal samples do not differ by more than 15%.

Differences in basal values between different groups of animals were analyzed by Student's t-test (for 2 groups) or by one-way-ANOVA for more than 2 groups.

The effect of various treatments was analyzed by ANOVA for repeated measures with treatments as between subjects factor and time as within subjects factor. Post-hoc comparisons between pre- and post-injection values and between treatments were made with Tukey-Kramer's test. Missing basal values because of occasional problems in sample collection or analysis were replaced by the mean of the samples immediately before and after or by the "last observation carried forward" method if the missing value is the last sample before drug injection. If two or more missing values occurred in the same mouse or missing values occurred after drug treatment the subject was not considered in the results.

The effects of a pharmacological treatment alone or in combination with other drugs on immobility time and locomotor activity were analyzed by two-way ANOVA followed by Tukey-Kramer's test.

Statistical analyses were done with the StatView 5.0 software (SAS Institute Inc., SAS Campus Drive, Cary, NC).

Chapter 3

STRAIN DIFFERENCES IN THE RESPONSE TO SSRIs: ROLE OF SEROTONIN

3.1 Introduction

Numerous studies have suggested associations between response to SSRIs, the mainstays in the treatment of depression, and genes modulating central serotonergic neurotransmission.

As discussed in *Chapter 1*, G1463A polymorphism of human brain tryptophan hydroxylase (TPH-2), the rate-limiting enzyme in the biosynthesis of 5-HT, is associated with less synthesis of 5-HT when the enzyme was expressed in PC-12 cells and with a poor response to SSRIs (Zhang et al., 2005).

The gene encoding mouse TPH-2 shows a single nucleotide polymorphism (C1473G) with a different allelic distribution in different strains of mice (***Table 3.1***; Zhang et al., 2004; Cervo et al., 2005; Kulikov et al., 2005; Jacobsen et al., 2008). DBA/2 and BALB/c mice are homozygous for the 1473G allele, linked to a lower 5-HT synthesis rate and no response to citalopram in the forced swimming test (FST; Cervo et al., 2005; Zhang et al., 2004).

In addition, citalopram produced a smaller reduction in accumulation of 5-HTP in DBA/2J and BALB/c than in C57BL/6J and 129/Sv mice, suggesting that it had less effect in mice with reduced rate of 5-HT synthesis (Cervo et al., 2005).

***Table 3.1** TPH-2 genotype at bp 1473 in several strains of mice*

STRAIN	1473C	1473G
C57BL/6J and C57BL/6N	+	
129/Sv and 129/SvJ	+	
BALB/c, BALB/cJ and BALB/cAnNCriBR		+
DBA/2J, DBA/2N and DBA/Ola		+
NMRI	+	
AKR/J	+	
PT/Y	+	
YT/Y	+	
C3H/HeJ	+	
DD/He	+	
A/J		+

p-Chlorophenylalanine (pCPA) is a selective inhibitor of 5-HT synthesis in the brain and periphery (Koe and Weissman, 1966). It binds and inactivates irreversibly tryptophan hydroxylase without affecting the activity of the closely related enzyme tyrosine hydroxylase.

The inhibition of 5-HT synthesis with pCPA supports to the role of 5-HT in the response to SSRIs, preventing the antidepressant-like effect of SSRIs in the mouse and rat FST and mouse TST (Page et al., 1999; Rodrigues et al., 2002; Gavioli et al., 2004; Cervo et al., 2005). Furthermore, the inhibition of 5-HT synthesis with pCPA or the 5-HT precursor, tryptophan (Trp) depletion reversed the effect of SSRIs in drug-remitted depressed patients, as described in *Chapter 1*.

Moreover, pCPA and depletion of Trp (Grahame-Smith, 1964; Weber and Horita, 1965) reduced the availability of extracellular 5-HT at central synapses (Oluyomi et al., 1994; Pozzi et al., 1999; Bel and Artigas, 1996) while Trp, leading to increased 5-HT synthesis, enhanced the release of the neurotransmitter (Carboni et al., 1989).

Therefore it is likely that genotype-dependent impairment of 5-HT synthesis influences the availability of extracellular 5-HT and the effect of drugs that act on extracellular 5-HT concentrations such as the SSRIs.

It is important to confirm the effect of citalopram in several brain regions and to assess if the failure of citalopram to reduce immobility time in DBA/2 and BALB/c mice, attributable to genotype-dependent impairment of 5-HT synthesis (Cervo et al., 2005), can be extended to other SSRIs. Hence, we assessed the effects of citalopram and paroxetine, respectively the most selective and the most potent SSRIs (Hyttel, 1977; Pozzi et al., 1999; Pollock, 2001; Rickels et al., 1992; Thomas et al., 1987) in the FST and on 5-HT synthesis in the whole brain and in several brain areas (brainstem, striatum, hippocampus, frontal cortex and rest of the cortex) of different strains of mice.

The characteristics of a mouse can vary depending on the particular substrain (Bryant et al., 2008), which is a strain of mouse that has diverged from its parent strain for 20 or more generations. The substrain may show residual heterozygosity left over from the time of separation or carry new mutations not found in the parent strain. Therefore, in order to assess the contribution of the different substrains background to the response to SSRIs, we compared different substrains of C57BL/6 (J and N) and DBA/2 (J and N) mice. "J" identifies mice substrains deriving from The Jackson Laboratory while "N" defines those from the National Institutes of Health (*www.criver.com*). Such comparison can help clarifying the relationship between the genotype-dependent impairment of 5-HT synthesis and the response to SSRIs.

Since there is evidence that non-serotonergic mechanisms may contribute to the antidepressant-like effects of SSRIs (Cryan et al., 2004), the involvement of noradrenaline and dopamine in the effect of SSRIs was tested by evaluating the effect of citalopram and paroxetine on catecholamine synthesis.

The role of 5-HT was further assessed by evaluating the response to paroxetine in mice with lowered (using pCPA) or boosted (with Trp) 5-HT synthesis. 5-HTP and DOPA accumulation, induced by decarboxylase inhibition, were used as an indicator respectively of 5-HT and catecholamines synthesis after various pharmacological treatments. To exclude that differences in Trp availability may account for the reduced 5-HT synthesis in mice carrying the mutated isoform of TPH-2, we also measured the level of Trp in several brain regions of DBA/2J and C57BL/6J mice.

Next we investigated whether genotype-dependent impairment of 5-HT synthesis influenced extracellular 5-HT and the ability of citalopram to raise extracellular levels of the neurotransmitter in the medial prefrontal cortex (mPFC) and dorsal hippocampus (DH), two brain regions representative of the serotonergic innervations arising respectively from the dorsal and median raphé nuclei (Azmitia and Segal, 1978).

Extracellular levels of 5-HT are mainly determined by the balance between the amounts of neurotransmitter released and taken up at nerve terminals. Thus, changes in both mechanisms might contribute to differences in the availability of extracellular 5-HT in different strains. To evaluate the

contribution of these factors, we used the method of the "zero-net-flux" that allows assessing the real extracellular concentration and provides an indirect estimate of reuptake functionality. Additionally, we evaluated [^{3}H]5-HT uptake in cortical and hippocampal synaptosomes of C57BL/6 and DBA/2J mice and the potency of citalopram in inhibiting [^{3}H]5-HT uptake.

3.2 Methods

Microdialysis and behavioural studies were conducted as described in *Chapter 2*.

3.2.1 5-HT and catecholamines synthesis

5-HT and catecholamines synthesis rate were assessed by measuring the levels of 5-hydroxytryptophan (5-HTP) and dihydroxyphenylalanine (DOPA), respectively the immediate precursor of 5-HT and catecholamines, after inhibition of aromatic L-aminoacid decarboxylase with m-hydroxybenzylhydrazine (NSD-1015; Carlsson and Lindqvist, 1978).

Thirty min after citalopram or paroxetine, mice were given 100 mg/kg NSD-1015 and killed by decapitation 30 min later.

Each brain was removed from the skull and laid on an ice-chilled dish; the cerebellum was removed and discarded. The brain was cut along the sagittal line. Half the brain was immediately frozen on dry ice. Brain areas including brainstem, striatum, hippocampus, cortex and frontal cortex were dissected out from the other half and frozen. Tissue sample were homogenized by sonication (output 2-3, 30-35 pulses with a Branson Sonifier, model 250; Branson Ultrasonic Corporation, Danbury, CT) in 10 volumes of ice-cold 0.1 M $HClO_4$. Homogenates were left at 4°C for 30 min to complete deproteinization, then centrifuged at 4800 x g for 10 min at 4°C. The clear supernatant was injected into the HPLC equipped with a 150 x 3.9 mm C18 reverse-phase column (Nova-pack, Waters, Italy) and coupled to an electrochemical detector (Coulochem II, ESA, USA) to

determine tissue concentration of 5-HTP and DOPA, according to the procedure described elsewhere (Lasley et al., 1984).

3.2.2 Tryptophan assay

The tissue concentration of Trp was determined in a separate group of mice according to the procedure described elsewhere (Lasley et al., 1984). The brain areas dissection and the preparation of tissue samples were performed as described in *section 3.2.1*. The clear supernatant was injected into the HPLC equipped with a 150 x 3.9 mm C18 reverse-phase column (Nova-pack, Waters, Milan, Italy) and coupled to the Coulochem II electrochemical detector equipped with a 5011 analytical cell (ESA Inc., Chelmsford, MA) at the following potentials: E1 +400 mV, E2 +520 mV. The mobile phase consisted of monochloroacetic acid 0.15 M, sodium octyl sulfate 1 mM, Na_2EDTA 0.01 mM and 150 mL/L methanol, pH 3 with 10 M NaOH, pumped at 1 mL/min.

3.2.3 Zero-net-flux protocol

The zero-net-flux method of quantitative microdialysis (Lönnroth et al., 1987) was used in the mPFC and DH of DBA/2J and C57BL/6J mice. Mice were perfused with aCSF at 1 µL/min and after 1-h equilibration, baseline samples were collected at 20-min intervals and analyzed by HPLC.

When dialysate 5-HT was stable, three concentrations of 5-HT, 0.5, 1.5 and 5 nM (C_{in}), dissolved in aCSF were perfused through the microdialysis probe. These concentrations (C_{in}) of standard solution of 5-HT were chosen taking into account the *in vitro* recovery of 5-HT across the cuprophan membrane, estimated as follows:

The dialysis probes were dropped into a 5-HT solution (100 fmol/20 µL) in aCSF. The probe was perfused with aCSF (1 µL/min), at room temperature. After 5 min washout, three consecutive 20-min samples were collected and the concentration of 5-HT in the dialysate is determined by HPLC as described in *Chapter 2*. The percentage recovery is calculated by comparing the mean concentrations of the neurotransmitter in the three samples of dialysate ($C_{dialysate}$) with that in the solution in which the probe was immersed (C_{out}) according to the formula $C_{dialysate}/C_{out}*100$ (Zetterström et al., 1983). The *in vitro* recovery of the neurotransmitter was expressed as Mean ± SEM of two different experiments.

In the zero-net-flux experiments, each concentration of 5-HT was perfused for 1 h through the probe (C_{in}). The first 20-min fraction was discarded and the mean 5-HT content in the second and third fractions corresponds to the C_{out} used to determine extracellular 5-HT and the extraction fraction (Ed). A linear regression plot was plotted with C_{in} on the x-axis and the difference between C_{in} and C_{out} on the y-axis. The point where the line crossed the x-axis was referred to as C_{ext}, where there is no net diffusion of 5-HT across the microdialysis membrane. The Ed corresponds to the slope of the linear regression.

3.2.4 Preparation of the synaptosomal fraction and [^{3}H]5-HT uptake

Hippocampi and frontal cortices of 4 mice (dissected out from C57BL/6J and DBA/2J mice as described in *section 3.2.1*) were homogenized in 40 volumes of ice-cold 0.32 M sucrose, pH 7.4, in a glass homogenizer with a Teflon pestle. The homogenates were centrifuged at 1000xg for 10 min at

4°C and the supernatants centrifuged again at 12,000xg for 20 min at 4°C to yield the crude synaptosomal pellet (Gray and Whittaker, 1962).

The pellets were resuspended in a buffer containing: NaCl (1.16 M); $NaHCO_3$ (0.25 M); NaH_2PO_4 (12 mM); KCl (0.59 M); MgSO4 (1); $CaCl_2$ (0.12 M); $MgSO_4$ (0.12 M); glucose (1.11 M); pargyline (0.25 mM); Hepes (20 mM); ascorbic acid (0.3 mM); pH 7.4. Samples of 500 µL were preincubated for 5 min at 30°C in a water bath. Uptake started by the addition of 100 µL of [^{3}H]5-HT (NEN, 21.4 Ci/mmol). For saturation studies 4 different concentrations of [^{3}H]5-HT were used (12.5, 25, 50 and 100 nM) and non-specific uptake was determined in parallel in the presence of 0.3 µM citalopram.

For inhibition studies, 25 nM [^{3}H]5-HT was added to the synaptosomal suspension containing 0, 3, 10, 30 or 300 nM of citalopram, in triplicate.

Uptake reactions were stopped after 5 min by adding 2 mL of ice-chilled buffer, followed by a rapid filtration through cellulose mixed ester filters (0.65 µm pore size, Millipore, Italy), which were washed with further 2 mL of buffer. The radioactivity trapped on the filters was counted in 4 mL of Ultima Gold MV (PerkinElmer Life and Analytical Sciences, Waltham, MA) in a Wallac 1409 liquid scintillation counter (PerkinElmer Life and Analytical Sciences, Waltham, MA) with a counting efficiency of about 60% (Gobbi et al., 2002).

Protein concentrations were assessed using bovine serum albumin as standard (Bradford, 1976) using the Bio-Rad protein assay reagent (Bio-Rad, Laboratories GmbH, Germany).

The saturation curves, i.e., in the presence of different concentrations of [^{3}H]5-HT, were fitted using the "one-site binding, hyperbola" equation

(GraphPad Prism 4.0a). This estimates the maximal uptake velocity (*V*max) and *K*m (affinity of [^{3}H]5-HT).

IC_{50} values were determined by fitting the concentration-response curves obtained in the presence of different concentrations of citalopram using the "one-site competition" equation built into Graph-Pad Prism 4.0a. Three independent experiments were carried out.

These experiments were done in collaboration with Dr. Marco Gobbi, Unit of Synaptic Transmission, Laboratory of Biochemistry and Protein Chemistry, Mario Negri Institute.

3.2.5 Drugs and drugs treatments

Citalopram hydrobromide (Tocris Cookson, Bristol, UK), paroxetine hydrochloride (GlaxoSmithKline, West Sussex, UK) and NSD-1015 (Sigma-Aldrich, Milan, Italy) were dissolved in 0.9% NaCl and injected intraperitoneally (i.p.) at the doses indicated. Control mice were injected with saline. The doses of citalopram (1.25-20 mg/kg) and time of treatment were in the range of those affecting immobility time in the FST and brain 5-HT synthesis (Cervo et al. 2005). Several doses of paroxetine (1.25-10 mg/kg), in the range of those selectively increasing extracellular 5-HT (David et al., 2003b), were used to evaluate the effect of the drug in the FST and open field activity.

pCPA ethyl ester (Sigma-Aldrich, Milan, Italy) was dissolved in sterile water. To facilitate the dissolution of Trp (Fluka, Buchs, Switzerland), 1.5 mL 1M NaOH were added to 16.95 mL of water. The pH of the solution was adjusted to 7.4 with 1.55 mL 1M HCl.

The drug solutions were freshly prepared immediately before use and injected in a volume of 10 mL/kg, with the exception of Trp that was given in 20 mL/kg. Doses of drugs were referred to the respective salts, except for doses of pCPA that were calculated as free base.

In the behavioural studies citalopram and paroxetine were injected intraperitoneally (i.p.) 30 min before the tests (Cervo et al., 2005).

To determine whether a treatment that boosts 5-HT synthesis (Cervo et al., 2005) reinstated the antidepressant-like effect of paroxetine in the FST, separate group of DBA/2 and BALB/c mice were given 300 mg/kg Trp or vehicle i.p. 30 min before 2.5 mg/kg paroxetine (inactive dose in the FST) or vehicle. Time of treatment and dose of tryptophan correspond to those previously shown to increase 5-HT synthesis and restore the antidepressant-like effect of citalopram (Cervo et al., 2005).

To demonstrate that a treatment that lowers 5-HT synthesis (Cervo et al., 2005) reduces the antidepressant-like activity of the SSRI, separate groups of C57BL/6N mice were given 100 mg/kg pCPA or vehicle orally for three consecutive days. This dose reduced brain 5-HT synthesis to the level observed in DBA/2 mice (Cervo et al., 2005). Twenty-four hours after the last dose, mice were given 2.5 and 5 mg/kg paroxetine (active doses in the FST) or vehicle and 30 min later their immobility time was evaluated in the FST.

Finally, the effect of tryptophan-induced reinstatement of paroxetine antidepressant-like activity was evaluated in DBA/2J mice in which 5-HT was completely depleted by 300 mg/kg pCPA (once daily for 3 days). This protocol allowed a marked reduction of brain 5-HT without affecting the levels of brain catecholamines. Separate groups of DBA/2J mice treated

with pCPA or vehicle as above, were given 300 mg/kg Trp or vehicle 30 min before receiving 2.5 mg/kg paroxetine or vehicle, given 30 min before testing.

To assess the effects of paroxetine and citalopram on 5-HT synthesis, separate group of mice were treated with the drug or vehicle (at the same doses used in the behavioural tests), 30 min later were given 100 mg/kg NSD-1015 and killed by decapitation after further 30 min, according to Carlsson et al. (1978). Another group of mice was injected with 300 mg/kg Trp 30 min before paroxetine or vehicle.

3.2.6 Statistics

Data were analyzed as described in *Chapter 2*.

In particular, to determine whether basal immobility time and locomotor activity differed between strains, data were analyzed by one-way ANOVA followed by Tukey-Kramer's or Dunnett's test. The effects of paroxetine on immobility time in different strains of mice given 2.5 and 5 mg/kg of the drug were compared by two-way ANOVA with strain and paroxetine as main factors. The effect of paroxetine in each strain was compared with that of vehicle by one-way ANOVA.

Finally, two-way ANOVA was used to analyze the effects of paroxetine in combination with tryptophan or pCPA on immobility time.

Basal 5-HTP and DOPA levels in different strains of mice were expressed in ng/g tissue. Regional differences in basal 5-HTP and DOPA across strains were compared by one-way ANOVA followed by Tukey-Kramer's test. The effects of citalopram and paroxetine on 5-HTP and DOPA accumulation in different strains were analyzed by two-way ANOVA, with strain and

treatment as main factors. To account for difference in basal 5-HTP levels, data were expressed as percentage increase of 5-HTP levels in vehicle groups. *Post-hoc* comparisons between strains and treatments were done by Tukey-Kramer's test.

The effect of the combination of paroxetine and Trp or pCPA on 5-HTP and DOPA levels was analyzed by two-way ANOVA.

Extracellular levels of 5-HT, uncorrected for *in vitro* recovery of the probe, were expressed as fmol/20 µL. Mean basal values of 5-HT in different strains of mice were compared by one-way ANOVA followed by Tukey-Kramer's test or by Student's t-test. All time-course data were analyzed by ANOVA for repeated measures with treatments and strain as between-subject factors and time as within-subjects factor. *Post-hoc* comparisons of pre- and post-injection values and comparisons between treatments were done with Tukey-Kramer's test. Regional differences in basal Trp across strains were compared by Student's t-test. Kinetic parameters (Vmax and Km) were compared by Student's t-test. The IC_{50} was analyzed by ANOVA.

3.3 Results

3.3.1 Effect of paroxetine on immobility time

No significant differences were found between experiments in basal immobility time in the various strains regardless of the number of vehicle injections [C57BL/6N, $F_{3,34}$= 0.3, p>0.05; BALB/c, t_{17}= 0.8, p>0.05; DBA/2J, $F_{2,24}$= 1.9, p>0.05; DBA/2N, $F_{4,42}$= 2.0, p>0.05]. When data were pooled, mean basal immobility time (s) in different strains was: C57BL/6J, 133 ± 19 (n= 9); C57BL/6N, 159 ± 6 (n= 38); DBA/2J 153 ± 4 (n= 27); DBA/2N 163 ± 4 (n= 47); BALB/c 155 ± 5 (n= 19). ANOVA showed no significant differences across strains [$F_{4,135}$ = 2.1, p>0.05].

The effect of paroxetine in the FST differed markedly across strains (***Figure 3.1***). Overall, two-way ANOVA indicated a significant effect of strain [$F_{4,136}$ = 19.2, p<0.01], paroxetine [$F_{2,136}$= 16.0, p<0.01] and their interaction [$F_{8,136}$= 4.5, p<0.01]. Paroxetine reduced immobility time in C57BL/6J mice [$F_{3,34}$= 4.7, p<0.01] and C57BL/6N [$F_{3,38}$= 13.3, p<0.01]. Post-hoc comparison showed a significant effect at 2.5 and 5 mg/kg whereas 1.25 mg/kg had no effect. Immobility time in DBA/2J [$F_{3,36}$= 0.8, p>0.05], DBA/2N [$F_{3,36}$= 0.8, p>0.05] and BALB/c [$F_{3,36}$= 0.3, p>0.05] mice was not affected by any dose.

As shown in ***Figure 3.2*** pCPA (100 mg/kg orally for 3 days) did not affect the immobility time in the FST, but it completely prevented the effect of 2.5 mg/kg paroxetine in C57BL/6N mice [$F_{1,35}$= 19.2, p<0.01], while it has no effect on the reduction of immobility time induced by 5 mg/kg paroxetine [$F_{1,35}$= 0.6, p>0.05].

As shown in ***Figure 3.3***, 2.5 mg/kg of paroxetine, which had no effect in DBA/2J, DBA/2N or BALB/c mice, significantly reduced immobility time in mice pre-treated with 300 mg/kg Trp [DBA/2N, $F_{1,33}$= 4.2, $p<0.05$; DBA/2J, $F_{1,24}$= 4.6, $p<0.05$; BALB/c, $F_{1,31}$= 20.1, $p<0.01$], a dose that by itself did not affect immobility in the three strains.

Figure 3.4 reports the effect of the combination of Trp and paroxetine in DBA/2N and DBA/2J mice pre-treated with pCPA (300 mg/kg orally for 3 days) or vehicle. pCPA completely prevented the effect of the combination of Trp and paroxetine in either strain [DBA/2N, $F_{1,33}$= 11.2, $p<0.01$; DBA/2J $F_{1,35}$= 22.1, $p<0.01$].

As shown in ***Figure 3.5***, 300 mg/kg Trp did not enhance the effect of an inactive dose of citalopram in C57BL/6N mice ($F_{1,34}$= 1.02, $P>0.05$).

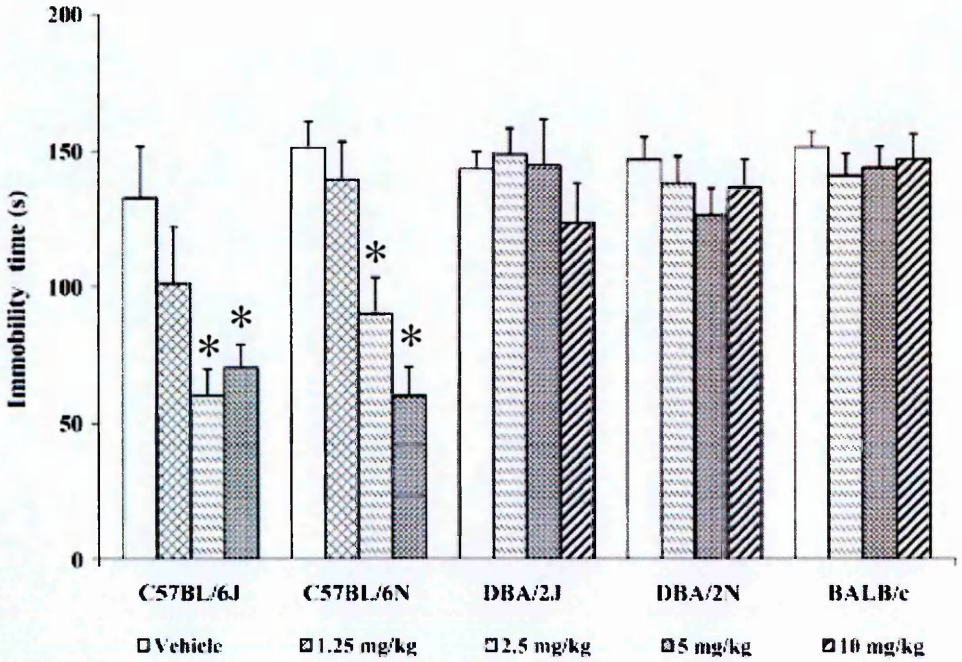


Figure 3.1 Strain differences in the effect of paroxetine in the FST. Mice received paroxetine i.p. 30 min before testing. Histograms are the mean ± SEM of 7-11 mice per group. *p<0.05 vs. vehicle, Dunnett's test.

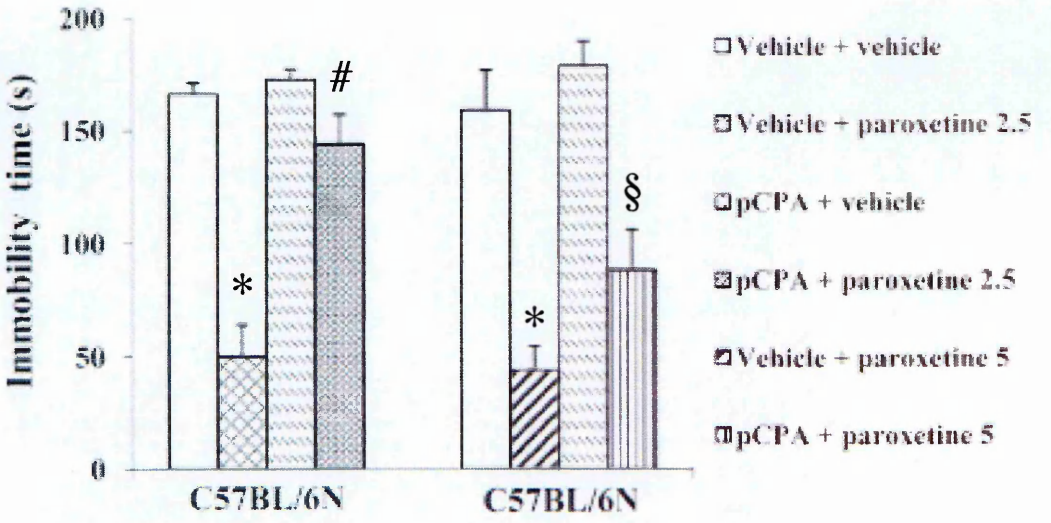


Figure 3.2 Effect of pCPA on paroxetine-induced reduction of immobility time in C57BL/6N mice. Mice were given vehicle or 100 mg/kg pCPA orally for three consecutive days. Twenty-four hours after the last dose they received 2.5 and 5.0 mg/kg paroxetine or vehicle 30 min before testing. Histograms are the mean ± SEM of 7-9 mice per group. *p< 0.05 vs. vehicle; #p<0.05 vs. paroxetine, §p<0.05 vs. pCPA (Tukey-Kramer's test).

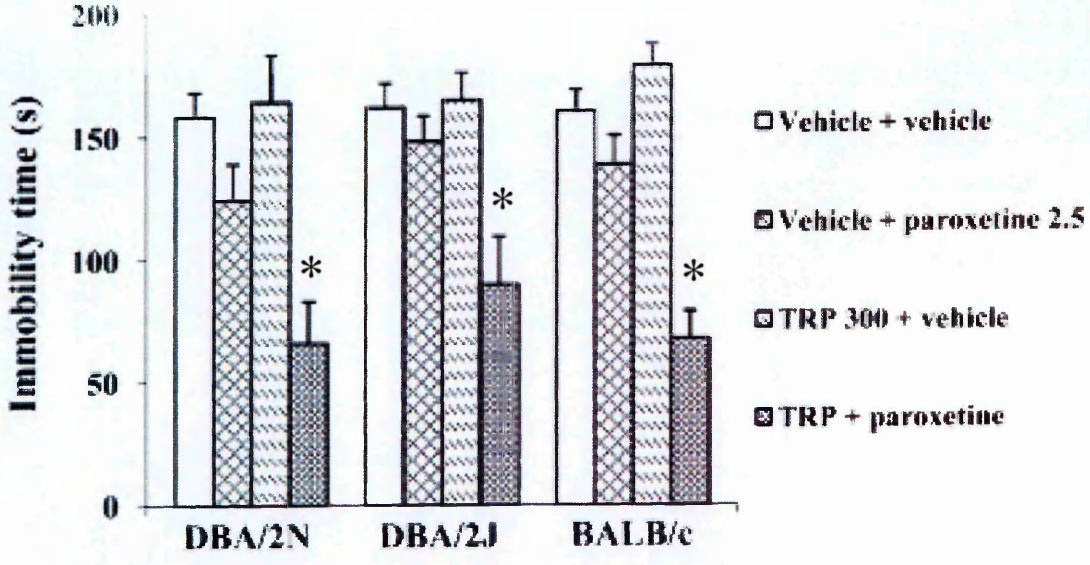


Figure 3.3 Effect of tryptophan (TRP) co-administered with paroxetine on immobility time of DBA/2N, DBA/2J and BALB/c mice. Mice received vehicle or 300 mg/kg i.p. TRP 30 min before 2.5 mg/kg i.p. paroxetine or vehicle given 30 min before testing. Histograms are the mean ± SEM of 7-10 mice per group. *$p<0.05$ vs. vehicle (Tukey-Kramer's test).

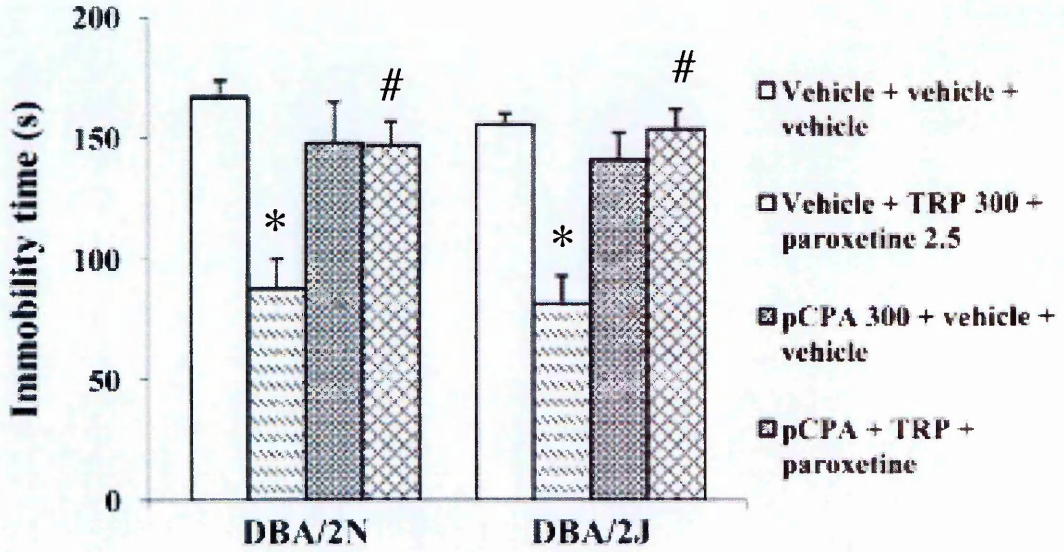


Figure 3.4 Effect of pCPA on reduction of immobility time by TRP co-administered with paroxetine in DBA/2N and DBA/2J mice. Mice were given vehicle or 300 mg/kg pCPA orally for three consecutive days. Twenty-four hours after the last dose they received vehicle or 300 mg/kg i.p. TRP 30 min before 2.5 mg/kg i.p. paroxetine or vehicle given 30 min before testing. Histograms are the mean ± SEM of 7-10 mice per group. *$p<0.05$ vs. vehicle; #$p<0.01$ vs. paroxetine (Tukey-Kramer's test).

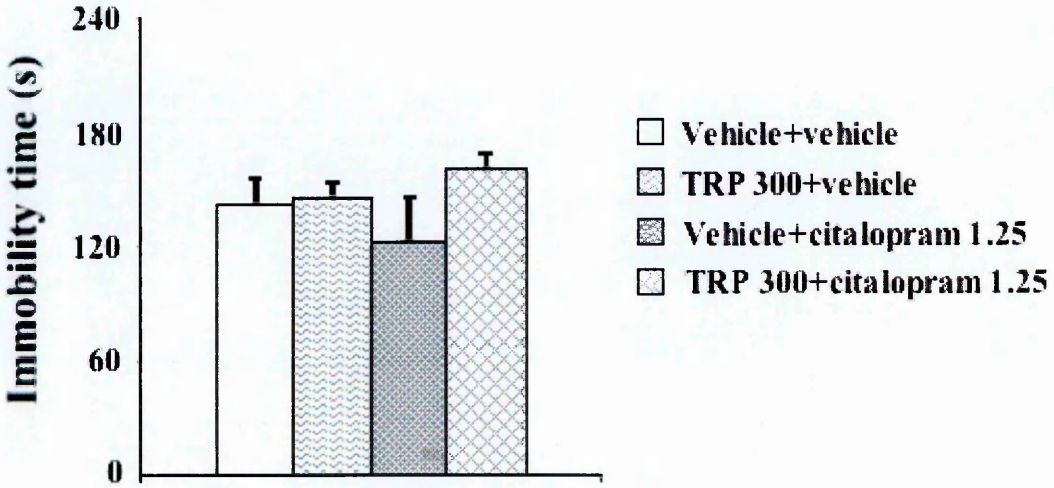


Figure 3.5 Immobility time in C57BL/6N mice given 1.25 mg/kg citalopram alone or in combination with 300 mg/kg tryptophan (TRP). TRP was injected intraperitoneally 1 h before citalopram. Mean± SEM of 9-10 mice per group.

3.3.2 Locomotor activity

The open field test showed significant differences in spontaneous locomotion, with BALB/c less active than C57BL/6 substrains and DBA/2J mice [$F_{3,30}$= 15.8, p<0.0001] (***Table 3.2***). As reported in ***Table 3.2a***, C57BL/6J and C57BL/6N mice showed no change in locomotor activity after doses of paroxetine that reduced immobility time in the FST [C57BL/6J, $F_{3,28}$= 0.5, p>0.05; C57BL/6N, $F_{3,32}$= 2.6, p>0.05].

Table 3.2b shows that tryptophan and paroxetine given singly had no effect but given together they reduced the locomotor activity of DBA/2J but not DBA/2N mice [DBA/2J: tryptophan, $F_{1,30}$= 2.7, p>0.05; paroxetine, $F_{1,30}$= 1.8, p>0.05; tryptophan x paroxetine interaction, $F_{1,30}$= 5.0, p<0.05. DBA/2N: tryptophan, $F_{1,28}$= 0.002, p>0.05; paroxetine, $F_{1,28}$= 3.2, p>0.05; tryptophan x paroxetine interaction, $F_{1,28}$= 0.0001, p>0.05]. In BALB/c mice tryptophan, paroxetine and the two together did not affect the open-field activity [tryptophan, $F_{1,28}$= 0.2, p>0.05; paroxetine, $F_{1,28}$= 1.6, p>0.05; tryptophan x paroxetine interaction, $F_{1,28}$= 0.1, p>0.05].

Table 3.2 Open-field activity in C57BL/6J, C57BL/6N, DBA/2N, DBA/2J and BALB/c mice.

Dose of paroxetine	C57BL/6J	C57BL/6N
(mg/kg)	Number of squares crossed in a 4-min period	
Vehicle	188 ± 10 (8)	169 ± 14 (9)
1.25	191 ± 9 (8)	138 ± 17 (9)
2.5	216 ± 29 (8)	137 ± 9 (9)
5.0	207 ± 21 (8)	181 ± 15 (9)

a) Effects of different doses of paroxetine or vehicle on open-field activity in C57BL/6J and C57BL/6N mice. Data are expressed as Mean ± SEM. The number of mice is indicated in parentheses.

Treatment	DBA/2J	DBA/2N	BALB/c
	Number of squares crossed in a 4-min period		
Vehicle + vehicle	153 ± 13 (9)	182 ± 10 (8)	80 ± 8# (8)
Vehicle + paroxetine	166 ± 18 (8)	163 ± 13 (8)	103 ± 25 (8)
Tryptophan + vehicle	162 ± 13 (9)	181 ± 3 (8)	91 ± 4 (8)
Tryptophan + paroxetine	110 ± 14* (8)	163 ± 12 (8)	104 ± 9 (8)

b) Effects of tryptophan (300 mg/kg), paroxetine (2.5 mg/kg) and vehicle singly or together on open-field activity in DBA/2N, DBA/2J and BALB/c mice. *$p<0.05$ vs. vehicle + vehicle; #$p<0.05$ vs. vehicle-treated mice of all other strains (Tukey-Kramer's test).

3.3.3 Brain 5-HTP

Basal levels of 5-HTP and DOPA

Table 3.3a shows brain and regional levels of 5-HTP in different strains of mice. Post-hoc comparisons indicated that brain and regional levels of 5-HTP in DBA/2J, DBA/2N and BALB/c mice were 20-40% lower than in C57BL/6J and C57BL/6N mice ($p<0.05$, Tukey-Kramer's test).

Overall, ANOVA indicates a significant difference across strains in basal 5-HTP levels in the brain [$F_{4,20}= 27.2$, $p<0.01$], hippocampus [$F_{4,20}= 18.1$, $p<0.0001$], striatum [$F_{4,20}= 8.1$, $p=0.0005$], brainstem [$F_{4,20}= 23.3$, $p<0.0001$], cortex [$F_{4,20}= 32.7$, $p<0.0001$] and frontal cortex [$F_{4,20}= 2.9$, $p<0.05$]. There were no significant differences between C57BL/6J and C57BL/6N or between DBA/2J and DBA/2N substrains in brain and regional 5-HTP levels.

There were no differences between strains in DOPA levels in the whole brain or in any of the regions examined with the exception of the frontal cortex of BALB/c mice where DOPA was significantly higher (38%) than in C57BL/6J mice (***Table 3.3b***; [frontal cortex: $F_{4,20}= 3$, $p=0.04$]).

Table 3.3 Basal 5-HTP and DOPA levels in C57BL/6J, C57BL/6N, DBA/2N, DBA/2J and BALB/c mice.

STRAIN	BRAIN	STRIATUM	HIPP	CX	FCX	BST
C57/6J	234.9±4.8	259.7±23.9	250.2±12.7	177.4±6.4	164.2±9.9	398.8±25.9
C57/6N	268.3±15.9	376.8±51.4	269.6±12.4	204.4±13.6	204.5±16.2	462.7±23.1
DBA/2J	173.1±4.3 *#	188.8±22.3 #	200.6±9.5 *#	129.1±4.1 *#	156.5±13.3	274±11.8 *#
DBA/2N	174.6±9.4 *#	159.0±33.5 #	196.8±9.4*#	109.3±5.7 *#	143.3±13.9 #	273.9±7.2 *#
BALB/c	160.4±4.8 *#	166.8±17.0 #	148.7±11.9*#§x	107.9±4.1 *#	156.5±14.4	300.9±12.2 *#

a) Basal level of 5-HTP. * $p<0.05$ vs. C57/6J; # $p<0.05$ vs. C57/6N; § $p<0.05$ vs. DBA/2J;x $p<0.05$ vs. DBA/2N (Tukey-Kramer's test). HIPP=hippocampus; CX=rest of the cortex; FCX=frontal cortex; BST=brainstem. Values are expressed as ng/g tissue (Mean ± SEM). The number of mice is 5 per group.

STRAIN	BRAIN	STRIATUM	HIPP	CX	FCX	BST
C57/6J	248.9±5.3	961.6±104.5	102.1±6.7	304.7±14.1	120.1±8.3	225.9±7.7
C57/6N	263.0±12.6	989.8±119.4	107.0±6.7	301.7±8.3	149.0±11.0	240.0±11.0
DBA/2J	251.2±8.6	1078.9±158.8	90.4±6.0	306.1±10.1	127.0±12.3	217.0±6.4
DBA/2N	265.3±13.9	830.7±76.7	110.4±6.6	292.8±15.3	128.3±15.2	221.4±5.6
BALB/c	327.0±36.7	854.6±246.5	94.5±6.5	336.6±14.5	193.2±29.8 *	249.7±9.0

b) Basal level of DOPA. * $p<0.05$ vs. C57/6J (Tukey-Kramer's test). Values are expressed as ng/g tissue (Mean ± SEM). The number of mice is 5 per group. See ***Table 3.3a*** for abbreviations.

Effects of paroxetine on 5-HTP levels

Table 3.4a shows the effect of paroxetine on brain 5-HTP levels in the various mouse strains in the whole brain and in different brain regions. Paroxetine had a significant effect on 5-HTP accumulation [$F_{3,79}$= 107.4, $p<0.01$] but the effect differed across strains [strain, $F_{3,77}$= 10.9, $p<0.001$]. As reported in ***Table 3.4b***, the effect of paroxetine was significant in all areas, indicating that paroxetine effectively reduced 5-HTP in all brain regions examined. Reduction ranged between 8% and 50% depending on the brain region and the dose. No clear dose-dependent effects were observed in the range of doses examined.

Maximal reduction was found in the hippocampus whereas paroxetine tended to have less effect in the brainstem and in the striatum.

Post-hoc comparison indicated that paroxetine induced significantly less reduction of 5-HTP levels in the striatum, hippocampus, cortex and frontal cortex of DBA/2J compared to C57BL/6J mice and in DBA/2J mice compared to C57BL/6N mice ($p<0.05$, Tukey-Kramer's test). No differences were found in 5-HTP levels in C57BL/6J and C57BL/6N and in DBA/2J and DBA/2N substrains.

Table 3.4 Effect of paroxetine on 5-HTP levels

DOSE	STRAIN	BRAIN	STRIATUM	HIPP	CX	FCX	BST
1.25 mg/kg	C57BL/6J	-21±6%*	-21±8%	-36±8% *	-32±5% *	-24±6% *	-22±3%*
2.5 mg/kg		-27±3%*	-42±3% *	-42±1% *	-33±5% *	-30±7% *	-26±6%*
5 mg/kg		-35±3%*	-40±4%*	-42±4% *	-42±2% *	-36±2% *	-27±2%*
1.25 mg/kg	C57BL/6N	-29±6%*	-28±6%	-43±3% *	-38±3% *	-31±3% *	-24±4%*
2.5 mg/kg		-39±7%*	-27±9%	-50±5% *	-38±9% *	-40±6% *	-29±9%*
5 mg/kg		-35±3%*	-8±9%	-46±3% *	-39±3% *	-32±3% *	-24±3%*
1.25 mg/kg	DBA/2J	#§ -6±8% *	-10±10%	-24±6% *	-15±5% *	-12±5%	-13±9%
2.5 mg/kg		#§ -20±3%*	# -23±2%	-36±2% *	-25±3% *	-22±5%	-28±4%*
5 mg/kg		#§ -16±6%*	-15±7%	-29±6% *	-25±1% *	-19±4%	-26±5%*
1.25 mg/kg	DBA/2N	§-17±3%*	-33±7%	-33±2% *	-24±5% *	§-26±4% *	-17±4%
2.5 mg/kg		§-22±3%*	-26±8%	-33±3% *	-28±3% *	§-17±1% *	-14±2%
5 mg/kg		§-25±6%*	-28±8%	-38±2% *	-41±8% *	§-25±4% *	-22±3%*

a) Effect of paroxetine on 5-HT synthesis in different strain of mice. Values are expressed as percentage of decrease ±SEM. * $p<0.05$ vs. vehicle (Tukey-Kramer's test); # $p<0.05$ vs C57BL/6J (Tukey-Kramer's test); §$p<0.05$ vs C57BL/6N (Tukey-Kramer's test). n=5. See ***Table 3.3a*** for abbreviations.

	STRAIN	PAROXETINE	INTERACTION
BRAIN	$F_{3,77}=10.9$ *	$F_{3,77}=52.1$ *	$F_{9,77}=1.8$
STRIATUM	$F_{3,74}=2.6$	$F_{3,74}=11.4$ *	$F_{9,74}=1.3$
HIPP	$F_{3,78}=6.9$ *	$F_{3,78}=85.1$ *	$F_{9,78}=1$
CX	$F_{3,78}=6.6$ *	$F_{3,78}=57.9$ *	$F_{9,78}=1.5$
FCX	$F_{3,78}=6.3$ *	$F_{3,78}=35.1$ *	$F_{9,78}=1.7$
BST	$F_{3,76}=1.5$	$F_{3,76}=24.7$ *	$F_{9,76}=0.7$

b) Statistical analysis of the effect of paroxetine of mice shown in ***Table 3.4a***. Two-way ANOVA: * $p<0.001$. See ***Table 3.3a*** for abbreviations.

Effects of citalopram on 5-HTP levels

Table 3.5a shows the effect of 5 and 20 mg/kg citalopram on 5-HTP levels in C57BL/6J and DBA/2J mice and the effect of 5 mg/kg citalopram in 129/Sv and BALB/c mice in the whole brain and in different brain regions. F values (ANOVA) are shown in ***Table 3.5b***. Citalopram had a significant effect on 5-HTP in all brain regions examined (percentage of reduction varied between 4% and 54% depending on the dose and brain region). Maximal reduction was found in hippocampus whereas citalopram tended to have less effect in the brainstem and striatum.

ANOVA revealed a significant difference between C57BL/6J and DBA/2J mice in the whole brain and in hippocampus (***Table 3.5b***).

The Tukey-Kramer test indicated that citalopram had significantly less effect in the hippocampus of DBA/2J compared to C57BL/6J mice. Neither citalopram (***Tables 3.6***) nor paroxetine (***Table 3.7***) had significant effects on brain and regional levels of DOPA ($p > 0.05$).

Table 3.5 Effect of citalopram on 5-HTP levels

DOSE	STRAIN	BRAIN	STRIATUM	HIPP	CX	FCX	BST
5 mg/kg	C57BL/6J	-37±5% *	-29±11%*	-42±8%*	-36±13%*	-33±7% *	-16±7%
20 mg/kg		-46±9% *	-33±7% *	-49±9 *	-31±10%*	-31±4% *	-11±10%
5 mg/kg	DBA/2J	-23±6% *	-14±1%	#{ -20±4%	-29±5% *	-22±7%	-21±9%
20 mg/kg		-23±4% *	-12±6%	#{ -4±9%	-19±8%	-20±2%	-17±4%
5 mg/kg	129/Sv	-42±8% *	-35±8% *	-54±5%*	-47±5%*	-43±5% *	n.d.
5 mg/kg	BALB/c	-26±3% *	-26±4% *	-41±3%*	-28±8%*	-28±3% *	n.d.

a) Effect of citalopram on 5-HT synthesis in different strain of mice. Values are expressed as percentage of decrease. * $p<0.05$ vs. vehicle (Tukey-Kramer's test); # $p<0.05$ vs C57BL/6J (Tukey-Kramer's test). n=5-7. n.d.: not determined
See ***Table 3.3a*** for abbreviations.

		STRAIN	CIT	INTERACTION
BRAIN	**C57BL/6J vs. DBA/2J**	$F_{1,30}=11.9$ #	$F_{2,30}=37.6$ *	$F_{2,30}=4$ §
	129/Sv vs. BALB/c	$F_{1,29}=2.1$	$F_{1,29}=44.7$ *	$F_{1,29}=2.2$
STRIATUM	**C57BL/6J vs. DBA/2J**	$F_{1,29}=6.4$ #	$F_{2,29}=9.6$ *	$F_{2,29}=1.8$
	129/Sv vs. BALB/c	$F_{1,29}=0.4$	$F_{1,29}=16.6$ *	$F_{1,29}=0.4$
HIPP	**C57BL/6J vs. DBA/2J**	$F_{1,27}=21.5$ *	$F_{2,27}=15.1$ *	$F_{2,27}=6.7$ #
	129/Sv vs. BALB/c	$F_{1,29}=0.7$	$F_{1,29}=42.9$ *	$F_{1,29}=0.7$
CX	**C57BL/6J vs. DBA/2J**	$F_{1,19}=1.2$	$F_{2,19}=13.1$ *	$F_{2,19}=0.4$
	129/Sv vs. BALB/c	$F_{1,27}=3$ #	$F_{1,27}=47.5$ *	$F_{1,27}=3.1$
FCX	**C57BL/6J vs. DBA/2J**	$F_{1,22}=1$	$F_{2,22}=6.3$ #	$F_{2,22}=0.3$
	129/Sv vs. BALB/c	$F_{1,25}=2.9$	$F_{1,25}=66$ *	$F_{1,25}=2.9$
BST	**C57BL/6J vs. DBA/2J**	$F_{1,31}=0.3$	$F_{2,31}=2.9$	$F_{2,31}=0.07$

b) Statistical analysis of the effect of citalopram (CIT). § $P<0.05$; # $p<0.01$; * $p<0.001$.
See ***Table 3.3a*** for abbreviations.

Table 3.6 Effect of citalopram on DOPA levels

STRAIN	CITALOPRAM	BRAIN	STRIATUM	CX	FCX	HIPP	BST
C57BL/6J	veh	238±5	1227±83	216±12	74±6	440±114	192±10
	5	242±10	1073±78	192±6	91±11	537±99	227±24
	20	227±10	1066±44	234±13	79±5	444±141	196±14
DBA/2J	veh	234±4	957±131	257±13	99±15	481±107	201±24
	5	232±9	940±34	291±60	84±10	614±185	188±19
	20	229±6	916±68	249±12	82±6	528±124	203±11
129/Sv	veh	245±23	894±80	218±15	82±4	65±6	n.d.
	5	218±8	1008±85	210±17	82±5	57±4	n.d.
BALB/c	veh	269±8	1087±60	239±6	123±6	73±2	n.d.
	5	170±10	908±83	247±10	101±6	69±2	n.d.

Effect of citalopram (5 and 20 mg/kg i.p.) on catecholamines synthesis in different strains of mice. Values are expressed as ng/g tissue (Mean±SEM). n=5-6 per group. n.d.= not determined. See ***Table 3.3a*** for abbreviations.

Table 3.7 Effect of paroxetine on DOPA levels

STRAIN	PAROXETINE	BRAIN	STRIATUM	CX	FCX	HIPP	BST
C57BL/6J	veh	256±19	648±9	126±13	129±10	285±10	220±35
	1.25	208±7	536±90	97±4	113±6	271±12	186±16
	2.5	210±6	620±51	101±8	110±11	320±36	183±14
	5	198±3	580±66	95±5	103±4	235±11	179±5
C57BL/6N	veh	192±8	729±48	81±4	87±9	208±16	235±12
	1.25	187±10	644±77	97±3	94±5	199±10	264±6
	2.5	185±10	660±63	80±9	84±9	198±12	255±27
	5	187±6	631±72	74±5	79±4	202±9	247±11
DBA/2J	veh	207±7	837±79	79±5	76±10%	215±15	305±24
	1.25	232±14	1114±83	89±5	87±5%	208±15	270±36
	2.5	204±4	1010±54	74±0	75±2%	182±8	270±9
	5	205±6	1090±56	73±4	77±3%	192±11	271±13
DBA/2N	veh	286±9	938±88	107±11	154±11%	306±10	409±13
	1.25	277v9	969±78	102±7	125±8%	303±11	446±6
	2.5	274±5	993±63	97±7	144±12%	294±10	434±13
	5	253±4	815±74	84±4	120±5%	268±10	417±16

Effect of paroxetine (1.25, 2.5 and 5 mg/kg i.p.) on catecholamines synthesis in different strains of mice. Values are expressed as ng/g tissue (Mean±SEM). n=5-6 per group. n.d.= not determined. See ***Table 3.3a*** for abbreviations.

Effect of tryptophan in combination with paroxetine or pCPA

At 300 mg/kg, Trp increased 5-HTP levels by about threefold in the brain of DBA/2J mice (***Table 3.8***). Paroxetine-induced reduction of 5-HTP in mice given Trp was not significantly different in any of the brain regions examined from 2.5 mg/kg of paroxetine alone (both 21% less than basal values in the brain; $p>0.05$, Student's t-test).

pCPA (300 mg/kg orally for three days) reduced brain 5-HTP levels by 77% in mice pre-treated with vehicle and significantly attenuated the rise of 5-HTP induced by 300 mg/kg Trp (***Table 3.9***). Two-way ANOVA showed significant effects of pCPA [$F_{1,19}= 65.8$, $p<0.0001$], Trp [$F_{1,19}= 158.0$, $p<0.0001$] and their interaction [$F_{1,19}= 4.5$, $p<0.05$].

Table 3.8 Effect of tryptophan on paroxetine-induced decrease of 5-HTP levels

Treatment	BRAIN	STR	HIPP	CTX	FCX	BST
	5-HTP (percentage of reduction below control levels)					
PRX	-20±3 (n=5)	-23±2 (n=5)	-36±2 (n=5)	-25±3 (n=5)	-22±5 (n=5)	-28±4 (n=5)
TRP+PRX	-21±4 (n=5)	-25±3 (n=6)	-35±2 (n=6)	-21±3 (n=6)	-19±4 (n=6)	-22±5 (n=5)

Percentage of reduction of 5-HTP levels in different brain areas of DBA/2J mice given tryptophan (TRP) plus paroxetine (PRX) or PRX alone. Absolute 5-HTP levels (ng/g) in mice given TRP+Veh were: brain, 594±20; striatum (STR), 296±27; hippocampus (HIPP), 482±39; rest of cortex (CTX), 368±8; frontal cortex (FCX), 315±12; brainstem (BST), 1163±61. See ***Table 3.3a*** for basal levels of 5-HTP in untreated DBA/2J mice. Data are Means±SEM. The number of mice per group is shown in parentheses.

Table 3.9 Effect of tryptophan on brain 5-HTP levels in DBA/2J mice pre-treated with pCPA

Pre-treatment	Treatment	5-HTP (ng/g ± SEM)
Vehicle	**Vehicle**	148 ± 11 (6)
Vehicle	**Tryptophan**	429 ± 37* (5)
pCPA	**Vehicle**	33 ± 4* (6)
pCPA	**Tryptophan**	233 ± 16# (6)

The number of mice per group is shown in parentheses.*$p<0.05$ vs. vehicle + vehicle; #$p<0.05$ vs. vehicle + tryptophan (Tukey-Kramer's test).

Basal levels of tryptophan in the brain and in several brain regions

As reported in ***Table 3.10***, there were no differences in basal level of tryptophan between C57BL/6J and DBA/2J mice in any of the brain regions examined (all $p>0.05$, Student's t-test).

Table 3.10 Tissue levels of tryptophan (μM) in the brain, striatum, hippocampus (HIPP), cortex, frontal cortex (FCX) and brainstem (BST) of C57BL/6J and DBA/2J mice.

Strain	BRAIN	STRIATUM	HIPP	CORTEX	FCX	BST
C57BL/6J	11.2±0.6	16.0±1.1	16.1±0.8	11.3±0.4	14.2±0.8	14.1±1.5
DBA/2J	11.4±1.0	16.3±1.4	13.3±1.3	10.5±1.0	12.7±1.3	11.1±0.7

Data are Mean±SEM of 5 mice per group.

3.3.4 Extracellular 5-HT

Basal level of 5-HT

Since basal extracellular 5-HT levels in each strain did not differ significantly across experiments, the values for each strain were pooled. As shown in ***Figure 3.6a***, mean basal extracellular 5-HT in the mPFC of DBA/2J, DBA/2N and BALB/c mice was significantly lower (about 40%) than in C57BL/6J and C57BL/6N mice [$F_{4,94}$= 34, $p<0.0001$]. Basal extracellular 5-HT in the DH of DBA/2J, DBA/2N and BALB/c mice was 20-30% lower than in C57BL/6J and C57BL/6N mice [$F_{4,89}$= 24.6, $p<0.0001$] (***Figure 3.6b***). No significant differences in basal extracellular 5-HT were found between C57BL/6N and C57BL/6J substrains and across DBA/2J, DBA/2N and BALB/c mice (all comparisons $p>0.05$; Tukey-Kramer's test).

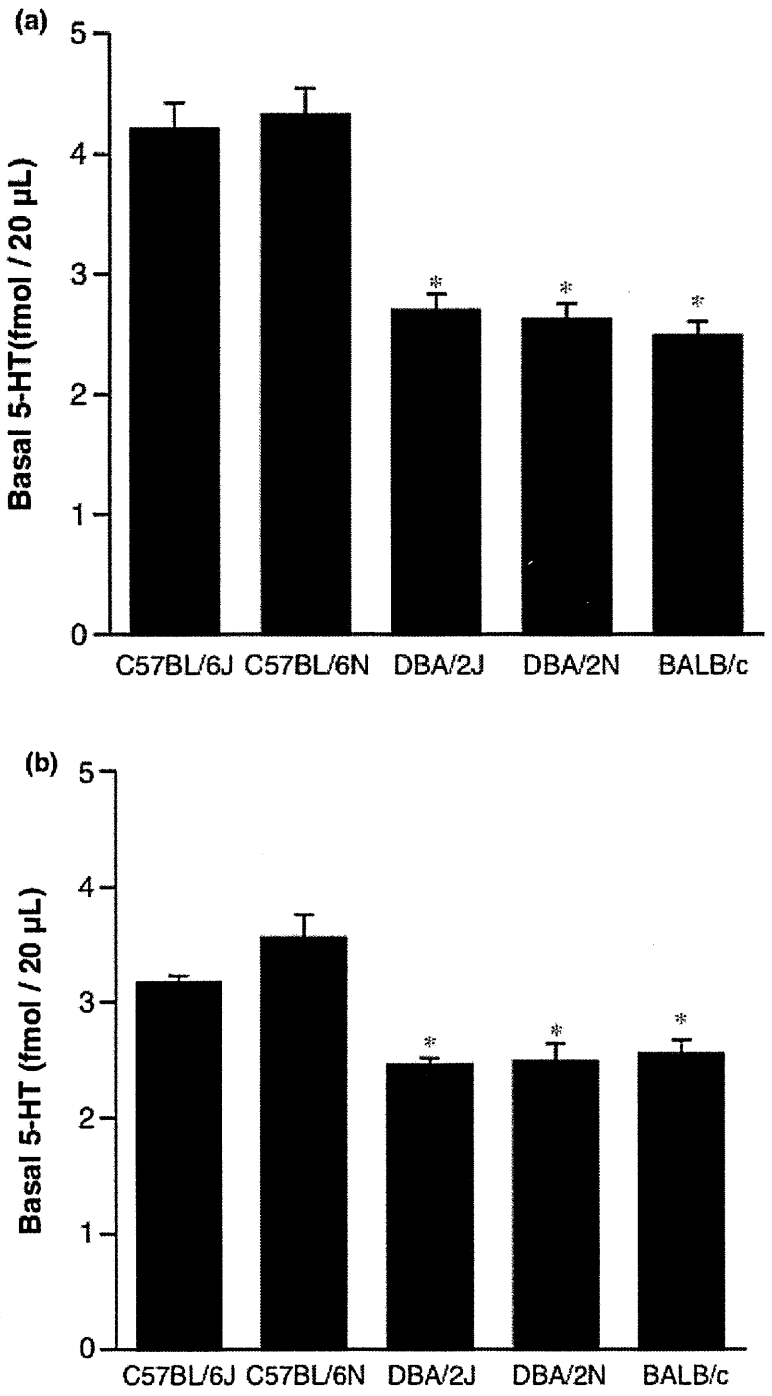


Figure 3.6 a) Basal extracellular 5-HT in the mPFC of C57BL/6J (n=19), C57BL/6N (n=9), DBA/2J (n=42), DBA/2N (n=9) and BALB/c (n=20) mice. ***b)*** Basal extracellular 5-HT in the DH of C57BL/6J (n=21), C57BL/6N (n=11), DBA/2J (n=42), DBA/2N (n=10) and BALB/c (n=10).
*$p<0.05$ vs. C57BL/6J and C57BL/6N mice (Tukey-Kramer's test).

Extracellular 5-HT in the medial prefrontal cortex and dorsal hippocampus of C57BL/6J and DBA/2J mice using the zero-net-flux method

The *in vitro* relative recovery of 5-HT through 1 mm and 2 mm length of cuprophan membrane was 7.3±0.5% and 19.4±0.1% (Mean±SEM) respectively. ***Table 3.11*** shows the extracellular concentrations of 5-HT and the extraction fraction (Ed) calculated from the zero-net-flux plot of the changes in dialysate 5-HT (C_{in}–C_{out}) as a function of 5-HT concentration infused through the probe (C_{in}) using a linear relationship between C_{in} and C_{in}-C_{out} with correlation coefficients (r^2) exceeding 0.99 in the mPFC and DH of C57BL/6J and DBA/2J mice (***Figure 3.7***). Consistent with the results of conventional microdialysis, extracellular 5-HT in the mPFC and DH of DBA/2J mice was significantly lower than in C57BL/6J mice (respectively 51% and 28%). No differences between genotypes were observed in the cortical and hippocampal Ed.

Table 3.11 Mean extracellular concentration of 5-HT (C_{ext}), and in vivo extraction fraction (Ed)

	mPFC		DH	
Strain	C_{ext} (nM)	Ed (%)	C_{ext} (nM)	Ed (%)
C57BL/6J	0.41±0.06	61±6	0.29±0.02	64±4
DBA/2J	0.21±0.01*	53±4	0.21±0.02*	55±4

Mean±SEM. n=4. *p<0.05 vs. C57BL/6J mice (Student's t-test).

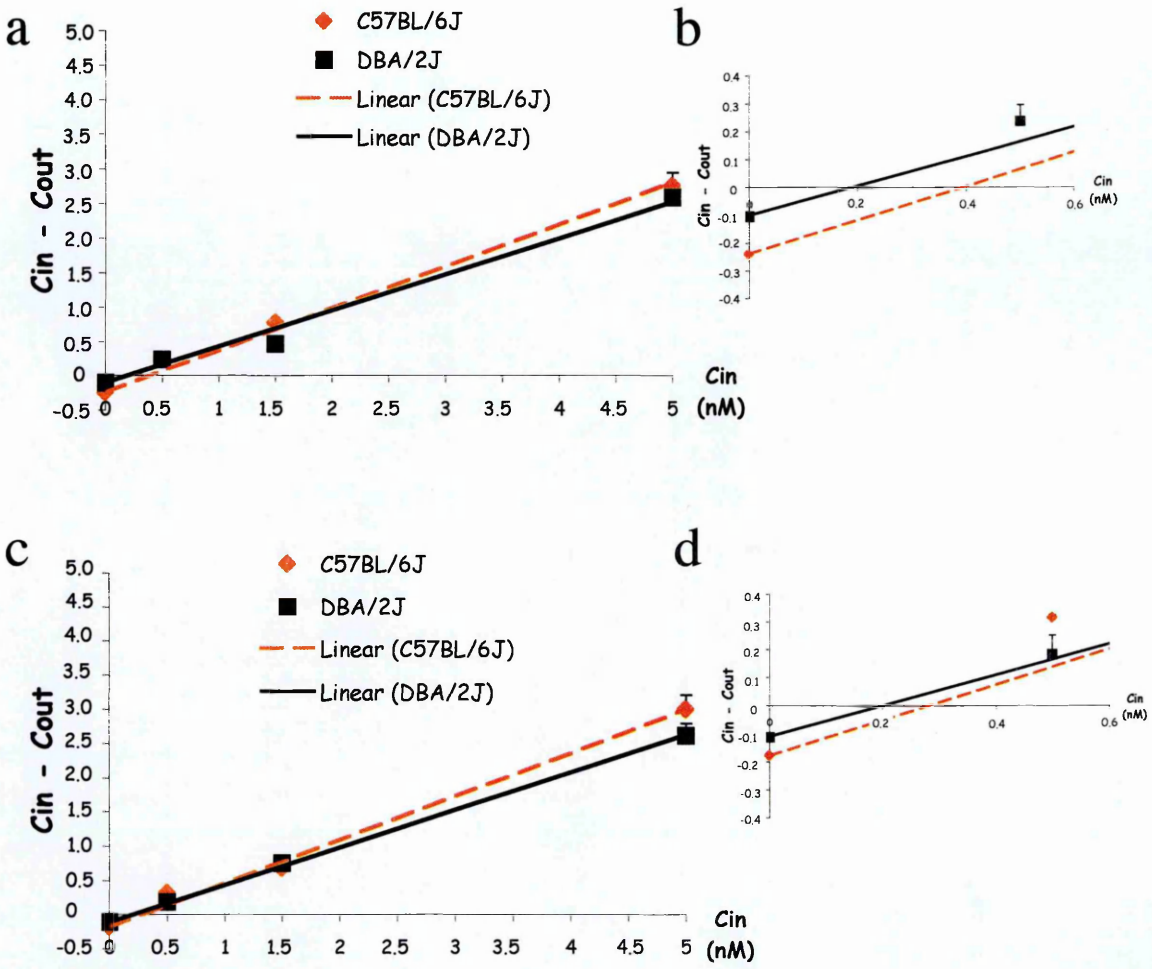


Figure 3.7 Linear regression plot of the mPFC (***a*** and ***b***) and DH (***c*** and ***d***) obtained with the zero net flux C57BL/6J and DBA/2J mice. The point where the line crosses the x-axis (see the enlargements **b** and **d**) is referred to as Cext, which is the point where there is zero net diffusion across the membrane. The Ed is the slope of the linear regression and has been shown to provide an estimate of changes in 5-HT uptake. The dialysate concentration is represented by the y-intercept. Mean±SEM. n=4.

[^{3}H]5-HT uptake into hippocampal and frontocortical synaptosomes and inhibitory potency of citalopram

The kinetic parameters of [^{3}H]5-HT uptake were measured in synaptsomes. The Vmax and Km values (***Table 3.12***) were very similar in synaptosomes obtained from brain regions of C57BL/6N and DBA/2N mice ([frontal cortex, FCX: t_4= -0.8, p=0.5]; [hippocampus, HIPP: t_4= -0.1, p=0.9]). The IC_{50} of citalopram for inhibition of [^{3}H]5-HT uptake (***Figure 3.8***) was very similar in the two mice strains [$F_{3,8}$= 0.3, p=0.8].

***Table 3.12** Synaptosomal [^{3}H]5-HT uptake in the frontal cortex and hippocampus of C57BL/6J and DBA/2J mice*

	FRONTAL CORTEX		HIPPOCAMPUS	
	C57BL/6J	**DBA/2J**	**C57BL/6J**	**DBA/2J**
Km (nM)	41.7±9.2	45.7±6.0	37.1±6.6	34.5±6.6
Vmax (pmol/min/mg proteins)	3.7±0.2	3.8±0.3	3.6±0.5	4.2±0.3

[^{3}H]5-HT uptake was assessed with 4 concentration of [^{3}H]5-HT raging from 12.5 to 100 nM. Data are Means±SEM of 3 different experiments.

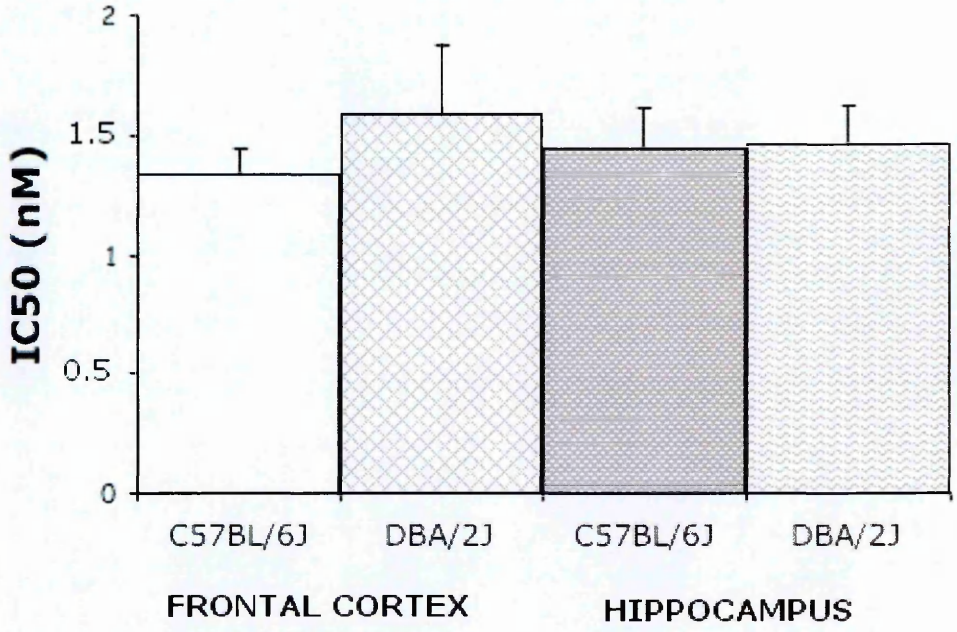


Figure 3.8 Potency of citalopram as [^{3}H]5-HT uptake inhibitor in the hippocampus and frontal cortex of C57BL/6J and DBA/2J mice.
Data are Means±SEM of 3 different experiments.

Effect of citalopram on extracellular 5-HT in the medial prefrontal cortex

As shown in ***Figure 3.9a*** saline had no effect on extracellular 5-HT in any strain of mouse [$F_{6,60}$= 0.7, p>0.05]. Citalopram dose-dependently increased extracellular 5-HT in the mPFC. At 1.25 mg/kg it significantly increased extracellular 5-HT in the mPFC of C57BL/6J mice (157% of basal values) but had no effect in DBA/2J and BALB/c mice (***Figure 3.9b***; [strain, $F_{2,12}$= 25.7, p<0.0001; time, $F_{6,72}$= 4.4, p<0.001; time x strain, $F_{12,72}$= 3.2, p<0.001]). As shown in ***Figure 3.9c***, 5 mg/kg citalopram significantly increased extracellular 5-HT in the mPFC of all strains [strain, $F_{2,14}$= 23.3, p<0.0001; time, $F_{6,84}$= 16.7, p<0.0001; time x strain, $F_{12,84}$= 2.9, p<0.01] but with a significant less effect in DBA/2J and BALB/c mice. In C57BL/6J mice 5-HT reached 13.9 fmol/20 µL after 40 min (285%) while the peaks of extracellular 5-HT in DBA/2J and BALB/c mice were respectively 5.9 and 5.4 fmol/20 µL (209-248% of basal values). Extracellular 5-HT was maximally increased by 5 mg/kg citalopram. No further increase was observed in any strain at 20 mg/kg (***Figure 3.9d***; [strain, $F_{2,13}$= 6, p<0.01; time, $F_{6,78}$= 9.5, p<0.0001; time x strain, $F_{12,78}$ = 2, p<0.05]).

The effect of 5 mg/kg citalopram on extracellular 5-HT in DBA/2 and C57BL/6 substrains (***Figure 3.9e*** and ***3.9f***) was not significantly different ([DBA/2, $F_{1,11}$= 0.5, p>0.05]; [C57BL/6, $F_{1,10}$= 0.4, p>0.05]).

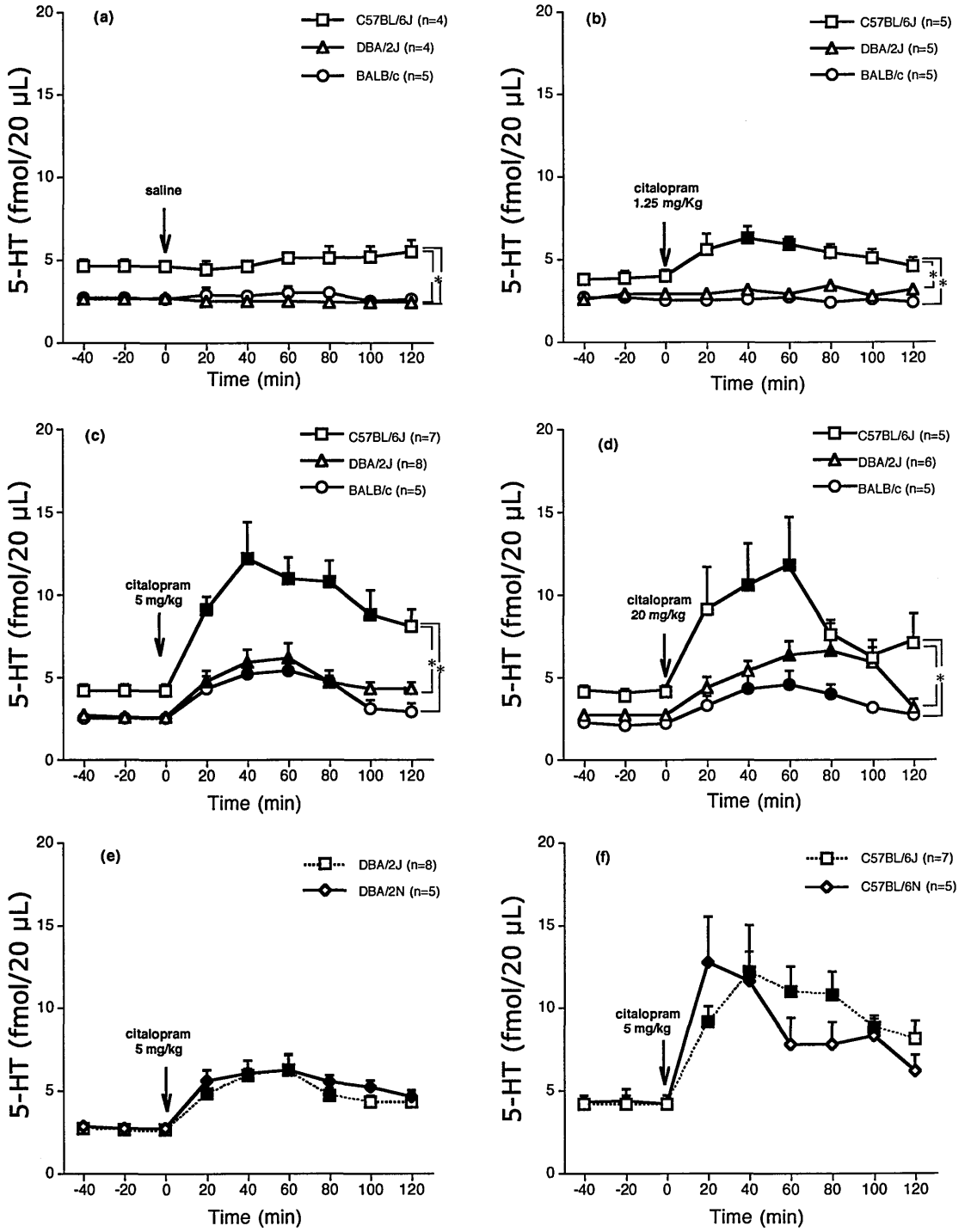


Figure 3.9 Effect of saline (**a**) and 1.25 mg/kg (**b**), 5 mg/kg (**c**) and 20 mg/kg (**d**) citalopram on extracellular 5-HT in the mPFC of C57BL/6J, DBA/2J and BALB/c mice. Arrows indicate the injection of citalopram. Mean ± S.E.M. The number of mice for each group is indicated in parentheses. *p<0.05 (Tukey-Kramer's test). Solid symbols indicate $p < 0.05$ vs. basal values (Tukey-Kramer's test). Panels (**e**) and (**f**) show the effect of 5 mg/kg of citalopram on extracellular 5-HT in the mPFC of DBA/2N and C57BL/6N mice with the values for DBA/2J and C57BL/6J mice.

Effect of citalopram on extracellular 5-HT in the dorsal hippocampus

As shown in ***Figure 3.10a***, saline had no effect on extracellular 5-HT in either strain of mouse [time, $F_{6,60}$= 1, p>0.05]. At 1.25 mg/kg citalopram increased extracellular 5-HT in the DH of C57BL/6J mice (138%) but had no effect in DBA/2J mice (***Figure 3.10b***; [strain, $F_{1,9}$= 21.4, p<0.001; time, $F_{6,54}$= 6.7, p<0.0001; time x strain, $F_{6,54}$ = 8.7, p<0.001]). Five mg/kg citalopram significantly raised extracellular 5-HT in both strains (***Figure 3.10c***; [strain, $F_{1,8}$= 3.4, p>0.05; time, $F_{6,48}$= 35.4, p<0.0001; time x strain, $F_{6,48}$= 5.6, p<0.001]). However, it had significantly less effect in DBA/2J mice. Extracellular 5-HT reached 8.6 and 5.6 fmol/20 µL in C57BL/6J (270% of basal values) and DBA/2J mice (232% of basal values). No further increase was observed with the dose of 20 mg/kg. Extracellular 5-HT rose significantly more in response to 20 mg/kg citalopram in C57BL/6J mice (***Figure 3.10d***; [strain, $F_{1,9}$= 6.4, p<0.05; time, $F_{6,54}$= 8.6, p<0.0001; time x strain, $F_{6,54}$= 1.7, p>0.05]).

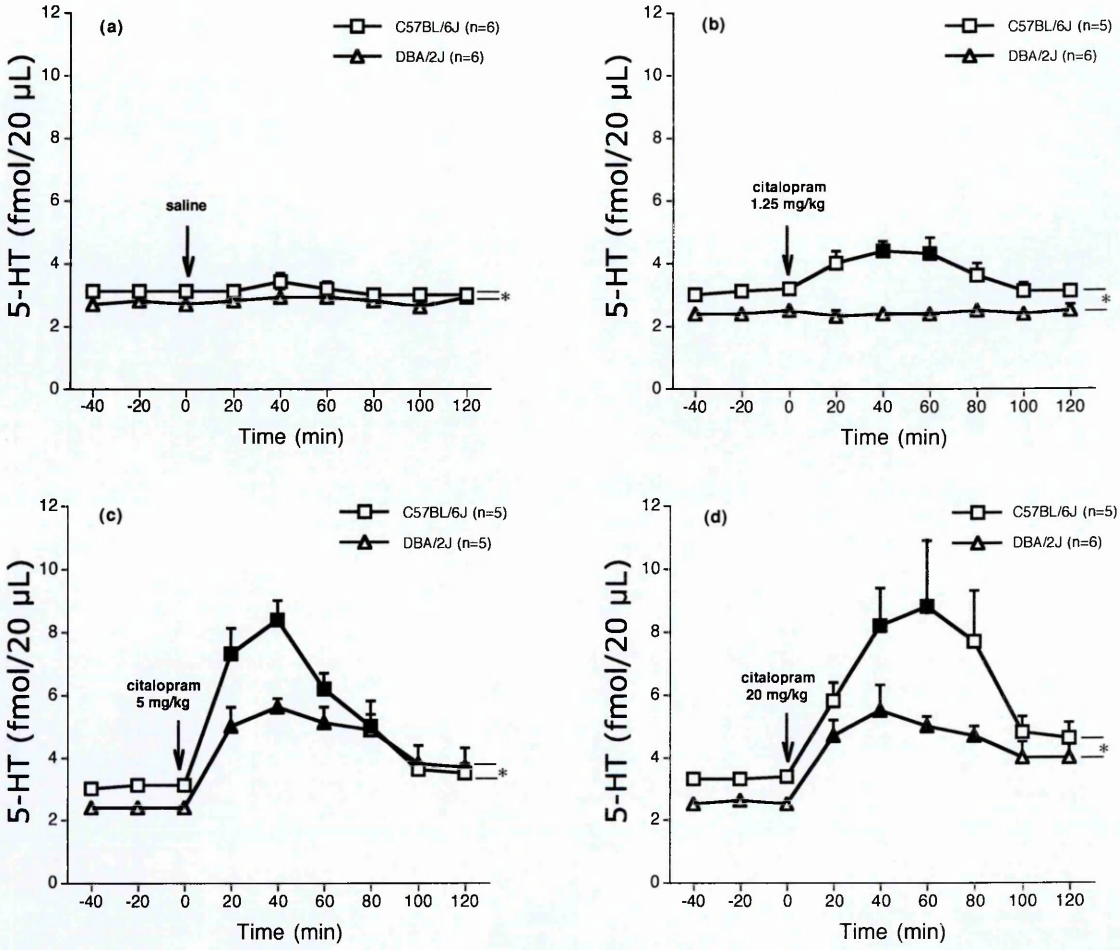


Figure 3.10 a Effect of saline (***a***) and 1.25 mg/kg (***b***), 5 mg/kg (***c***) and 20 mg/kg (***d***) citalopram on extracellular 5-HT in the dorsal hippocampus of C57BL/6J and DBA/2J mice. Arrows indicate the injection of citalopram. Mean ± S.E.M. The number of mice for each group is indicated in parentheses. *$p<0.05$ (Tukey-Kramer's test). Solid symbols indicate $p<0.05$ vs. basal values (Tukey-Kramer's test).

3.4 Discussion

The results obtained provide the first description of how the blockade of 5-HT reuptake affects both 5-HT synthesis and the *in vivo* release in mice differing in the activity of TPH-2, the rate-limiting enzyme in brain 5-HT synthesis. The results confirmed that DBA/2N, DBA/2J and BALB/c inbred mice that share the same allele of TPH-2 (1473G), have lower brain 5-HT synthesis than mice homozygous for the 1473C allele (Zhang et al., 2004; Cervo et al., 2005). Moreover, we showed that citalopram and paroxetine reduced immobility time in C57BL/6 mice ("responders"), but had no effect in DBA/2 and BALB/c mice (Cervo et al., 2005; Guzzetti et al., 2008). Furthermore "non-responder" strains had lower basal and citalopram-induced rise of extracellular 5-HT in the medial prefrontal cortex (mPFC) and dorsal hippocampus (DH) than "responders" strains (Calcagno et al., 2007).

Therefore it is likely that the lack of effect of SSRIs in the FST in mice with the mutated enzyme is due to impairment of 5-HT synthesis and release.

Both citalopram and paroxetine inhibited brain 5-HT synthesis in almost all brain regions of the strains examined but they had a significantly less effect in most brain regions of DBA/2J, DBA/2N and BALB/c mice. The fact that SSRIs had no effect on the levels of DOPA indicates the selectivity for serotonin of these drugs at the doses used and suggests that catecholamine synthesis is not involved in the response to SSRIs in the FST.

Effect of SSRIs in the FST in different strains of mice

Previous studies showed strain differences in the response to SSRIs in the mouse FST that are only partially compatible with the above findings. Consistent with the present results, which define C57BL/6 mice as "responders" and DBA/2 and BALB/c mice as "non-responders", paroxetine and citalopram did not affect immobility time in the FST in DBA/2J mice (David et al., 2003). However other studies have shown that BALB/c and DBA/2J mice are highly responsive to citalopram in the TST (Crowley et al., 2005). Moreover, fluoxetine showed anti-immobility effects in BALB/c and DBA/2J but had no effect in C57BL/6J mice (Lucki et al., 2001). In addition paroxetine did not reduce immobility time in a substrain of the C57BL/6 strain (C57BL/6Rj; David et al., 2003). Therefore there is no clear consensus between this study and similar ones.

The different substrain of C57BL/6 mice used between the various studies may be a factor: the C57BL/6Rj mice may have a low sensitivity to antidepressant drugs since imipramine too had no effect in this strain (David et al., 2003) whilst it reduced immobility time in C57BL/6 mice (Bai et al., 2001). Also, the basal immobility time in different strain of mice is influenced by the tank diameter. The present study used a 10 cm diameter tank, in comparison to a 21 cm diameter tank used by Lucki et al. (2001). Immobility was strongly reduced in DBA/2J mice by increasing the diameter of the tank from 10 to 20 cm (Cervo et al., 2005). However, even if "false positives" in the rat FST are reduced by using a larger tank (Sunal et al., 1994), this factor did not influence the strain-dependent response to paroxetine and citalopram. In fact, under the same experimental conditions,

paroxetine and citalopram reduced the immobility time of C57BL/6 mice but had no effect in DBA/2 and BALB/c mice regardless of the size of the tank (Cervo et al., 2005).

Role of 5-HT synthesis and TPH-2

Comparing the strain-dependent effect of citalopram in the mouse TST no association was found between the C1473G polymorphism of TPH-2 and the response in the TST (Crowley et al., 2005). Also, no clear association was found between TPH-2 genotype and citalopram-induced suppression of food-intake in acutely food deprived mice (Crowley et al., 2005). Other studies have shown that the C1473G SNP of TPH-2 is associated to enhanced aggressive behaviour (Kulikov et al., 2005) but not with impulsive behaviour on a delayed reinforcement task in the mouse (Isles et al., 2005).

As in the human studies, controversy also exists around the importance of the TPH-2 polymorphism in mice. It should also be considered that genotype-environment interaction might determine differences in behavioural phenotype that makes problematic the comparison across laboratories. Overall, other important genetic or neurochemical interactions are also obviously at play in influencing the serotonergic system and how it responds to antidepressants in the behavioural tests.

As well as the TPH-2 polymorphism, the mouse strains used in the present study differ in several neurochemical parameters potentially involved in the antidepressant response. These include the content of brain NA (Kempf et al., 1974), the number of NA uptake sites in the locus ceruleus (Hwang et

al., 1999), and the density of hippocampal glutamate, GABA, and 5-HT receptors (Zilles et al., 2000). Although we cannot exclude an effect of these differences, the results suggest that the failure of citalopram and paroxetine to reduce immobility in DBA/2 and BALB/c mice is mainly attributable to the genotype-dependent impairment of 5-HT synthesis and release.

In support of the importance of 5-HT in the response to SSRIs, dose of pCPA, inhibiting 5-HT synthesis in "responder" mice to the level observed in DBA/2 and BALB/c mice, completely prevented the effect of SSRIs in the FST (Cervo et al., 2005; Guzzetti et al., 2008). These findings are in accordance with previous studies that have demonstrated that depletion of 5-HT in rodents prevented the effect of SSRIs in the mouse and rat FST and TST (Gavioli et al., 2004; Page et al., 1999; O'Leary et al., 2007) and support the role of 5-HT synthesis in the therapeutic effect of SSRIs.

Inhibition of 5-HT synthesis with doses of pCPA which depleted tissue 5-HT by about 90% reduced basal levels and completely prevented the citalopram- and fluoxetine-induced rises of extracellular 5-HT in the rat mPFC (Pozzi et al., 1999). Similarly, inhibition of 5-HT release by the sodium channel blocker tetrodotoxin or stimulation of 5-HT_{1A} autoreceptors strongly attenuated the rise of extracellular 5-HT produced by SSRIs (Kalén et al., 1988; Gundlah et al., 1997; Rutter and Auerbach, 1993).

Furthermore a delivery of tryptophan to DBA/2 and BALB/c mice enhanced brain 5-HTP, restoring 5-HT synthesis, and reversed the resistance of these strains in the FST to citalopram (Cervo et al., 2005) and paroxetine (Guzzetti et al., 2008), without enhancing the effect of an inactive dose of

citalopram in C57BL/6N mice (Calcagno et al., 2007). Substrate availability does not contribute to strain differences in synthesis (Zhang et al., 2004; Cervo et al., 2005) or basal extracellular concentrations of 5-HT (Calcagno et al., 2007; Isles et al., 2005). Brain and regional Trp concentrations were similar in C57BL/6J and DBA/2J mice and clearly below the Km of the wild-type and mutated TPH-2 isoforms (Sakowski et al., 2006). Consequently, under physiological conditions the enzyme's activity is limited by the availability of the substrate and it is possible to raise 5-HT synthesis administering 300 mg/kg Trp (Cervo et al., 2005; Guzzetti et al., 2008). The same dose of Trp reinstated the anti-immobility effect of paroxetine in DBA/2N, DBA/2J and BALB/c mice and this effect was completely abolished by pCPA, suggesting that 5-HT mechanisms play a major role in this effect.

Extracellular 5-HT is tightly coupled to the activity of brain TPH. This is supported by studies showing that loading with Trp enhances 5-HT synthesis and extracellular concentrations, whereas depletion leads to a large reduction (Carboni et al., 1989; Sarna et al., 1991; Westerink and De Vries, 1991; Bel and Artigas, 1996).

The data supported the notion that this TPH-2 polymorphism influenced sensitivity to SSRIs in the FST and suggest that tryptophan augmentation could be a useful strategy to enhance SSRIs' effect in treatment-resistant patients, particularly in those with low 5-HT synthesis determined by functional polymorphisms of TPH-2. Even if some clinical studies indicated some benefit from therapy with tryptophan, unfortunately its use as a dietary supplement was discontinued in 1989 due to an outbreak of

eosinophilia-myalgia syndrome that was traced to a contaminated synthetic Trp.

In C57BL/6N mice pCPA pre-treatment abolished the anti-immobility effect of 2.5 mg/kg but not 5 mg/kg paroxetine. This suggests a contribution of non-serotonergic mechanisms to the effect of the higher dose of paroxetine. Previous studies reported that higher doses also increased extracellular NA in the frontal cortex of Swiss mice (David et al., 2003b) and the genetic deletion of dopamine β-hydroxylase, the enzyme responsible for the NA synthesis, abolished the anti-immobility effect of 5-20 mg/kg paroxetine and other SSRIs in the tail suspension test but did not affect that of citalopram (Cryan et al., 2004).

pCPA did not change basal immobility time in C57BL/6N mice. This finding is consistent with previous studies reporting no effects of pCPA or the selective destruction of serotonergic neurons with the neurotoxin 5,7-dihydroxytryptamine in mice and rats (Borsini, 1995; Cervo and Samanin, 1991; Gavioli et al., 2004; Page et al., 1999; Redrobe et al., 1998; Rodrigues et al., 2002). Moreover congenic mice carrying the mutant isoform of TPH-2 and TPH-2 knockout mice showed no or marginal changes in immobility time compared to control genotypes confirming that reduced synthesis did not affect basal immobility time in the FST (Tenner et al., 2008; Savelieva et al., 2008). Taken together, these findings suggest that the increase or reduction of 5-HT synthesis may be not sufficient by itself to affect immobility time in the FST.

Differences in the response to paroxetine or citalopram across strains cannot be attributed to pharmacokinetic factors since brain and plasma levels of the drug (Cervo et al., 2005; Guzzetti et al., 2008) in the same

animals at the end of the behavioural studies were essentially similar in the five strains given SSRIs alone or combined with treatments affecting 5-HT synthesis.

The fact that doses of paroxetine, Trp, pCPA or their combinations did not affect locomotor activity makes it unlikely that motor performance was involved in the antidepressant-like effects in the FST. The lower basal motor activity of BALB/c mice compared to other strains had no influence on basal immobility time and is unlikely to account for the lack of effect of paroxetine in the FST.

Role of extracellular 5-HT

Neurochemical consequences due to variation in the C1473G allele of TPH-2 are not limited to an impairment of 5-HT synthesis but also involve reduced release of 5-HT. Our results are consistent with previous microdialysis studies showing that basal extracellular 5-HT in several regions of mice carrying the 1473G allele of TPH-2 are lower than in mice with the "C" allele (Isles et al., 2005). In addition, the results showed that extracellular 5-HT was similar in C57BL/6J and C57BL/6N mice and there were no differences in extracellular 5-HT across DBA/2J, DBA/2N and BALB/c strains. Furthermore, citalopram increases cortical and hippocampal extracellular 5-HT in DBA/2J, DBA/2N and BALB/c less than in C57BL/6 substrains (Calcagno et al., 2007).

Extracellular levels of 5-HT are mainly determined by the balance between the amounts of neurotransmitter released and taken up at nerve terminals. Therefore, changes in both mechanisms might contribute to the lower availability of extracellular 5-HT in the brain of DBA/2J and BALB/c mice.

Conventional microdialysis measures the concentration of 5-HT in the dialysate. Although dialysate 5-HT reflects the extracellular concentration, the actual extracellular concentration is more accurately determined by quantitative microdialysis techniques such as the zero-net-flux method which also gives an estimate of the extraction fraction (Ed) or probe recovery *in vivo* (Lönnroth et al., 1987; Cosford et al., 1996).

The *in vivo* Ed is affected by changes in 5-HT uptake but not by changes of release or metabolism (Cosford et al., 1996). Using this method we found that DBA/2J mice had less cortical and hippocampal extracellular 5-HT than C57BL/6J mice, confirming the results obtained with the conventional microdialysis. As we found no differences in Ed between genotypes, differences in 5-HT reuptake are unlikely to account for strain differences in extracellular 5-HT. These results were confirmed evaluating [^{3}H]5-HT uptake in cortical and hippocampal synaptosomes of C57BL/6J and DBA/2J mice and the data are in accordance with a previous report that found no significantly difference between these strains (Jazrawi et al., 1987). Although the uptake of [^{3}H]5-HT in the raphé of BALB/c mice was higher than in C57BL/6 mice, no information is available for the cortex or hippocampus (Daszuta et al., 1982). Moreover the potency of citalopram to inhibit [^{3}H]5-HT uptake in FCX and hippocampus was similar between C57BL/6J and DBA/2J mice.

These findings suggest that reduced release of 5-HT, probably caused by impairment of 5-HT synthesis, mainly contributed to the low extracellular concentrations of 5-HT found in the mPFC and DH of DBA/2 and BALB/c mice.

C57BL/6J mice given citalopram had significantly higher mPFC extracellular 5-HT than DBA/2J and BALB/c mice. Likewise, citalopram increased extracellular 5-HT more in the DH of C57BL/6J mice than in DBA/2J mice. These findings indicate that the drug's ability to boost the extracellular availability of the neurotransmitter is impaired in mice carrying the 1473G allele of TPH-2. The association between the effects of citalopram on cortical extracellular 5-HT and C1473G SNP is strengthened by the fact that it had similar effects in C57BL/6N and C57BL/6J mice, which share the same allelic isoform of TPH-2. Similarly, there were no differences in its effect on extracellular 5-HT across DBA/2J, DBA/2N and BALB/c strains.

Strain differences in the response to citalopram were particularly evident with the low dose (1.25 mg/kg) which raised extracellular 5-HT in the mPFC and DH of C57BL/6J mice but had no such effect in DBA/2J and BALB/c mice. The lower sensitivity of DBA/2J and BALB/c mice to citalopram is unlikely to be due to less 5-HT reuptake inhibition since with higher doses - 5 or 20 mg/kg - which maximally increased extracellular 5-HT in C57BL76J mice, extracellular 5-HT in the mPFC of DBA/2J and BALB/c still remained lower than in C57BL/6J mice.

The limited effect of citalopram on extracellular 5-HT in DBA/2J and BALB/c mice is quite likely attributable to the impaired TPH-2 activity and consequently 5-HT synthesis. However, we cannot exclude that besides TPH-2 allelic polymorphism, the background strain has influenced the response to SSRIs (Tenner et al., 2008). Studies in TPH-2 knockout mice or congenic lines of mice carrying the allelic variants of TPH-2 on a common genetic background are needed to clarify this point.

In conclusion, these findings increase the likelihood that strain-dependent effects of paroxetine and citalopram (Calcagno et al., 2007; Cervo et al., 2005; Guzzetti et al., 2008) are based on their ability to enhance 5-HT transmission and not on some secondary effects of the drugs or genetic differences not related to serotonergic transmission.

In summary, comparing mouse strains differing in their ability to synthesize and release 5-HT, and using pharmacological interventions aimed at enhancing or reducing 5-HT synthesis, we showed the fundamental role of 5-HT in SSRIs' effect in the FST. The results raised the question whereas an enhanced serotonergic tone corresponds to a restored antidepressant-like effect in the FST. Therefore comparisons of mice carrying allelic variants of TPH-2 may serve as a good model for testing pharmacological strategies aimed at improving the effect of SSRIs.

Chapter 4

ENHANCEMENT OF EXTRACELLULAR 5-HT RESTORES THE ANTIDEPRESSANT-LIKE EFFECT OF CITALOPRAM IN NON-RESPONDER MICE

4.1 Introduction

DBA/2N, DBA/2J and BALB/c inbred mice carry a mutated allele of TPH-2 (C1473G) and have lower brain 5-HT synthesis and release than mice homozygous for the 1473C allele such as C57BL/6N and C57BL/6J strains (Zhang et al., 2004; Calcagno et al., 2007). As shown in *Chapter 3*, citalopram and paroxetine reduced immobility time in C57BL/6 mice ("responder"), but had no such effect in DBA/2 and BALB/c mice ("non-responders"). The 5-HT precursor tryptophan (Trp) restored the anti-immobility effect of SSRIs in "non-responder" strains (Cervo et al., 2005; Guzzetti et al., 2008). Interestingly, these mice have lower citalopram-induced rise of extracellular 5-HT in the medial prefrontal cortex (mPFC) and dorsal hippocampus (DH) than "responders" mice (Calcagno et al., 2007). This suggests that insufficient 5-HT at its sites of action might explain why SSRIs do not reduce immobility time in DBA/2 and BALB/c mice.

Thus, we assessed whether intervention aimed at enhancing the effect of citalopram on extracellular 5-HT restored its ability to reduce immobility time in the mouse FST.

Microdialysis studies showed that the inhibition of 5-HT synthesis with pCPA and depletion of the 5-HT precursor, Trp, reduced the availability of extracellular 5-HT at central synapses (Oluyomi et al., 1994; Bel and Artigas, 1996; Pozzi et al., 1999) while Trp, by stimulating 5-HT synthesis, enhanced the release of the neurotransmitter (Carboni et al., 1989).

Therefore we investigated whether boosting 5-HT synthesis with Trp enhanced the effect of citalopram on extracellular 5-HT.

Although Trp is essential for 5-HT synthesis, as shown in ***Figure 4.1***, of the dietary tryptophan that is not used in protein synthesis, 99% is metabolized along the kynurenine pathway (*for review see* Stone and Darlington, 2002). An alternative pathway is involved in the conversion of Trp to 5-HT and subsequently to melatonin. Both kynurenines and melatonin have been implicated in antidepressant actions; therefore it cannot be excluded that these metabolites might contribute to the antidepressant-like effect of Trp in the FST.

Melatonin is synthesized from Trp via 5-HT through two enzymatic steps (***Figure 4.1***) involving the N-acetylation through serotonin N-acetyltransferase to obtain N-acetylserotonin, which is methylated by hydroxyindole-O-methyltransferase to form melatonin (Weissbach et al., 1960; Axelrod and Weissbach, 1961). Exogenous melatonin, with few exceptions (Bourin et al., 2004; Dubocovich et al., 1990), reduces immobility time in the rat and mouse FST (Micale et al., 2006; Raghavendra et al., 2000; Wong and Ong, 2001) and mouse TST (Prakhie and Oxenkrug, 1998). In addition, Trp markedly increased the levels of circulating (Esteban et al., 2004; Jaworek et al., 2004; Leja-Szpak et al., 2004) and brain (Crespi et al., 1994) melatonin in the rat. Thus, we also addressed the role of this hormone in tryptophan-induced rescue of the behavioural effect of paroxetine by combining inactive doses of paroxetine and melatonin and measuring melatonin levels in the brain of DBA/2N mice given Trp alone or

in association with other treatments as an indicator of the conversion of Trp into melatonin.

The ability of SSRIs to raise extracellular 5-HT, and possibly their clinical effect, is limited by the simultaneous activation of the autoinhibitory feedback controlling the activity of 5-HT neurons and the release of the neurotransmitter mainly through the activation of 5-HT_{1A} auto- and post-synaptic receptors (Artigas et al., 2001; Artigas et al., 1996; Invernizzi et al., 1992; Invernizzi et al., 1997). Blockade of 5-HT_{1A} receptors, preventing activation of the inhibitory feedback, enhances the increase of extracellular 5-HT caused by SSRIs in several brain regions (Artigas et al., 1996; Gartside et al., 1995; Hjorth, 1993; Hjorth and Auerbach, 1994; Invernizzi et al., 1992; Invernizzi et al., 1996; Invernizzi et al., 1997). Accordingly, the non-selective 5-$HT_{1A/1B}$ and β-adrenoceptor antagonist pindolol accelerated the effect of SSRIs in depressed patients, although some studies did not confirm this finding (Artigas et al., 2001).

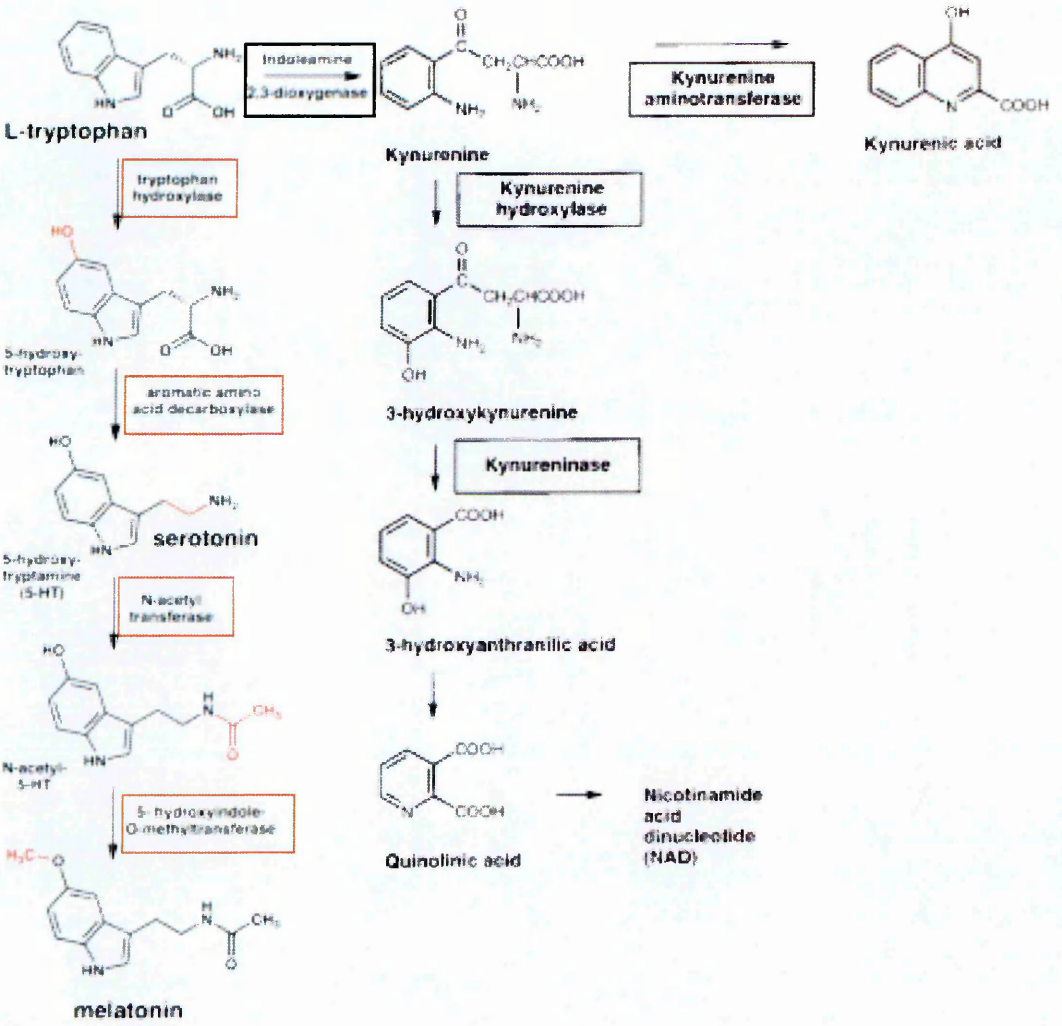
5-HT_{2C} receptors are also involved in the feedback control of 5-HT neurons (Sharp et al., 2007). 5-HT_{2C} receptor antagonists potentiate the effect of SSRIs on immobility time in the TST and enhance the effect of citalopram on extracellular 5-HT (Boothman et al., 2006a; Cremers et al., 2004). Desensitization of rat brain 5-HT_{2C} receptors after long-term treatment with antidepressant drugs (Kennett et al., 1994) suggests that adaptive changes of these receptors might contribute to the development of the antidepressant action.

Recently the editing of 5-HT2CR mRNA has been reported to participate in

the pathogenesis of depressive disease. Post-mortem studies showed abnormal mRNA editing of the 5-HT_{2C} receptor, favouring the expression of less constitutively active receptor isoforms in the brain of depressed suicide victims (Englander et al., 2005; Gurevich et al., 2002b), while chronic administration of SSRIs to mice induced opposite effect (Gurevich et al., 2002a). Thus, the 5-HT_{2C} receptor offers a promising target for enhancing the efficacy of SSRIs.

Therefore this study also examined whether WAY100635 and SB242084, respectively a selective 5-HT_{1A} and 5-HT_{2C} receptor antagonist, enhanced the effect of citalopram on cortical and hippocampal extracellular 5-HT in "non-responder" DBA/2N mice and rescued its effect in the FST.

***Figure 4.1** Pathways of tryptophan metabolism*



modified from Stone and Darlington, 2002.

4.2 Methods

Microdialysis studies and the behavioural experiments were carried out as described in *Chapter 2*. The brain areas dissection and the preparation of tissue samples were performed as described in *section 3.2.1*.

4.2.1 Melatonin assay

Tissue levels of melatonin in the brain of DBA/2N mice were determined by HPLC coupled to a fluorometric detector essentially according to Drijfhout et al (1993). Separation was achieved through a reverse phase analytical column (Supelcosil LC18-DB 3 µm, 150 x 4.6 mm; Supelchem, Milan, Italy). The mobile phase consisted of 10 mM Na_2HPO_4, 0.01 mM EDTA, 20% CH_3CN, adjusted to pH 4 with 85% H_3PO_4 and pumped at 1 mL/min with a LC-10ADvp pump (Shimadzu, Milan, Italy). Melatonin was detected by a SP2020 plus, scanning fluorescence detector (Jasco, Tokyo, Japan) using an excitation wavelength of 280 nm and an emission wavelength of 345 nm. The assay was calibrated daily with 10 fmol/20 µL melatonin standard made up in $HClO_4$ 0.1 M. The detection limit was 1 fmol melatonin on column (signal-to-noise ratio = 2).

The identity of the melatonin peak in samples was confirmed by comparing retention times and excitation/emission spectra of samples and pure melatonin solution, spiking the sample with pure melatonin (***Figure 4.2***) and changing the percentage of CH_3CN (12-20%) in the mobile phase. Moreover, we confirmed that N-acetyl-5-HT, 6-hydroxymelatonin, 5-HT, 5-HIAA, NA, DA, GABA, GLU, tyrosine and tryptophan do not interfere with the melatonin peak.

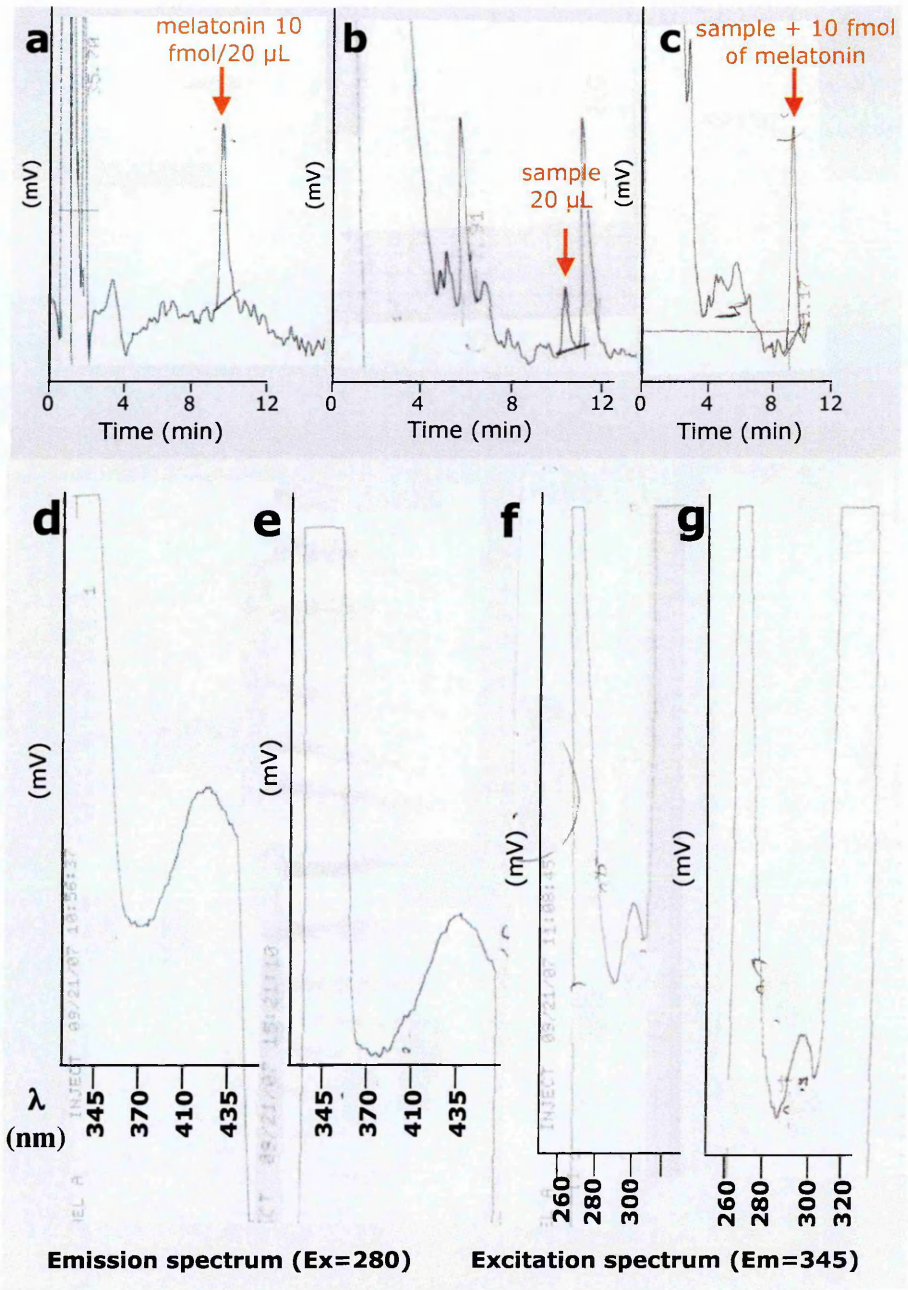


Figure 4.2 ***a)*** Chromatogram of melatonin standard (10 fmol/20 µL).

b) Chromatogram of sample (20 µL) from a brain of DBA/2N mouse.

c) Chromatogram of same sample spiked with 10 fmol melatonin standard.

d) Standard emission spectrum.

e) Sample emission spectrum.

f) Standard excitation spectrum.

g) Sample excitation spectrum.

4.2.2 Drug treatments

Citalopram hydrobromide (Tocris Cookson, Bristol, UK) paroxetine hydrochloride (GlaxoSmithKline, West Sussex, UK) and the $5HT_{1A}$ receptor antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxenide trihydrochloride) (WAY100635; Pharmacia and Upjohn, Nerviano, Italy) were dissolved in saline (NaCl 0.9%; 10 mL/kg) and injected respectively i.p. and subcutaneously (s.c.) at the doses indicated. The dose of citalopram used in the present study maximally increased extracellular 5-HT in the mPFC and DH of DBA/2N and C57BL/6 mice (Calcagno et al., 2007) and maximally reduced immobility time in C57BL/6 mice (Cervo et al., 2005).

The $5\text{-}HT_{2C}$ receptor antagonist, 6-chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB242084; GlaxoSmithKline, Harlow, UK), was dissolved in DMSO:4M tartaric acid:water (50:1:49) and injected s.c. Control mice received an injection of the vehicle (10 mL/kg).

Tryptophan, 300 mg/kg, was dissolved as described in *Chapter 3*.

Tryptophan, WAY100635 and SB242084 were injected 20 min before citalopram.

Doses of the 5-HT receptor antagonists were selected on the basis of blockade of the effects of selective agonists (Boothman et al., 2006b; O'Neill and Conway, 2001).

In behavioural experiments, mice were given the 5-HT receptor antagonists 20 min before citalopram and were submitted to the FST or open field test 30 min after the last injection. Microdialysis and behavioural studies were done on separate groups of mice.

Melatonin (Fluka, Milan, Italy) 50, 100 and 200 mg/kg was dissolved in 1 mL dimethylsufoxide (DMSO) and diluted 1:10 with sterile water. Control mice received 10% DMSO in water. In the dose-response experiment, melatonin was administered i.p. 30 min before testing. In the combination study, 100 mg/kg melatonin and 2.5 and 5.0 mg/kg paroxetine were given i.p. 30 minutes before the test session.

4.2.3 Data analysis

Extracellular levels of 5-HT, not corrected for *in vitro* recovery of the probe, were expressed as fmol/20 µL. Basal values of 5-HT in different experiments and in different strains of mouse were compared by one-way analysis of variance (ANOVA) or Student's t-test. All time-course data were analyzed by ANOVA for repeated measures with treatments as between-subjects factor and time as within-subjects factor. *Post-hoc* comparisons between pre- and post-injection values and comparisons between treatments were done with Tukey-Kramer's test.

The effects of citalopram alone or with WAY100635 or SB242084 on immobility time and locomotor activity were analyzed by two-way ANOVA followed by Tukey-Kramer's test.

Finally, two-way ANOVA was used to analyze the effects of paroxetine in combination with melatonin on immobility time.

4.3 Results

4.3.1 Basal level of extracellular 5-HT

Mean (±SEM) basal extracellular 5-HT in the mPFC and DH of DBA/2N mice was 2.5±0.1 (n=44) and 2.5±0.1 (n=45) fmol/20 µL, respectively and for DBA/2J mice was 2.7±0.1 (n=20) and 2.4±0.1 (n=19) fmol/20 µL, respectively (not corrected for probe recovery). No significant differences were found across different experiments (DBA/2N: mPFC, [$F_{7,36}$= 2.3, p>0.05]; DH, [$F_{7,37}$= 1.5, p>0.05] and DBA/2J: mPFC, [$F_{3,16}$= 0.25, p>0.05]; DH, [$F_{3,15}$= 1.6, p>0.05]) and across substrains (mPFC, [t_{62}= 1, p>0.05] and DH, [t_{62}= -1.1, p>0.05]).

Basal extracellular 5-HT in the mPFC and DH of C57BL/6N mice (respectively 3.6±0.1 fmol/20 µL, n=11 and 3.6±0.2 fmol/20 µL, n=11) was significantly higher than in DBA/2N mice ([mPFC: t_{53}= 6.4, p<0.0001] and [DH: t_{54}= 28.8, p<0.0001]). This confirms previous finding and is in accordance with the results of ZNF (see *Chapter 3*).

4.3.2 Effect of tryptophan on citalopram-induced rise of 5-HT in the medial prefrontal cortex and dorsal hippocampus of DBA/2J mice

As shown in ***Figure 4.3a***, 300 mg/kg Trp by itself significantly increased extracellular 5-HT in the mPFC (144%) but, given 20 min before citalopram, it had no effect on that drug's ability to raise extracellular 5-HT. ANOVA indicated a significant effect of time [$F_{7,112}$ = 16.6, p<0.0001] but no significant interaction between Trp and citalopram [$F_{1,16}$ = 0.3, p>0.05] or between these factors and time [$F_{7,112}$ = 1.3, p>0.05]. Trp by itself had no effect on extracellular 5-HT or on the citalopram-induced rise of extracellular

5-HT in the DH (***Figure 4.3b***; [interaction Trp and citalopram: $F_{1,15}$=0.004, $p>0.05$; time $F_{7,105}$=3.7, $p<0.01$; interaction between treatments and time: $F_{7,105}$=0.8, $p>0.05$]).

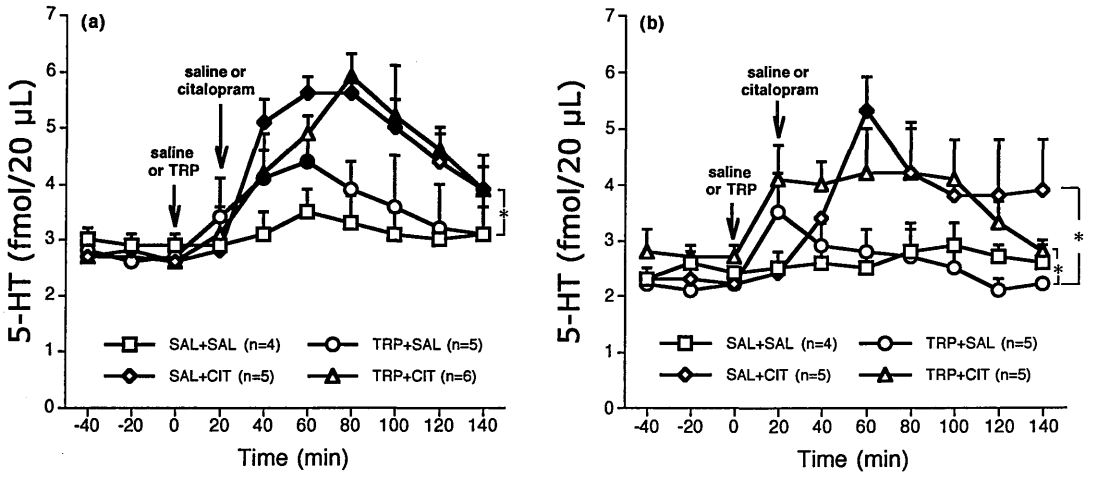


Figure 4.3 Effect of tryptophan on citalopram-induced rise of extracellular 5-HT in medial prefrontal cortex (***a***) and dorsal hippocampus (***b***) of DBA/2J mice. Mice received saline (SAL) or 300 mg/kg tryptophan (TRP) 20 min before saline or 5 mg/kg citalopram (CIT). Arrows indicate the injection. Mean ± S.E.M. The number of mice for each group is indicated in parentheses. *$p<0.05$ (Tukey-Kramer's test). Solid symbols indicate $p<0.05$ vs. basal values (Tukey-Kramer's test).

4.3.3 Effect of melatonin on immobility time

Melatonin significantly reduced immobility time in DBA/2N mice [$F_{3,36}$= 5.7, p<0.01]. Post-hoc comparison showed that the effect was significant at 200 mg/kg while lower doses had no significant effects (***Figure 4.4a***). Melatonin has a biphasic effect on immobility time in C57BL/6N mice. It reduced immobility time at 100 mg/kg while a significant increase was observed at 200 mg/kg [$F_{3,26}$= 17.1, p<0.0001]. Fifty mg/kg melatonin had no significant effects. ***Figure 4.4b*** shows that the combination of 2.5 and 5.0 mg/kg paroxetine with 100 mg/kg melatonin had no effect on immobility time in DBA/2N mice. ANOVA showed no significant effects of melatonin [$F_{1,42}$= 0.9, p>0.05], paroxetine [$F_{2,42}$= 1.5, p>0.05] and their interaction [$F_{2,42}$= 0.1, p>0.05]. Doses of melatonin reducing immobility time in the FST significantly reduced spontaneous locomotion in both strains. The number of crosses ±SEM was: C57BL/6N, Vehicle 152±6, Melatonin 100 mg/kg 74±14; DBA/2N, Vehicle 137±16, Melatonin 200 mg/kg 39±8 (both p<0.001, Student's t test).

4.3.4 Brain levels of melatonin

As shown in ***Table 4.1***, 30 min after the administration of 100 and 200 mg/kg melatonin to DBA/2N mice, the brain levels of the hormone were significantly increased while no significant changes were observed with 50 mg/kg melatonin [$F_{3,51}$= 96.6, p<0.0001]. Tryptophan, paroxetine and their combination at doses reducing immobility time in the FST did not affect brain melatonin content [Tryptophan, $F_{1,20}$= 0.3, p>0.05; paroxetine, $F_{1,20}$= 0.007, p>0.05; tryptophan x paroxetine, $F_{1,20}$= 0.007, p>0.05].

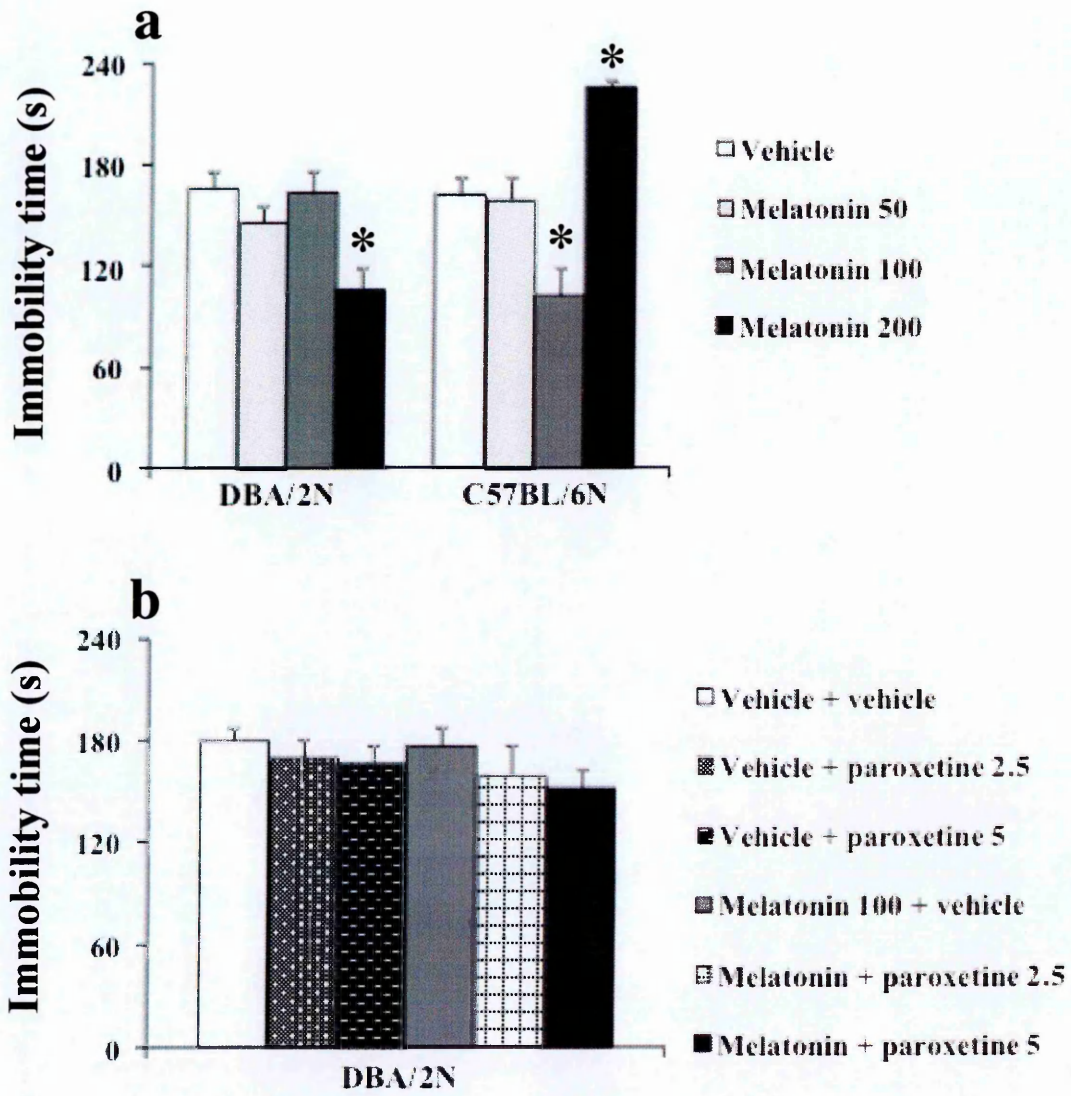


Figure 4.4 Effect of melatonin alone ***(a)*** and in combination with paroxetine ***(b)*** on immobility time in the FST. Melatonin (50-200 mg/kg) was given to C57BL/6N and DBA/2N mice 30 min before testing ***(a)***. A group of DBA/2N mice received 100 mg/kg melatonin alone or together with 2.5 and 5.0 mg/kg paroxetine 30 min before testing ***(b)***. Histograms are the mean ± SEM of 7-10 mice per group.

Table 4.1 Effect of melatonin (a), tryptophan and tryptophan plus paroxetine (b) on melatonin levels in the brain of DBA/2N mice

	melatonin (pg/g ± SEM)
a	
Vehicle	273 ± 37 (17)
Melatonin 50 mg/kg	409 ± 54 (10)
Melatonin 100 mg/kg	731 ± 74* (18)
Melatonin 200 mg/kg	2485 ± 205* (10)
b	
Vehicle + vehicle	410 ± 66 (6)
Tryptophan + vehicle	378 ± 83 (6)
Vehicle + paroxetine	410 ± 59 (6)
Tryptophan + paroxetine	366 ± 56 (6)

Mice were given 50, 100 and 200 mg/kg melatonin or vehicle (***a***) and 300 mg/kg tryptophan or vehicle 30 min before 2.5 mg/kg paroxetine or vehicle (***b***). Mice were killed by decapitation 30 min after the last injection. The number of mice per group is shown in parentheses. *p<0.001 vs. vehicle (Dunnett's test).

4.3.5 Blockade of 5-HT$_{1A}$ receptors enhanced the citalopram-induced rise of extracellular 5-HT in the mPFC

As shown in ***Figure 4.5a***, 5 mg/kg citalopram increased extracellular 5-HT in the mPFC of DBA/2N mice, reaching 5.2±0.3 fmol/20 µL of 5-HT at 60 min (210% of basal value). WAY100635 0.3 mg/kg potentiated the overall effect of citalopram on extracellular 5-HT, the levels peaking at 9.9±2.1 fmol/20 µL. ANOVA indicated a significant interaction between WAY100635, citalopram and time [$F_{7,119}$= 3.3, p=0.003] and a significant effect of citalopram [$F_{1,17}$= 42.0, p<0.0001], WAY100635 [$F_{1,17}$= 17.0, p=0.0007] and their interaction [$F_{1,17}$= 12.0, p=0.003]. WAY100635 by itself had no effect on extracellular 5-HT.

The rise of extracellular 5-HT in DBA/2N mice after WAY100635 plus citalopram was similar to that observed in C57BL/6N mice given citalopram alone (10.0±0.4 fmol/20 µL 40 min after citalopram; [$F_{1,10}$= 0.5, p=0.5).

Extracellular 5-HT in the DH reached its maximum increase 40 min after citalopram (5.8±1.8 fmol/20 µL; ***Figure 4.5b***). Blockade of 5-HT$_{1A}$ receptors had no significant effect by itself and did not boost the citalopram-induced rise of extracellular 5-HT. ANOVA indicated a significant effect of citalopram [$F_{1,19}$= 11.4, p=0.003] but not of WAY100635 [$F_{1,19}$= 1.5, p=0.24], WAY100635 x citalopram [$F_{1,19}$= 1.2, p=0.29] or the interaction between WAY100635, citalopram and time [$F_{7,133}$= 0.5, p=0.8].

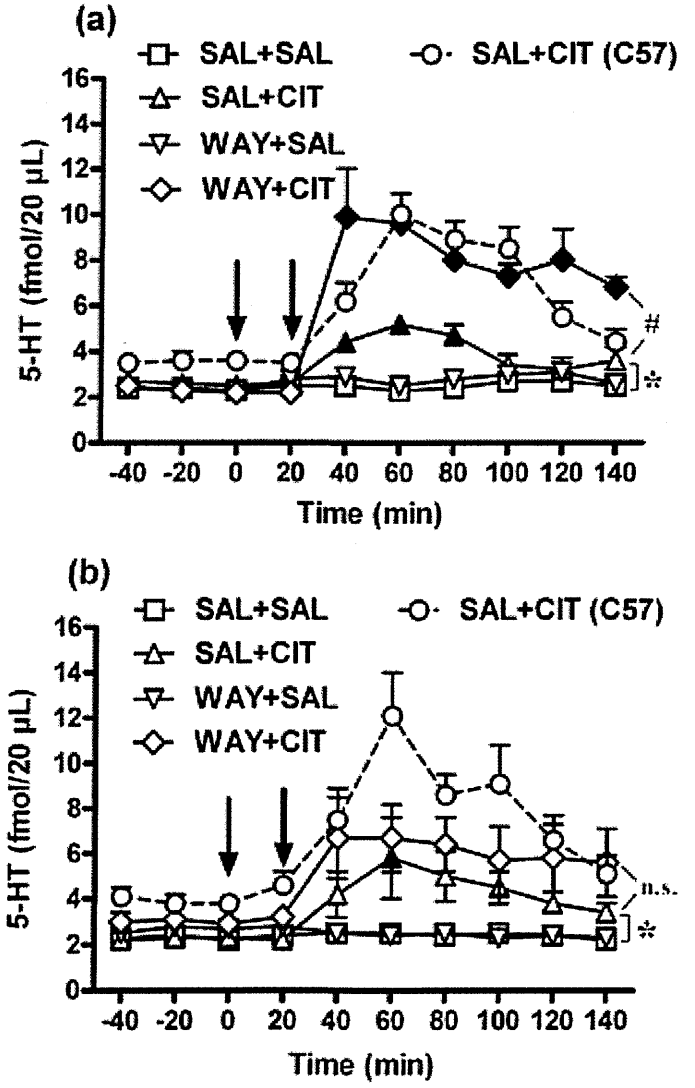


Figure 4.5 Effect of 0.3 mg/kg WAY100635 on citalopram-induced rise of extracellular 5-HT in the mPFC ***(a)*** and DH ***(b)*** of DBA/2N mice.
The first arrow indicates the injection of saline (SAL) or WAY100635 (WAY) and the second the injection of saline (SAL) or 5 mg/kg citalopram (CIT). The dashed line indicates the effect of 5 mg/kg citalopram in C57BL/6N mice (not included in the statistical analysis).
Mean basal levels of 5-HT in fmol/20 µL (±SEM) were: ***(a)*** SAL+CIT, 2.5±0.2 (n=5); WAY+SAL, 2.3±0.2 (n=5); WAY+CIT, 2.2±0.1 (n=6); SAL+SAL, 2.3±0.2 (n=5); SAL+CIT C57BL/6N mice 3.6±0.2 (n=6) ***(b)*** SAL+CIT, 2.3±0.2 (n=6); WAY+SAL, 2.7±0.1 (n=6); WAY+CIT, 2.9±0.3 (n=6); SAL+SAL, 2.2±0.2 (n=5); VEH+CIT C57BL/6N mice 3.8±0.3 (n=6).
Solid symbols indicate $p<0.05$ vs. basal values; *$p<0.05$ vs. SAL+SAL and #$p<0.05$ vs. SAL+CIT (Tukey-Kramer test).
n.s. not significant

4.3.6 Blockade of 5-HT$_{2C}$ receptors enhances the citalopram-induced rise of extracellular 5-HT in the mPFC

Blockade of 5-HT$_{2C}$ receptors with the selective antagonist SB242084 (1 mg/kg) enhanced the overall effect of citalopram on extracellular 5-HT in the mPFC from 5.7±0.7 to 7.6±1.0 fmol/20 µL at peak (***Figure 4.6a***). ANOVA indicated a significant effect of citalopram [$F_{1,19=}$ 34.4, $p<0.0001$] and the interaction between SB242084 and citalopram [$F_{1,19}$= 6.5, $p=0.02$]. The effect of SB242084 [$F_{1,19}$= 0.9, $p=0.35$] and the interaction between SB242084, citalopram and time was not significant [$F_{7,133}$= 0.8, $p=0.7$].

The rise of extracellular 5-HT in DBA/2N mice after SB242084 plus citalopram was similar to that in C57BL/6N mice given citalopram alone (9.4±1.3 fmol/20 µL at peak; [$F_{1,9}$= 2.7, $p=0.1$]).

Citalopram (5 mg/kg) raised extracellular 5-HT in the DH to 6.9±0.4 fmol/20 µL at peak (***Figure 4.6b***) and SB242084 did not significantly change this effect. ANOVA indicated a significant effect of citalopram [$F_{1,18}$= 42.2, $p<0.0001$] but not of SB242084 [$F_{1,18}$= 1.5, $p=0.2$], SB242084 x citalopram [$F_{1,18}$= 0.5, $p=0.5$] or the interaction between these factors and time [$F_{7,126}$= 0.9, $p=0.5$]. SB242084 by itself had no effect on extracellular 5-HT in the mPFC and DH.

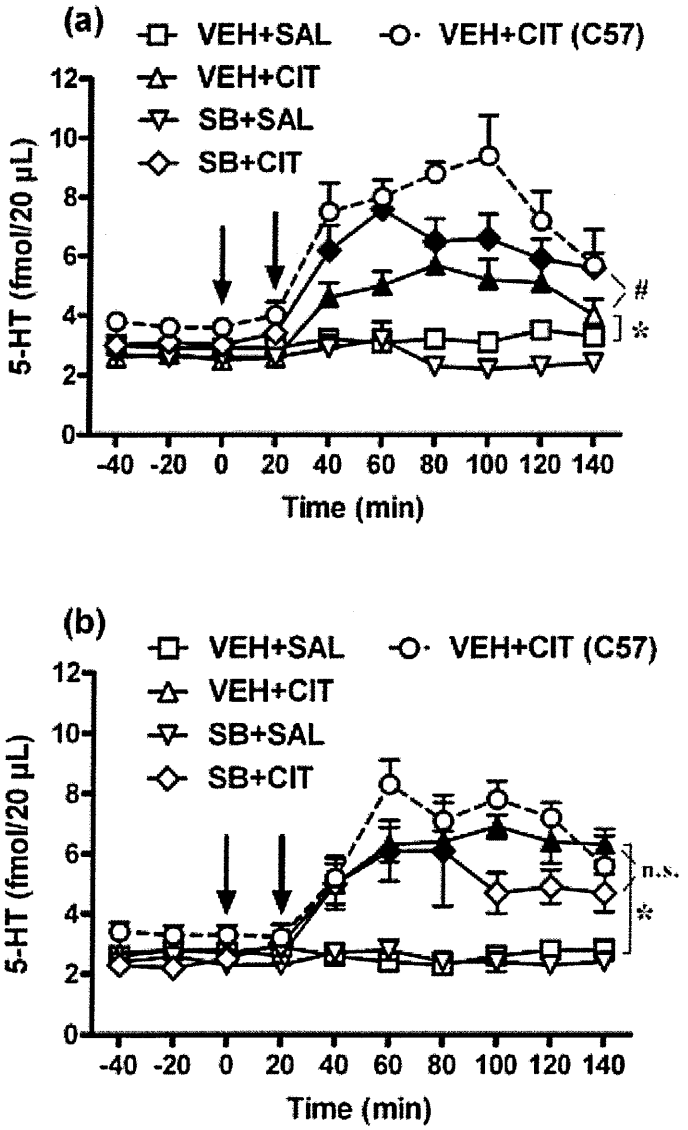


Figure 4.6 Effect of 1 mg/kg SB242084 on citalopram-induced rise of extracellular 5-HT in the mPFC (***a***) and DH (***b***) of DBA/2N mice.
The first arrow indicates the injection of vehicle (VEH) or SB242084 (SB) and the second the injection of saline (SAL) or citalopram (CIT). The dashed line indicates the effect of 5 mg/kg citalopram in C57BL/6N mice (not included in the statistical analysis).
Mean basal levels of 5-HT in fmol/20 µL (±SEM) were: (***a***) VEH+CIT, 2.5±0.2 (n=6); SB+SAL, 2.6±0.3 (n=6); SB+CIT, 3.0±0.2 (n=6); VEH+SAL, 2.9±0.2 (n=5); VEH+CIT C57BL/6N mice 3.6±0.1 (n=5) (***b***) VEH+CIT, 2.8±0.2 (n=5); SB+SAL, 2.3±0.2 (n=6); SB+CIT, 2.5±0.2 (n=6); VEH+SAL, 2.7±0.2 (n=5); VEH+CIT C57BL/6N mice 3.3±0.3 (n=5).
Solid symbols indicate $p<0.05$ vs. basal values; *$p<0.05$ vs. VEH+SAL and #$p<0.05$ vs. VEH+CIT (Tukey-Kramer test).
n.s. not significant

4.3.7 Effect of citalopram alone and in combination with WAY100635 and SB242084 on immobility time in the FST

As shown in ***Figure 4.7***, 5 mg/kg citalopram and 0.3 mg/kg WAY100635 by themselves did not affect immobility time in DBA/2N mice, but together they significantly reduced it (WAY100635, [$F_{1,28}$= 9.1, p=0.0054]; citalopram, [$F_{1,28}$= 8.2, p=0.0078] and WAY100635 x citalopram interaction, [$F_{1,28}$= 4.3, p=0.048]; two-way ANOVA).

SB242084 (1 mg/kg) significantly reduced the immobility time in mice given 5 mg/kg citalopram (***Figure 4.8***). ANOVA indicated a significant effect of SB242084 [$F_{1,28}$= 5.2, p=0.03], citalopram [$F_{1,28}$= 17.2, p=0.0003] and their interaction [$F_{1,28}$= 5.3, p=0.029]. SB242084 had no real effect by itself.

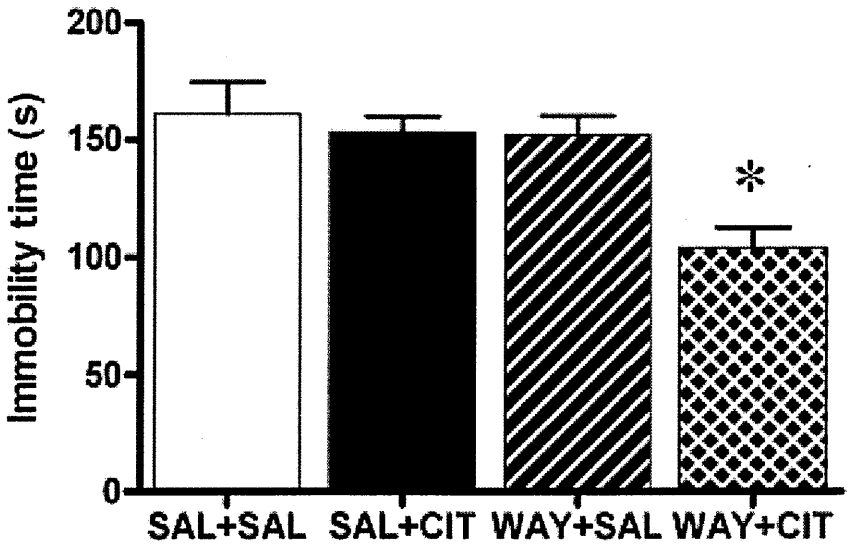


Figure 4.7 Effect of 0.3 mg/kg WAY100635 (WAY) on immobility time in the FST in DBA/2N mice given 5 mg/kg citalopram (CIT). Mean ± SEM of 8 mice per group.
*$p<0.05$ vs. SAL+SAL, SAL+CIT and WAY+SAL (Tukey-Kramer test).

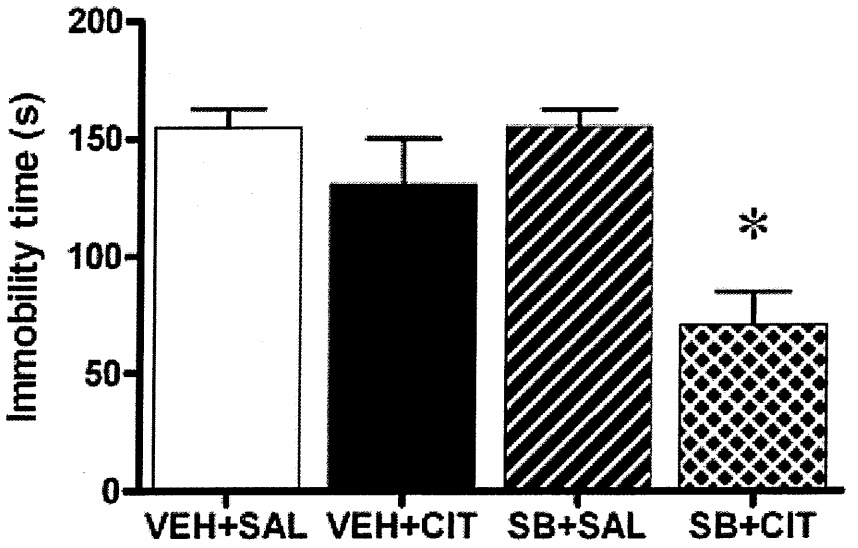


Figure 4.8 Effect of 1 mg/kg SB242084 (SB) on immobility time in the FST in DBA/2N mice given 5 mg/kg citalopram (CIT). Mean ± SEM of 8 mice per group.
*$p<0.05$ vs. VEH+SAL, VEH+CIT and SB+SAL (Tukey-Kramer test).

4.3.8 Open field activity

As reported in ***Table 4.2***, citalopram and WAY100635 alone or in combination did not affect the open-field activity of DBA/2N mice. The vehicle used to dissolve SB242084 strongly suppressed locomotor activity ($p<0.05$ vs. Saline+saline; Student's t-test). Overall, locomotor activity in mice given SB242084 was lower than in those given vehicle [$F_{1,28}= 6.6$, $p=0.02$]. However, post-hoc comparisons showed no significant differences between SB242084+saline and vehicle+saline or SB242084+citalopram and vehicle+citalopram (***Table 4.2***). Citalopram had no effect by itself [$F_{1,28}= 0.26$, $p=0.6$] or in combination with SB242084 [$F_{1,28}= 1.4$, $p=0.2$].

Table 4.2 Open-field activity of DBA/2N mice given citalopram alone and in combination with WAY100635 or SB242084.

Treatment	OPEN-FIELD ACTIVITY
	Squares crossed/4 min
(a)	
Saline+saline	210.4±22.5
Saline+citalopram	187.4±20.0
WAY100635+saline	165.5±11.0
WAY100635+citalopram	181.1±19.2
(b)	
Vehicle+saline	63.5±14.7*
Vehicle+citalopram	92.9±27.7
SB242084+saline	39.8±5.6
SB242084+citalopram	28.0±13.3

Mice were given 0.3 mg/kg WAY100635 (***a***), 1 mg/kg SB242084 (***b***) or respective vehicles 20 min before receiving 5 mg/kg citalopram.
Mean±SEM; 8 animals/group. *$p<0.05$ vs. saline+saline (Student's t-test).

4.4 Discussion

The present study addressed two strategies potentially useful to restore the antidepressant-like response of SSRIs: the augmentation with tryptophan (*Chapter 3*) and the blockade of the negative feedback loops involved in the control of serotonergic neurotransmission.

The administration of the 5-HT precursor, tryptophan, and the blockade of 5-HT_{1A} and 5-HT_{2C} receptors restored the response to citalopram in mice "non-responder" to the SSRI alone.

The results suggest that both strategies may be useful to rescue the antidepressant-like effect of SSRIs and support the importance of enhancing 5-HT transmission. The potential contribution of non-serotonergic mechanisms in the effects of tryptophan is also discussed.

Effects of tryptophan

Tryptophan restored citalopram and paroxetine antidepressant effect on immobility time in the FST (Cervo et al., 2005; Guzzetti et al., 2008). This effect was abolished by inhibiting 5-HT synthesis with pCPA (*Chapter 3*), suggesting that 5-HT mechanisms play a major role. However, a tryptophan dose that stimulates 5-HT synthesis compensated the differences in basal extracellular 5-HT between C57BL/6J and DBA/2J mice, albeit transiently, but had no effect on the ability of citalopram to raise extracellular 5-HT in the mPFC and DH of DBA/2J mice. This suggests that the stimulation of 5-HT synthesis is not sufficient to compensate for the reduced effect of citalopram in DBA/2J mice. As shown in *Chapter 3*, brain and regional tryptophan concentrations were similar in C57BL/6J and DBA/2J mice. Thus,

substrate availability does not contribute to strain differences in synthesis and basal extracellular concentrations of 5-HT.

A possible explanation for the apparent difference between extracellular 5-HT levels and the behavioural effects of tryptophan is that the stimulation of 5-HT synthesis by tryptophan which enhances 5-HT release may in turn suppresses the firing activity of 5-HT neurons of the raphé through the activation of 5-HT_{1A} autoreceptors (Liu et al., 2005). Thus, the suppression of 5-HT cell firing might mask the ability of tryptophan to enhance the effect of citalopram on extracellular 5-HT particularly in brain regions innervated by the dorsal raphé nucleus such as the mPFC in which the SSRI-induced rise of extracellular 5-HT is strongly limited by the activation of 5-HT_{1A} autoreceptors (Invernizzi et al., 1992; Invernizzi et al., 1997; Romero and Artigas, 1997; Hervás et al., 2000; He et al., 2001; Bortolozzi et al., 2004).

In addition, it cannot be excluded that non-serotonergic mechanisms contribute to the effect of tryptophan. Tryptophan is a precursor, via 5-HT, of melatonin (Weissbach et al., 1960) and administration of tryptophan increases circulating melatonin in human and rat blood (Hajak et al., 1991; Esteban et al., 2004; Jaworek et al., 2004; Leja-Szpak et al., 2004) and in the rat brain (Crespi et al., 1994). We found that melatonin reduced immobility time in the FST in C57BL/6N and DBA/2N mice, respectively at 100 and 200 mg/kg, a findings consistent with studies showing an antiimmobility effect of melatonin in the mouse FST and TST (Prakhie and Oxenkrug, 1998; Raghavendra et al., 2000; Wong and Ong, 2001).

Melatonin

A recent study (Jimenez-Jorge et al., 2007) confirmed the presence of

melatonin in the rat brain, and showed that the hormone is synthesized in the brain. However, to the best of our knowledge, information on the levels of melatonin in the mouse brain is not available. Using an HPLC-fluorometric method similar to that used by these authors, we found measurable, albeit low, levels of endogenous melatonin in the brain of DBA/2N mice and showed that the administration of melatonin at doses reducing immobility time in the FST strongly increased brain levels of this hormone. On the contrary, tryptophan alone or in combination with paroxetine had no effect indicating that these treatments unlikely affect melatonin synthesis in the brain of DBA/2N mice.

The finding that melatonin plus paroxetine did not affect the immobility time in DBA/2N mice, argue against the involvement of endogenous melatonin. However we note that in line with previous findings (Wong and Ong, 2001), we found that melatonin markedly reduced locomotor activity in C57BL/6N and DBA/2N mice, except at the highest dose (200 mg/kg) where it increased immobility. We cannot exclude that sedation might have contributed to the increase of immobility time observed after the injection of 200 mg/kg melatonin in C57BL/6N mice in the ability of tryptophan to rescue the anti-immobility effect of SSRIs

Kynurenines

Tryptophan is transformed into kynurenines by indoleamine 2,3-dioxygenase (Schwarcz, 2004) and kynurenic acid, one of the endogenous metabolites of tryptophan, is a non-selective antagonist of excitatory amino acid receptors with high affinity for the glycineß co-agonist site of the NMDA

receptor that exert antidepressant-like activity in the FST. Thus, blockade of NMDA receptors could contribute to the ability of tryptophan to restore the anti-immobility effect of paroxetine in "non responder" mice. Further studies with selective inhibitors of the key enzymes in the synthesis of kynurenines are needed to clarify whether kynurenines have any role in the mechanism by which tryptophan enhances the anti-immobility effect of SSRIs in DBA/2 mice.

5HT receptors

WAY100635 and SB242084, blocking respectively 5-HT_{1A} and 5-HT_{2C} receptors, enhanced the citalopram-induced rise of cortical extracellular 5-HT and restored the anti-immobility effect of citalopram in DBA/2N mice that do not respond to the SSRI alone (*section 4.3*). We used doses of WAY100635 and SB242084 similar to or lower than those antagonising the effects of 5-HT_{1A} and 5-HT_{2C} agonists in behavioural, biochemical and electrophysiological studies (Di Matteo et al., 2000; Kennett et al., 1997; Moser and Sanger, 1999; O'Neill and Conway, 2001; Pozzi et al., 2002; Trillat et al., 1998; Queree and Sharp, 2006). In addition, WAY100635 and SB242084 belong to different chemical classes and are among the most selective antagonists available for each respective receptor subtype (Forster et al., 1995; Kennett et al., 1997). This increases the likelihood that the behavioural and biochemical effects obtained combining citalopram with these two drugs reflects the selective involvement of 5-HT_{1A} and 5-HT_{2C} receptors.

The enhancement of the effect of citalopram on extracellular 5-HT by 5-HT_{1A} and 5-HT_{2C} receptor antagonists agrees with previous findings that

pharmacological blockade and/or genetic deletion of these receptors suppressed the inhibitory feedback regulating the activity of 5-HT neurons and enhanced the effects of SSRIs on extracellular 5-HT in several regions of the rat and mouse brain (Cremers et al., 2004; Invernizzi et al., 1992; Invernizzi et al., 1997; Piñeyro and Blier, 1999).

The observation that doses of WAY100635 and SB242084 enhancing the citalopram-induced rise of extracellular 5-HT in the mPFC but not in the DH reinstated the effect of citalopram in the FST supports the fundamental role of 5-HT in the anti-immobility effect of SSRIs and the preferential involvement of DR-mPFC 5-HT neurons in this effect.

First, DBA/2N mice have a lower brain synthesis rate and extracellular levels of 5-HT (Zhang et al., 2004; Cervo et al., 2005) and less effect of citalopram on extracellular 5-HT in the mPFC and DH than mice responding to citalopram in the FST (Zhang et al., 2004; Cervo et al., 2005; Calcagno et al., 2007; Jacobsen et al., 2008). Second, 5-HT synthesis inhibition with pCPA abolished the anti-immobility effect of citalopram and paroxetine in "responder" strains (Cervo et al., 2005; Guzzetti et al., 2008). Third, boosting 5-HT synthesis with the 5-HT precursor tryptophan restored the antidepressant-like effect of SSRIs in DBA/2N, DBA2J and BALB/c mice, which synthesize less 5-HT than C57BL/6J and C57BL/6N mice (Cervo et al., 2005; Guzzetti et al., 2008). This latter finding was confirmed by a recent study showing that the insensitivity of NMRI mice to SSRIs in the tail suspension test was associated with a reduction of tissue and extracellular 5-HT in the mPFC and was reversed by the 5-HT precursor 5-hydroxytryptophan (Jacobsen et al., 2008). Thus, it is quite likely that the

reduction of immobility time obtained by combining citalopram and WAY100635 or SB242084 is due to the fact that both these antagonists raise the effect of citalopram on extracellular 5-HT in the mPFC to the level reached in "responder" mice.

Differences in the behavioural response between mice given citalopram alone or with 5-HT_{1A} or 5-HT_{2C} receptor antagonists cannot be attributed to pharmacokinetic factors (Calcagno et al., 2009a). In fact, brain levels of citalopram at the end of the behavioural tests were essentially similar in mice given citalopram alone or with the 5-HT receptor antagonists. The fact that citalopram alone or combined with WAY100635 or SB242084 did not increase locomotor activity makes it unlikely that motor performance was involved in the effect of these treatments in the FST.

A clear reduction of open field activity was found in mice receiving the SB242084 vehicle. The fact that reduced locomotor activity was found in all groups receiving SB242084 vehicle, while immobility time was only reduced in mice given SB242084+citalopram makes it unlikely that the anti-immobility effect of this drug combination reflects changes in locomotor activity.

The blockade of 5-HT_{1A} and 5-HT_{2C} receptors with the antagonists had no effect by itself on extracellular 5-HT and immobility time, confirming that these receptors do not exert tonic control on the activity of serotonergic neurons (Adell et al., 2002; Boothman et al., 2006a; Cremers et al., 2004; Gartside et al., 1995; Invernizzi et al., 1997) and behaviour in the FST (Cremers et al., 2004; O'Neill and Conway, 2001; Tatarczynska et al., 2004).

One of the salient points of this study is that we use a mouse strain that does not respond to citalopram in the FST, to show that the response can be restored by enhancing the effect of citalopram on serotonergic transmission. Previous studies aimed at improving the antidepressant response were mostly done in rats or mice already responding to the antidepressant alone, and had given conflicting results. WAY100635 did not further reduce immobility time in the FST in mice and rats responding to SSRIs alone (Moser and Sanger, 1999; Guilloux et al., 2006). Genetic deletion or pharmacological blockade of 5-HT_{2C} receptors, that clearly enhanced the effect of SSRIs on extracellular 5-HT, only marginally enhanced their anti-immobility effect in the mouse TST (Cremers et al., 2004). In contrast, the present study clearly found that blockade of 5-HT_{1A} or 5-HT_{2C} receptors restores the antidepressant-like effect of citalopram in mice 'non-responder' to the drug alone.

Mechanisms underlying 5HT_{1A} effects

The observation that the 5-HT_{1A} antagonist, WAY100635, had no effect on the citalopram-induced rise of extracellular 5-HT in the DH is in line with previous reports that 5-HT_{1A} receptors exert strong inhibitory control over the activity of 5-HT neurons arising from the DR, such as the mPFC. In contrast, the DH, an area innervated by 5-HT neurons arising from the MR (Azmitia and Segal, 1978; Kosofsky and Molliver, 1987) contains far fewer 5-HT_{1A} receptors and 5-HT uptake sites than the DR (Adell et al., 2002; Hrdina et al., 1990; Lechin et al., 2006; Weissmann-Nanopoulos et al., 1985) and is less affected by the 5-HT_{1A} receptor or not at all (Beck et al., 2004; Sinton and Fallon, 1988; Lorens and Guldberg, 1974; Invernizzi et

al., 1991). Thus, 5-HT_{1A} receptor antagonists preferentially enhanced the effects of SSRIs on extracellular 5-HT in brain regions innervated by the DR (Casanovas and Artigas, 1996; Invernizzi et al., 1997).

Although the enhancement of extracellular 5-HT likely plays a major role in the ability of WAY100635 and SB242084 to reinstate the anti-immobility effect of citalopram, different mechanisms are likely involved in the action of these drugs. WAY100635 blocks 5-HT_{1A} receptors that are expressed by 5-HT neurons of the raphé (Miquel et al., 1992) and directly inhibit the activity of these cells by inducing membrane hyperpolarization through an action on potassium and calcium channels (Aghajanian and Lakoski, 1984; Penington and Fox, 1994). Post-synaptic 5-HT_{1A} receptors located in the mPFC are also involved in the auto-regulation of 5-HT neurons through a long feedback loop (Ceci et al., 1994; Celada et al., 2001; Hajós et al., 1999) and may contribute to the action of WAY100635.

Mechanisms underlying $5HT_{2c}$ effects

The fact that SB242084 enhanced the citalopram-induced rise of extracellular 5-HT in the mPFC but not DH suggests that 5-HT_{2C} receptors preferentially regulate 5-HT neurons arising from the DR.

To our knowledge, the anatomical distribution and cellular localization of 5-HT_{2C} receptors and the susceptibility of DR and MR 5-HT neurons to 5-HT_{2C} receptor regulation in the mouse brain have not been studied. Therefore, the clear-cut difference in the effects of SB242084 on the citalopram-induced rise of 5-HT in the mPFC and DH remains to be investigated.

5-HT_{2C} receptors are essentially localized on non-serotonergic neurons. Immunocytochemical studies showed that 5-HT_{2C} receptors are expressed in glutamic acid decarboxylase-positive, GABAergic neurons in the raphé and other brain regions (Pazos et al., 1985; Serrats et al., 2005). GABAergic neurons synapse upon 5-HT cells in the raphé (Wang et al., 1992) and control their activity (Gallager and Aghajanian, 1976). Moreover 5-HT applied to rat brain slices containing the DR caused a GABA- and 5-HT_{2C}-mediated inhibition of 5-HT neurons (Liu et al., 2000) suggesting that SB242084 might enhance the effect of citalopram by acting on 5-HT_{2C} receptors on GABAergic neurons intrinsic to the raphé that in turn inhibit 5-HT cells. Thus, 5-HT_{2C} receptors of the DR are probably mainly involved in the mechanism by which SB242084 restored the anti-immobility effect of citalopram in DBA/2N mice. However, extra-raphé 5-HT_{2C} receptors may also contribute (Cremers et al., 2007; Sharp et al., 2007). This issue is discussed in *Chapter 5*.

Altogether these findings suggest that strategies aimed at enhancing 5-HT transmission might be more effective in improving the antidepressant efficacy in subjects with hypofunctioning brain 5-HT transmission such as DBA/2N mice. The results also show that enhancing the effect of SSRIs on extracellular 5-HT confers sensitivity to citalopram in the FST to DBA/2N mice, which are otherwise insensitive to citalopram alone.

Augmentation of the response with 5-HT_{1A} and/or 5-HT_{2C} receptor antagonists may be useful to restore the antidepressant response to SSRIs, particularly in treatment-resistant depression associated with hypofunctioning 5-HT neurotransmission.

Chapter 5

BLOCKADE OF 5-HT_{2C} RECEPTORS IN THE DR ENHANCES THE EFFECT OF SSRIS ON 5-HT TRANSMISSION BY REDUCING GABAergic INHIBITION

5.1 Introduction

The failure of SSRIs to reduce immobility time in DBA/2 mice is at least partly due to a reduced 5-HT synthesis likely involving more complex changes in 5-HT transmission leading to an insufficient availability of 5-HT at its sites of action.

Our findings (*Chapter 4*) confirm that 5-HT_{2C} receptors are involved in the neurochemical and antidepressant-like effects of SSRIs. Given that 5-HT_{2C} receptors are not located on 5-HT neurones (Clemett et al., 2000), an indirect control of 5-HT neurotransmission is probably implicated. Several evidences suggest the involvement of GABAergic mechanism.

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain and GABA-mediated neurotransmission regulates many physiological and psychological processes (*see review* Brambilla et al., 2003). There are two major classes of GABA receptors: ionotropic GABA-A (and GABA-C receptors) and metabotropic GABA-B receptors. Although depression is seen largely as a dysfunction in monoamine neurotransmission and all antidepressant strategies focus largely on monoamines, clinical and preclinical evidences implicate dysfunction of the GABA system in depression (Krystal et al., 2002; Brambilla et al., 2003; Shiah and Yatham, 1998). Lower GABA levels in cerebrospinal fluid and plasma have been found in depressed patients compared to controls. Recently, studies using proton magnetic resonance spectroscopy, found decreased GABA concentrations in the occipital cortex of depressed patients and subsequent studies demonstrated that these low levels of GABA were normalized after

antidepressant treatments (Sanacora et al., 2002; Sanacora et al., 2003; Sanacora et al., 2004).

The decrease in GABA observed in depressed patients does not appear to be associated with changes in GABA uptake in the frontal cortex and cingulate gyrus, and neither GABA-B receptors nor glutamic acid decarboxylase (GAD; the biosynthetic enzyme for GABA) activity have been found to be altered whereas GABA-A receptor binding in frontal cortex was increased in depressed suicide victims (Brambilla et al., 2003; Schechter et al., 2005; Kalueff and Nutt, 2007). Additional evidence is provided by the findings of lower platelet GABA transaminase (a catabolic enzyme for GABA) and plasma GAD activity in depressed patients (Brambilla et al., 2003).

Collectively, these findings have raised interest in the role of GABA as a potential target for treating depression.

It has been shown that chronic administration of several antidepressant drugs reduces the levels of GABA-A receptors in rat brains, in regions such as the cortex, hippocampus, and hypothalamus. Additionally, several studies reported increased GABA-B receptor binding sites in frontal cortex and hippocampus in rats, after chronic treatment with various antidepressants (Gray et al., 1987; Lloyd et al., 1985; Pratt and Bowery, 1993; Sands et al., 2004).

More recently, there has been more emphasis on antagonism of GABA-B receptors as a potential therapeutic strategy for depression (Cryan and Kaupmann, 2005). In support of this, GABA-B(1) subunit knockout mice display antidepressant-like phenotype in the FST (Mombereau et al., 2004). Furthermore, chronic treatment with the GABA-B receptor antagonist CGP36742 has an antidepressant-like response in the learned helplessness

model (Nakagawa et al., 1999). Moreover, other GABA-B antagonists, CGP56433A and CGP55845A, also showed antidepressant-like effects in the rat FST, decreasing immobility and increasing swimming, a profile comparable with SSRIs (Slattery et al., 2005; Cryan et al., 2002). Depletion of 5-HT prevents the effect of CGP56433A and CGP55845A in the FST, suggesting a role of 5-HT in their mechanism.

Neurochemical and electrophysiological studies indicate that 5-HT neurons are subject to GABAergic inhibitory regulation (Gallager and Aghajanian, 1976; Wang and Aghajanian, 1977b; Gervasoni et al., 2000; Tao and Auerbach, 2003).

GABA neurones in the dorsal raphé (DR) synapse onto 5-HT neurones and exert an inhibitory effect (Wang et al., 1992; Liu et al., 2000; Varga et al., 2001).

GABA-A agonists, such as muscimol, and the non-selective agonist, progabide, appear to reduce 5-HT synthesis in rat brains (Nishikawa and Scatton, 1983; Nishikawa and Scatton, 1985) probably through GABA-A receptors located in the raphé nuclei. Local infusion of the GABA-A receptor antagonist, bicuculline, increases 5-HT release in the DR, indicating that GABA afferents exert a tonic inhibitory influence on 5-HT neurones (Tao and Auerbach, 2003).

There are considerable data supporting a relationship between GABAergic and serotonergic systems in the CNS. However, this relationship is complex. Anatomical studies demonstrate reciprocal synaptic interactions between 5-HT and GABA neurones in the DR. The GABA-B receptor agonist, baclofen, given systemically, increases 5-HT release in the DR and in the striatum

(Abellán et al., 2000). In addition, baclofen inhibited GABA release in raphé slice (Bagdy et al., 2000). Therefore GABA-B stimulation affected 5-HT neurones both directly via GABA-B receptors located on 5-HT neurones and indirectly through GABA-B autoreceptors. Moreover, it has been shown that also 5-HT_{1A} and 5-HT_{1B} agonists decrease 5-HT and GABA release in rat raphé nuclei (Bagdy et al., 2000), suggesting a reciprocal control between GABAergic and serotonergic neurons.

The presence of 5-HT_{2C} receptors on GABA neurones in the DR has been reported (Serrats et al., 2005). Activation of 5-HT_{2C} receptors indirectly attenuates the activity of serotonergic neurones probably acting through GABAergic interneurones in the raphé nuclei (Singewald and Sharp, 2000; Boothman et al., 2003; Boothman and Sharp, 2005; Boothman et al., 2006b; Liu et al., 2000) or through a long inhibitory feedback loop originating in projection areas (Cremers et al., 2007). Recent evidence suggests that 5-$HT_{2A/C}$ receptors activate GABAergic interneurons in rat hippocampus, PFC and DR (Shen and Andrade, 1998; Abi-Saab et al., 1999; Liu et al., 2000). In vivo electrophysiological findings showed that the phenethylamine derivatives, DOM and DOI, both non-selective 5-HT_2 receptor agonists, inhibit the firing of DR 5-HT neurones (Aghajanian et al., 1970; Garratt et al., 1991). In particular, DOI-induced inhibition of 5-HT cell firing, was blocked by the 5-HT_{2A} receptor antagonist M100907 but was also attenuated by the 5-$HT_{2B/2C}$ receptor antagonist SB206553 (Boothman et al., 2003). Moreover WAY161503, a selective 5-HT_{2C} receptor agonist, caused inhibition of 5-HT cell firing which was reversed by the selective 5-HT_{2C} receptor antagonist, SB242084 (Boothman et al., 2003; Boothman et al., 2006b). In addition, DOI and WAY161503 increase the expression of the

immediate early gene c-fos in GAD positive DR neurones and DR GAD positive neurones expressed 5-HT_{2C} receptor immunoreactivity (Boothman et al., 2006b; Boothman and Sharp, 2005).

Thus, a model of 5-HT feedback control has been proposed in which 5-HT_{2C} receptors activate DR GABA neurones to inhibit 5-HT neuronal activity (***Figure 5.1***).

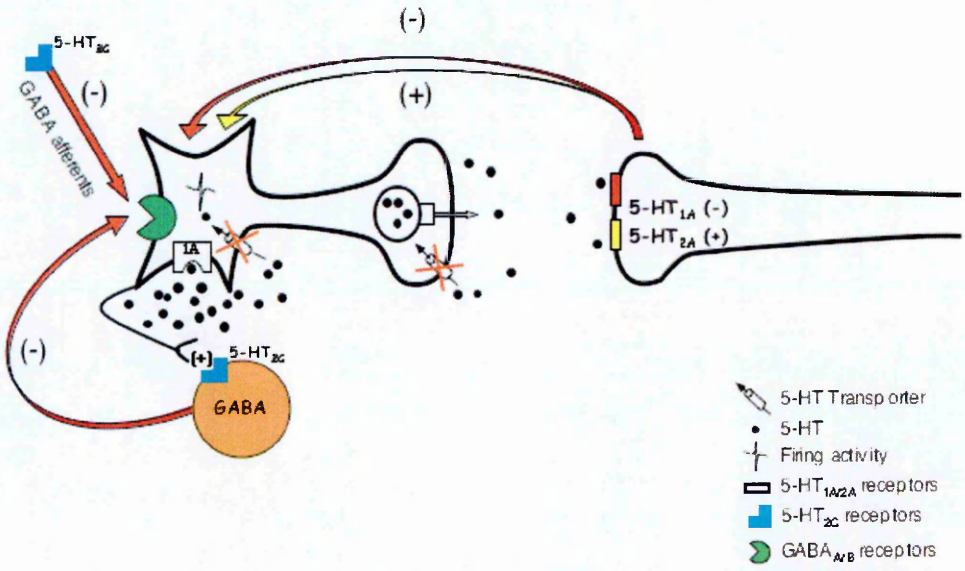


Figure 5.1 **Scheme of the putative role of 5-HT_{2C} receptors**

Glutamatergic cells in the vicinity of the DR also have a stimulatory influence on serotonergic neurons (Jolas and Aghajanian, 1997). The activation of DR serotonergic neurons in response to phasic sensory stimuli is dependent on glutamate (GLU; Levine and Jacobs, 1992) and this might be mediated by glutamatergic afferents from the habenula and cerebral cortex (Celada et al., 2001; Kalén et al., 1989; Wang and Aghajanian, 1977b).

A study by Martin-Ruiz (2001) has shown that modulation of glutamatergic neurotransmission by 5-HT_2 receptors might be involved in control of 5-HT release. The non-selective 5-HT_2 agonist, DOI, stimulated the impulse flow in pyramidal neurons projecting to the DR through 5-HT_{2A} receptors (but not 5-HT_{2C} receptors), thus resulting in an increased serotonergic activity and 5-HT release in mPFC. This effect was blocked by the AMPA–Kainate/glutamate receptor antagonist, NBQX, and likely depends on the release of GLU and stimulation of AMPA–Kainate receptors. Moreover, glutamatergic afferents from PFC also act indirectly through GABAergic neurons to inhibit 5-HT release and this may play a role in long-feedback loop inhibition of serotonergic neurons (Hajós et al., 1998; Celada et al., 2001; Varga et al., 2001).

Thus, the aim of this part of the study was to assess the role of GABA and GLU of the DR in controlling the effect of SSRIs on extracellular 5-HT in "responder" and "non-responder" mice. Therefore, I assessed if citalopram activates local GABAergic inputs to 5-HT cells in the DR evaluating the effect of this drug on extracellular GABA, GLU and 5-HT.

The inhibitor of 5-HT synthesis, p-chlorophenylalanine (pCPA), was used to evaluate the role of endogenous 5-HT in the effects of citalopram. Finally, I assessed if 5-HT_{2C} receptors control GABA and GLU release in the DR and their role in the effect of citalopram.

Since the measure of extracellular GABA and GLU with microdialysis does not always fulfil the classic criteria for exocytotic release (Timmerman and Westerink, 1997; van der Zeyden et al., 2008), preliminary studies were devoted to examine the effect of K^+ and the sodium channel blocker tetrodotoxin (TTX) on extracellular GABA, GLU and 5-HT in the DR under current experimental conditions.

5.2 Methods

Microdialysis procedures have been accomplished as described in *Chapter 2*.

5.2.1 GABA and GLU assay

GABA and GLU were measured after derivatization with o-phthalaldehyde (OPA; Sigma-Aldrich, Milan, Italy) based reagent according to Donzanti and Yamamoto (1988). Stock derivatizing reagent was prepared by dissolving 27 mg OPA in 1 mL methanol, followed by 5 µL β-mercaptoethanol and 9 mL 0.1 M sodium tetraborate buffer (pH 9.3) prepared by dissolving 0.62 g boric acid in about 80 mL ultrapure water (MilliQ, Millipore, USA). pH was adjusted to 9.3 with 2-3 mL 5 M NaOH and the final volume brought to 100 mL with water. Stock reagent solution was maintained at room temperature in a darkened bottle for one week. Derivatizing reagent was prepared by diluting stock solution 1:4 with 0.1 M sodium tetraborate borate buffer, 24 h before use.

5 µL of derivatizing reagent were added to 5 µL sample to measure GLU or GABA, thoroughly mixed and immediately injected into the HPLC by a refrigerated Midas autosampler (Spark-Holland, Emmen, The Netherlands) set at 4°C.

GABA and GLU were separated through a 4.6 x 80 mm C18 reverse-phase column (HR-80, ESA, Chelmsford, MA). New Guard RP-18 guard column (3.2 x 15 mm; Perkin-Elmer, USA) was used to protect the analytical column. The mobile phase for GABA was as follows: 0.05 M Na_2HPO_4, 35%

methanol, pH 6.25 with 85% phosphoric acid, pumped at 1.2 mL/min with a LC10-ADvp HPLC pump (Shimadzu, Milan, Italy).

The mobile phase for GLU separation contained 0.05 M Na_2HPO_4, 28% methanol, pH 6.4 with 85% phosphoric acid at a flow rate of 1 mL/min with a LC20-AD HPLC pump (Shimadzu, Milan, Italy).

GABA and GLU were measured by a fluorescence detector (Jasco SP2020, Tokyo, Japan). Excitation and emission wavelengths were 335 and 450 nm for both aminoacids. Assays were calibrated daily by injecting 0.2, 0.4 and 0.8 pmol/20 μL GABA or 1, 5 and 10 pmol/5 μL GLU, made up freshly in aCSF. Detection limits were 0.025 pmol/20 μL for GABA and 0.1 pmol/5 μL for GLU (signal-to-noise ratio = 2).

5.2.2 Drugs and reagents

Citalopram hydrobromide (Tocris Cookson, Bristol, UK) was dissolved in saline (10 mL/kg) and injected i.p. at 1.25, 5 and 20 mg/kg or dissolved in aCSF and perfused through the probe at the concentrations indicated. Control mice were injected with saline.

pCPA ethyl ester (Sigma-Aldrich, Milan, Italy) was prepared as described in *section 3.2.5*.

The 5-HT_{2C} receptor antagonist, 6-chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB242084; GlaxoSmithKline, Harlow, UK), was dissolved in DMSO: 4M tartaric acid: water (50: 1: 49) and injected s.c. or through the probe 20' before citalopram. SB242084 (1 mM), prepared as above, was further diluted in aCSF to the final concentration (0.1 μM) and perfused through the probe. Ro 60-0175, [(s)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine hydrochloride]

(Hoffmann-La Roche, Basel, Switzerland), the 5-HT_{2C} agonist, was dissolved in saline and injected i.p. at the doses indicated.

aCSF containing 60 mM KCl was prepared as follow: NaCl 87 mM; KCl 60 mM; $CaCl_2$ 1.26 mM and $MgCl_2$ 1 mM were dissolved in ultra pure water and buffered at pH 7.4 with 2 mM sodium phosphate buffer. High K^+ aCSF was perfused through the probe for 20 min to stimulated the exocytotic release of GABA, 5-HT and GLU, and after 1 h washout with "normal" aCSF, 1 µM tetrodotoxin (TTX), dissolved in sodium citrate buffer (pH 4.5), diluted to 1 µM with aCSF and perfused through the probe for 1 h to verify the action-potential dependent release of the neurotransmitters.

All drugs or vehicles were injected after the collection of 4 baseline samples. Only animals with basal samples not differing by more than 20% were considered in the results.

5.2.3 Data analysis

The chromatographic peaks of GABA, GLU and 5-HT were quantified based on the calibration curve calculated on three different standard concentrations by a chromatography software (Azur, Datalys, Saint Martin d'Heres, France). ***Figure 5.2*** shows representative chromatograms of GABA and GLU in standards and DR dialysate samples.

Basal values of the neurotransmitters, uncorrected for *in vitro* recovery of the probe, in different experiments and in different strains of mouse were expressed as pmol/20 µL (GABA and GLU) or fmol/20µL (5-HT) and compared by one-way ANOVA or Student's t-test. To facilitate comparisons, the effects of treatments on GABA, 5-HT and GLU were expressed as percentage of basal value.

The effect of perfusion with KCl and TTX was analyzed using the paired Student's t-test comparing the effect of elevated KCl 20 min after its perfusion with basal value and the effect of TTX at 1 h with basal value.

All time-course data were analyzed by ANOVA for repeated measures with treatments as between subjects factor and time as within subjects factor. *Post-hoc* comparisons between pre- and post-injection values and comparisons between treatments were done with Tukey-Kramer's test.

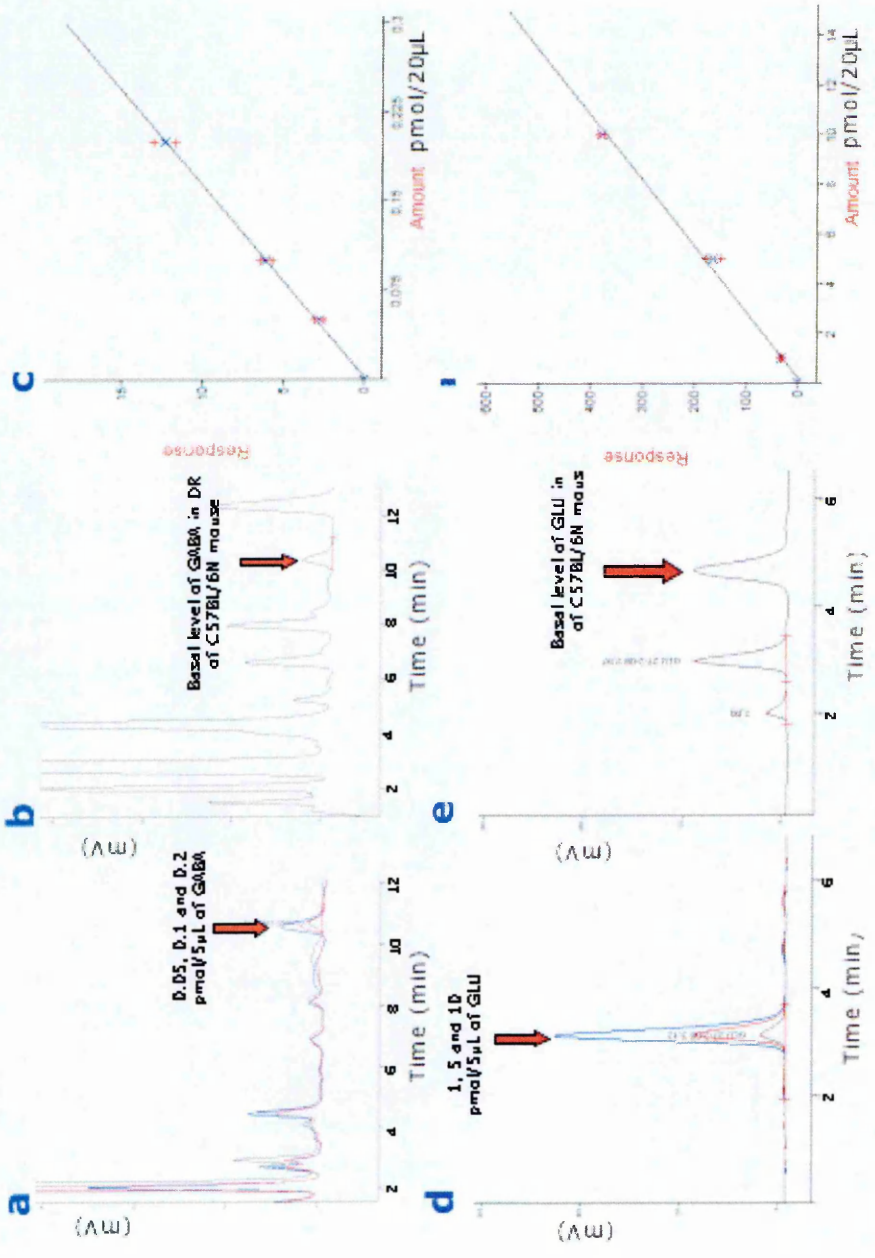


Figure 5.2 **a)** Chromatogram of 0.05,0.1 and 0.2 pmol/5 µL GABA standard
b) Chromatogram of DR GABA sample (basal; 5 µL) from a C57BL/6N mouse
c) example of calibration curve for GABA
d) Chromatogram of 1, 5 and 10 pmol/5 µL GLU standard
e) Chromatogram of DR GLU sample (basal; 5 µL) from a C57BL/6N mouse
f) example of calibration curve for GLU

5.3 Results

5.3.1 Basal extracellular levels of GABA, 5-HT and GLU in DBA/2N and C57BL/6N mice and effect of pCPA

Mean basal 5-HT, GABA and GLU concentrations in the DR of DBA/2N mice, obtained by pooling basal values of different experimental groups, were respectively: 6.7±0.2 fmol/20 µL (n=108); 0.29±0.01 pmol/20 µL (n=99); 20.9±0.7 pmol/20 µL (n=106).

Mean basal 5-HT, GABA and GLU concentrations in the DR of C57BL/6N mice, obtained by pooling basal values of different experimental groups, were respectively 19.1±1.0 fmol/20 µL (n=56); 0.85±0.04 pmol/20 µL (n=55); 24.6±1.3 pmol/20 µL (n=64).

As shown in ***Figure 5.3***, basal extracellular 5-HT, GABA and GLU in the DR of DBA/2N mice were significantly lower (respectively by about 65%; 66% and 15%) than in C57BL/6N mice [5-HT: t_{162}= 16, $p<0.0001$; GABA: t_{152}= 16.5, $p<0.0001$; GLU: t_{168}= 2.8, $p<0.01$)].

pCPA (300 mg/kg orally for three days) drastically reduced extracellular 5-HT in DBA/2N mice (by about 83%; [t_{120}= 8.7, $p<0.0001$]) without affecting basal levels of GABA and GLU [respectively t_{108}= 0.4, $p>0.05$; t_{116}= 0.8, $p>0.05$].

Mean basal 5-HT concentration in the mPFC of DBA/2N mice, obtained by pooling basal values of different experimental groups, was 2.9±0.2 fmol/20 µL (n=19).

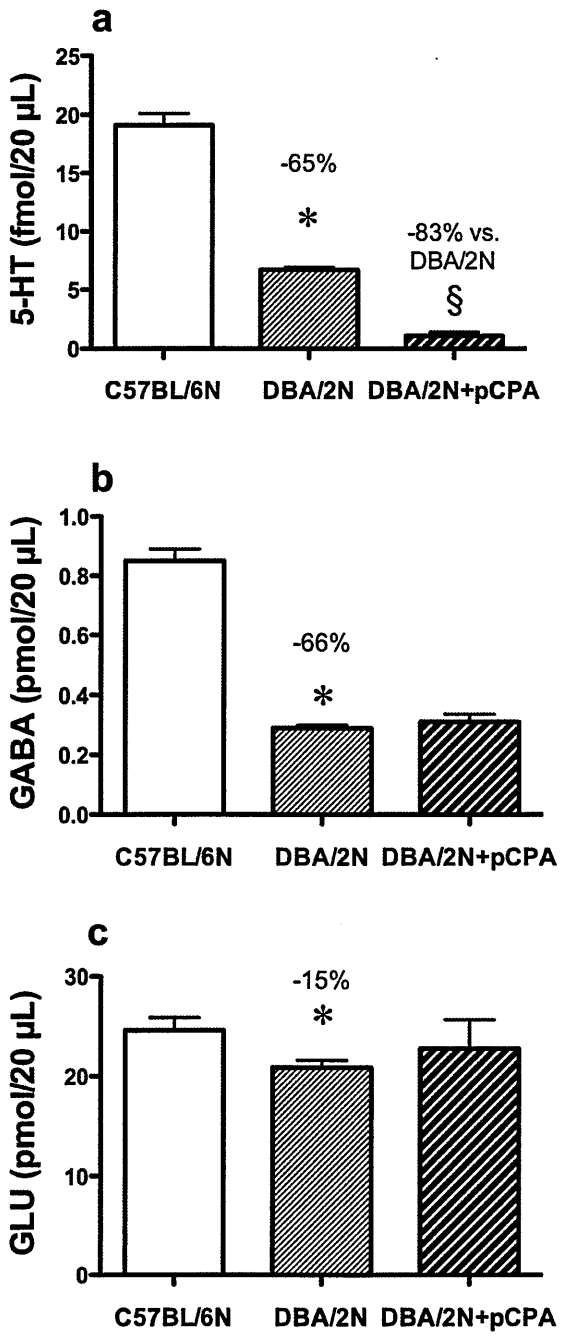


Figure 5.3 Basal extracellular 5-HT (**a**), GABA (**b**) and GLU (**c**) in the DR of C57BL/6N and DBA/2N mice. Results are Mean±SEM. The last column of each panel indicates the effect of pCPA in DBA/2N mice.
Mean basal concentrations in the DR of C57BL/6N, DBA/2N mice and DBA/2N mice receiving pCPA were respectively: 5-HT, 19.1±1.0 fmol/20 µL (n=56), 6.7±0.2 fmol/20 µL (n=108) and 1.1±0.3 fmol/20 µL (n=14); GABA, 0.85±0.04 pmol/20 µL (n=55), 0.29±0.01 pmol/20 µL (n=99) and 0.31±0.03 pmol/20 µL (n=11); GLU, 24.6±1.3 pmol/20 µL (n=64), 20.9±0.7 pmol/20 µL (n=106) and 22.8±2.9 pmol/20 µL (n=12).
*$p<0.05$ vs. C57BL/6N mice and §$p<0.05$ vs. DBA/2N mice (Tukey-Kramer's test).

5.3.2 Neuronal origin of extracellular GABA, 5-HT and GLU in the dorsal raphé: effect of elevated KCl and TTX

Mice were perfused through the probe with 60 mM KCl for 20 min to stimulate exocytotic neurotransmitter release. After 1 h wash-out with normal aCSF, animals were perfused with 1 µM TTX for 1 hour to verify the neuronal origin of the basal extracellular neurotransmitters in the DR. ***Figure 5.4*** shows the effect of 20-min perfusion with KCl and the effect of 1 hour perfusion with TTX.

Elevated KCl increased extracellular 5-HT by about 15-fold [t_7= 5.7, $p<0.001$] in DBA/2N mice and 5-fold [t_6= 6.7, $p<0.001$] in C57BL/6N mice. Extracellular GABA reached 172% and 218% of basal level after KCl respectively in DBA/2N [t_4= 3.3, $p<0.05$] and in C57BL/6N mice [t_6= 2.4, $p=0.055$]. Twenty-min infusion with 60 mM KCl increased extracellular GLU by 2.1-fold [t_7= 3.2, $p=0.01$] in DBA/2N mice and 2.4-fold [t_7= 2.2, $p=0.06$] in C57BL/6N mice.

One µM TTX significantly reduced extracellular 5-HT in both strains respectively by about 64 and 38% (DBA/2N: [t_7= 9.7, $p<0.0001$]; C57BL/6N: [t_6= 3.7, $p=0.01$]). GABA concentrations were lowered by 1 µM TTX by about 60% in DBA/2N mice [t_4= 8.2, $p=0.001$] and 53% in C57BL/6N mice [t_6= 4.5, $p<0.01$] whereas TTX had no significant effect on extracellular GLU in both strains (DBA/2N: [t_7= 0.07, $p>0.05$]; C57BL/6N: [t_7= 1.1, $p>0.05$]).

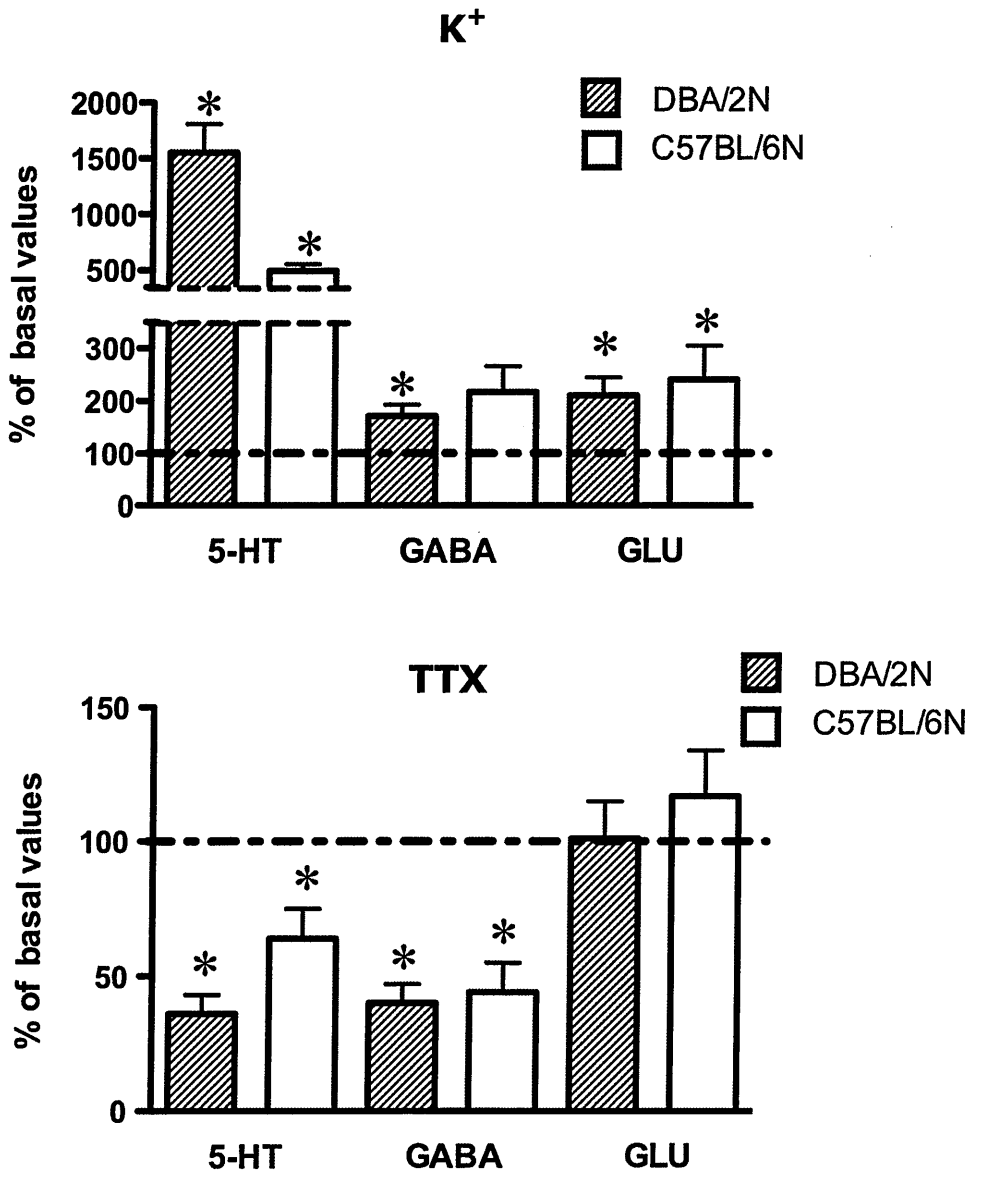


Figure 5.4 Effect of 60 mM KCl (upper panel) and 1 µM TTX (lower panel) on extracellular 5-HT, GABA and GLU in the DR of DBA/2N and C57BL/6N mice.
The dashed lines indicate basal values.
Results are Mean±SEM and are expressed as percentage of basal values. Basal levels of 5-HT were 5.0±0.6 fmol/20 µL (n=8) for DBA/2N mice and 19.6±1.4 fmol/20 µL (n=7) for C57BL/6N mice. Basal values of GABA and GLU in pmol/20 µL were respectively: 0.41±0.04 (n=5) for DBA/2N mice and 0.78±0.05 (n=7) for C57BL/6N mice and 18.9±2.2 (n=8) for DBA/2N mice and 19.4±3.0 (n=8) for C57BL/6N mice.
*$p < 0.05$ vs. basal values (Paired Student's t-test).

5.3.3 The stimulation of 5-HT_{2C} receptors reduced extracellular 5-HT and raised extracellular GABA in the DR of DBA/2N mice

As shown in ***Figure 5.5a***, the 5-HT_{2C} receptor agonist, Ro 60-0175, at 300 µg/kg i.p. significantly reduced extracellular 5-HT by about 35% ($p<0.05$, Tukey Kramer's test) whereas at lower dose (30 µg/kg, data not shown) had no effect.

On the contrary, 300 µg/kg Ro 60-0175 significantly raised extracellular GABA ($p<0.05$, Tukey Kramer's test). The maximum increase (220%) was reached 100 min after the injection (***Figure 5.5b***).

SB242084, 1 mg/kg s.c., had no significant effects by itself both on 5-HT and GABA levels (see ***Figure 5.9a*** and ***b***) but, given 20 min before Ro 60-0175, completely antagonized the effects of Ro 60-0175. ANOVA indicated a significant interaction between treatment and time respectively for 5-HT [$F_{7,70}= 2.3$, $p=0.03$] and GABA [$F_{7,77}= 2.2$, $p=0.04$].

Figure 5.5c shows that both Ro 60-0175 and the combination of Ro 60-0175 and SB242084 had no effect extracellular GLU [treatment: $F_{1,12}= 0.07$, $p=0.8$; time: $F_{7,84}= 1.9$, $p=0.08$; treatment x time: $F_{7,84}= 0.8$, $p=0.6$].

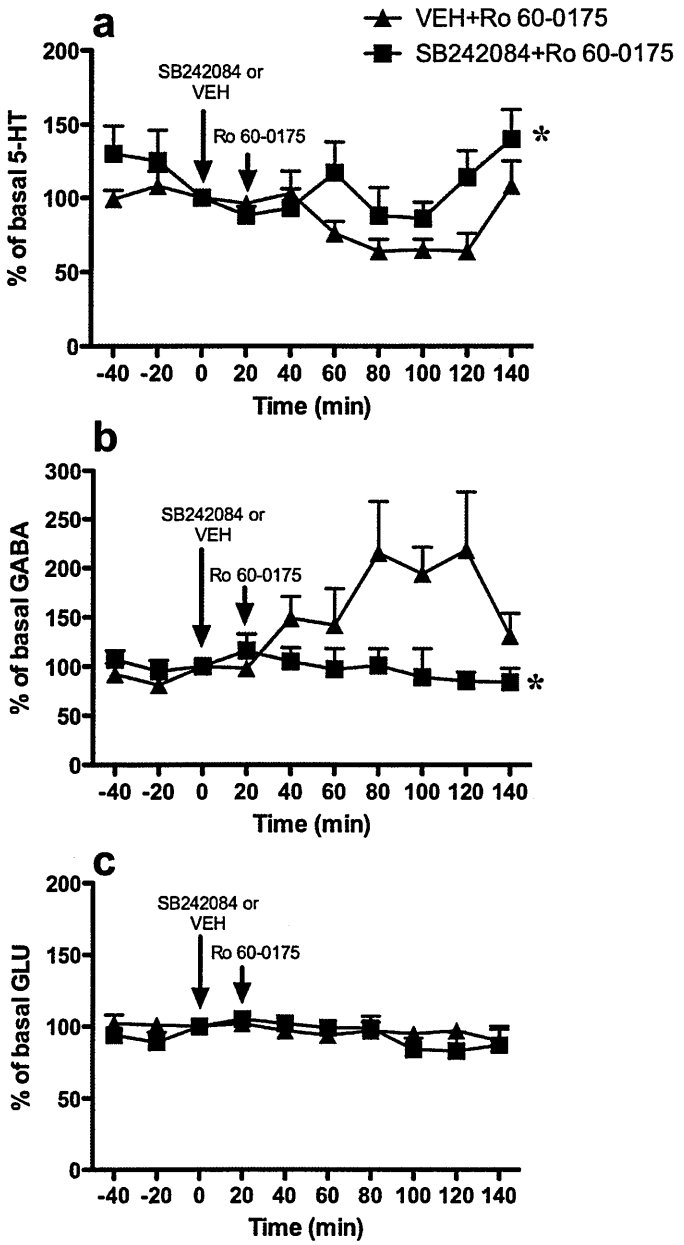


Figure 5.5 Effect of Ro 60-0175 on extracellular 5-HT (***a***), GABA (***b***) and GLU (***c***) in the DR.
Arrows indicate the injection of SB242084 or vehicle (VEH) and the injection of Ro 60-0175.
Results are Mean±SEM and are expressed as percentage of basal levels. Basal levels of 5-HT were 7.4±0.6 fmol/20 µL (n=7) for VEH+Ro 60-0175 and 6.8±2 fmol/20 µL (n=5) for SB242084+Ro 60-0175. Basal levels of GABA and GLU in pmol/20 µL were respectively: 0.27±0.02 (n=7) for VEH+Ro 60-0175 and 0.25±0.03 (n=6) for SB242084+Ro 60-0175 and 22.1±2.1 (n=7) for VEH+Ro 60-0175 and 21.3±1.9 (n=7) for SB242084+Ro 60-0175.
*$p<0.05$ SB242084+Ro 60-0175 vs. VEH+Ro 60-0175 (Tukey-Kramer's test).

5.3.4 Effect of citalopram on extracellular 5-HT, GABA and GLU in DBA/2N mice

As shown in ***Figure 5.6***, saline had no effect on extracellular 5-HT, GABA and GLU.

Citalopram at 1.25 mg/kg had no significantly effect on extracellular 5-HT in the DR of DBA/2N mice whereas at 5 and 20 mg/kg it significantly increased extracellular 5-HT (***Figure 5.6a***). ANOVA indicated a significant effect of citalopram [$F_{3,20}$= 4.4, $p<0.05$], time [$F_{6,120}$= 7.2, $p<0.0001$] and time x citalopram [$F_{18,120}$= 3.1, $p=0.0001$]. Extracellular 5-HT was maximally increased by 5 mg/kg citalopram, reaching 25 fmol/20 µL (300%), 40 min after citalopram. No further increase was observed with 20 mg/kg.

The infusion of citalopram through the probe significantly increased extracellular 5-HT in the DR (***Figure 5.6b***; [$F_{9,63}$= 21.1, $p<0.0001$]). Post-hoc comparisons indicated a significant effect of 1 µM (510% of basal value) and 10 µM citalopram (760% of basal value).

As shown in ***Figure 5.6c***, 5 and 20 mg/kg citalopram significantly increased extracellular GABA (390% of basal value, respectively 60 and 80 min after citalopram) whereas 1.25 mg/kg had no effect [citalopram: $F_{3,20}$= 13.3, $p<0.0001$; time: $F_{6,120}$= 5.1, $p=0.0001$; time x citalopram: $F_{18,120}$= 2.3, $p=0.004$].

The intra-raphé infusion of 10 µM citalopram significantly increased extracellular GABA [$F_{9,45}$= 8.1, $p<0.0001$]. Lower concentrations (0.1 and 1 µM) had no significant effect even if at 1 µM citalopram extracellular GABA reached about 300% of basal value.

Both systemic and local citalopram had no effect on extracellular GLU at any doses and concentration used (***Figure 5.6e***: [$F_{18,108}$= 1.2, p>0.05; ***Figure 5.6f***: $F_{9,54}$= 1.1, p>0.05]).

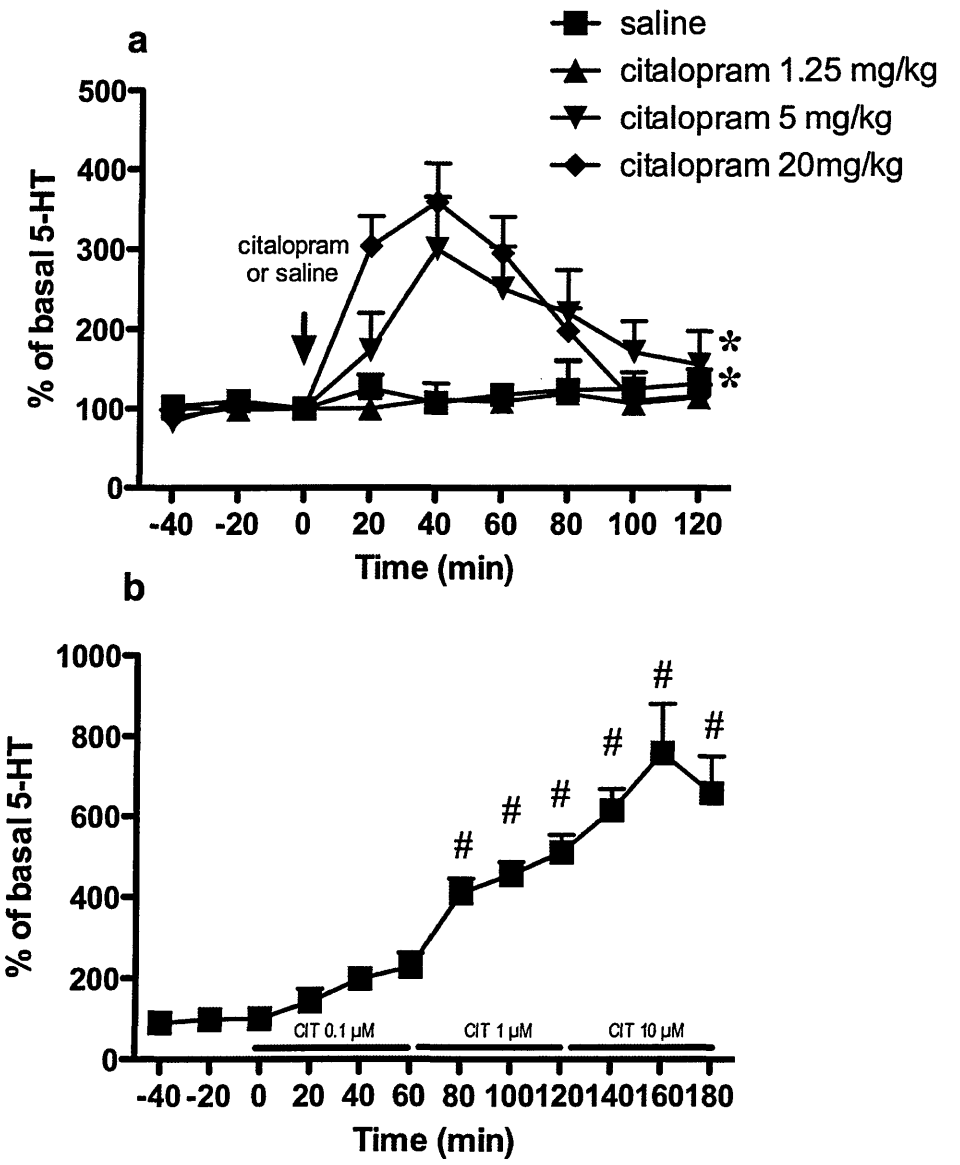


Figure 5.6a and ***b*** Effect of citalopram on extracellular 5-HT in the DR of DBA/2N mice.

Arrow indicates the injection of citalopram or saline. Horizontal bar indicates the citalopram infusion through the probe. Each concentration was infused for 1 h.
Results are Mean±SEM and are expressed as percentage of basal levels. Basal levels of 5-HT in fmol/20 µL were: saline 8.7±0.7 (n=5); citalopram 1.25 mg/kg: 4.7±0.8 (n=5); citalopram 5 mg/kg: 8.0±0.8 (n=8); citalopram 20 mg/kg: 5.4±0.6 (n=6) and local citalopram: 7.6±1.1 (n=8).
*$p<0.05$ citalopram 5 and 20 mg/kg vs. saline; # $p<0.05$ vs basal levels (Tukey-Kramer's test).

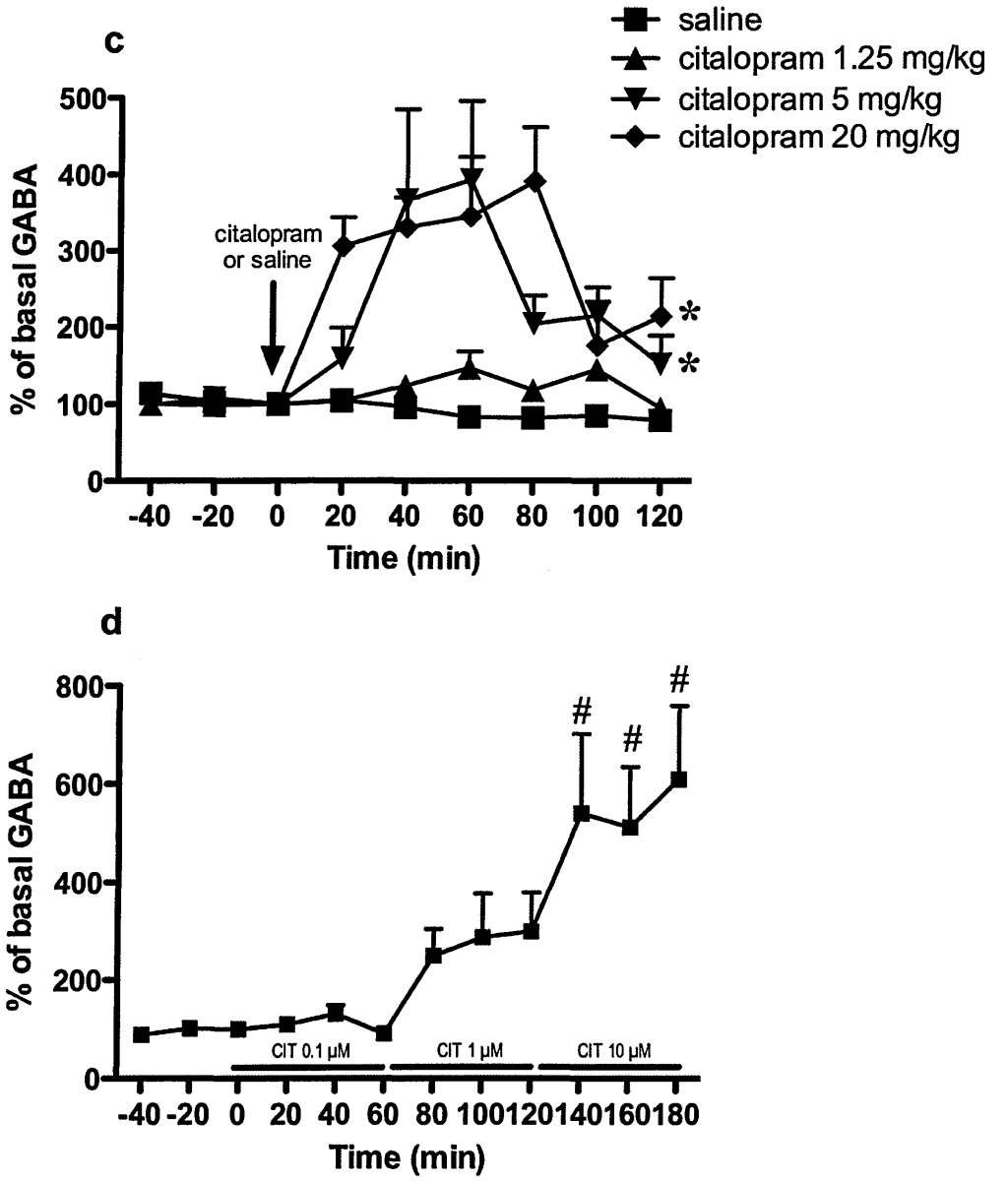


Figure 5.6c and ***d*** Effect of citalopram on extracellular GABA in the DR of DBA/2N mice.

Arrow indicates the injection of citalopram or saline. Horizontal bar indicates the citalopram infusion through the probe. Each concentration was infused for 1 h.
Basal levels of GABA in pmol/20 µL were: saline 0.40±0.15 (n=6); citalopram 1.25 mg/kg: 0.34±0.05 (n=5); citalopram 5 mg/kg: 0.26±0.04 (n=7); citalopram 20 mg/kg: 0.29±0.03 (n=6) and local citalopram: 0.30±0.07 (n=6).
*$p<0.05$ citalopram 5 and 20 mg/kg vs. saline; # $p<0.05$ vs basal levels (Tukey-Kramer's test).

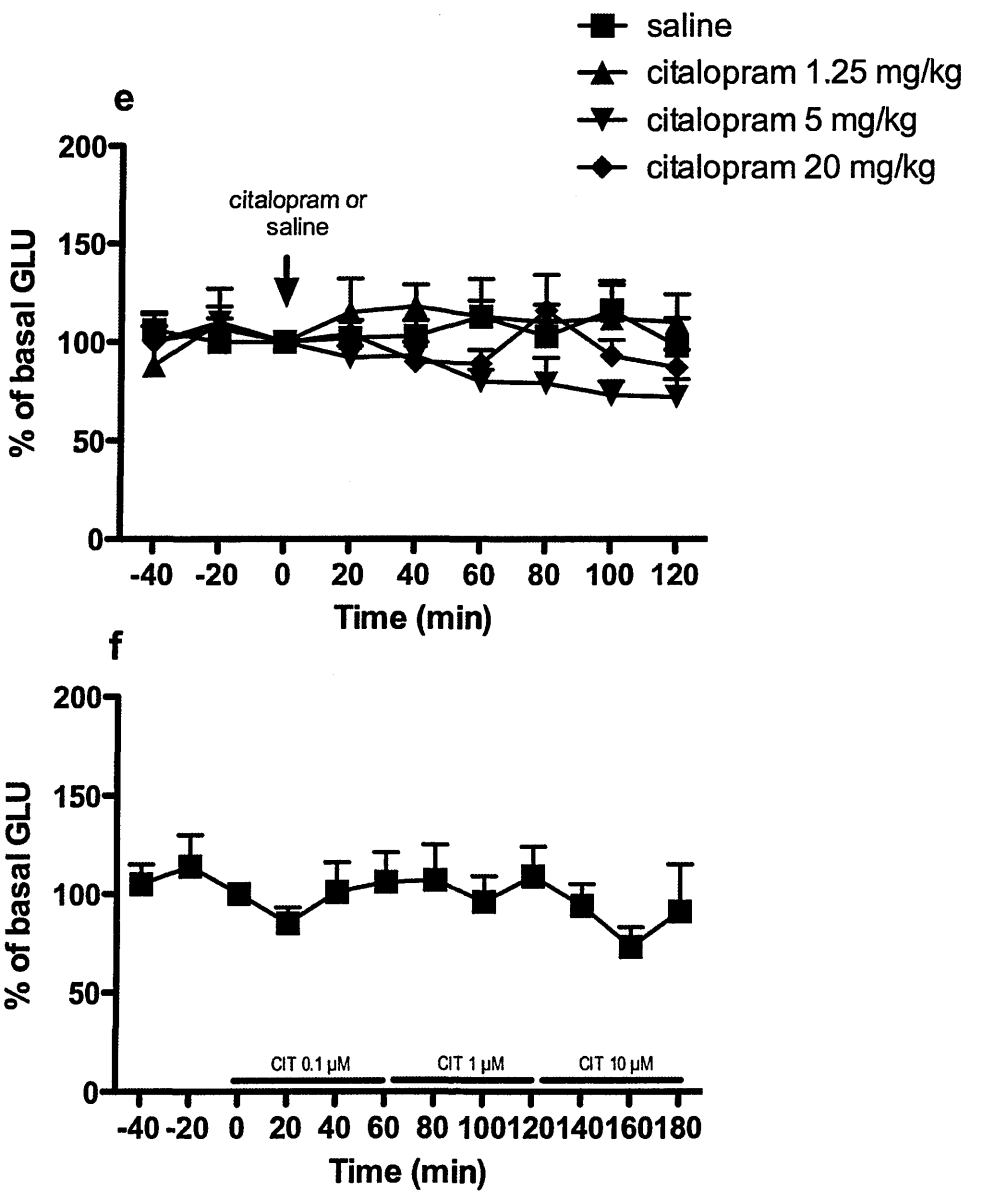


Figure 5.6e and ***f*** Effect of citalopram on extracellular GLU in the DR of DBA/2N mice.

Arrow indicates the injection of citalopram or saline. Horizontal bar indicates the citalopram infusion through the probe. Each concentration was infused for 1 h.
Basal levels of GLU in pmol/20 µL were: saline 19.2±2.4 (n=5); citalopram 1.25 mg/kg: 24.4±2.7 (n=5); citalopram 5 mg/kg: 27.0±3.4 (n=6); citalopram 20 mg/kg: 26.7±3 (n=6) and local citalopram: 19.9±4.4 (n=7).

5.3.5 Effect of 5-HT depletion on citalopram-induced rise of extracellular 5-HT and GABA in DBA/2N mice

As shown in ***Figure 5.3***, 300 mg/kg pCPA reduced extracellular levels of 5-HT in the DR of DBA/2N mice by about 83% without affecting basal level of GABA and GLU.

The pre-treatment with pCPA completely abolished the effect of 5 mg/kg citalopram on the raise of extracellular 5-HT (***Figure 5.7a***) and GABA (***Figure 5.7b***) (5-HT: citalopram, $F_{2,19}$= 4.3, p=0.03; time, $F_{6,114}$= 2.5, p=0.03; time x citalopram, $F_{12,114}$= 1.6, p>0.05; GABA: citalopram, $F_{2,15}$= 11.6, p<0.001; time, $F_{6,90}$= 2.0, p>0.05; time x citalopram, $F_{12,90}$= 2.4, p<0.001). Post-hoc comparisons indicate no differences both in extracellular 5-HT and GABA between animals treated with pCPA receiving saline or citalopram.

There were no significant differences in extracellular GLU between mice injected with citalopram, pre-treated or not with pCPA (***Figure 5.7c***: [citalopram: $F_{2,15}$= 2.9, p>0.05; time: $F_{6,90}$= 0.3, p>0.05; time x citalopram: $F_{12,90}$= 0.9, p>0.05]).

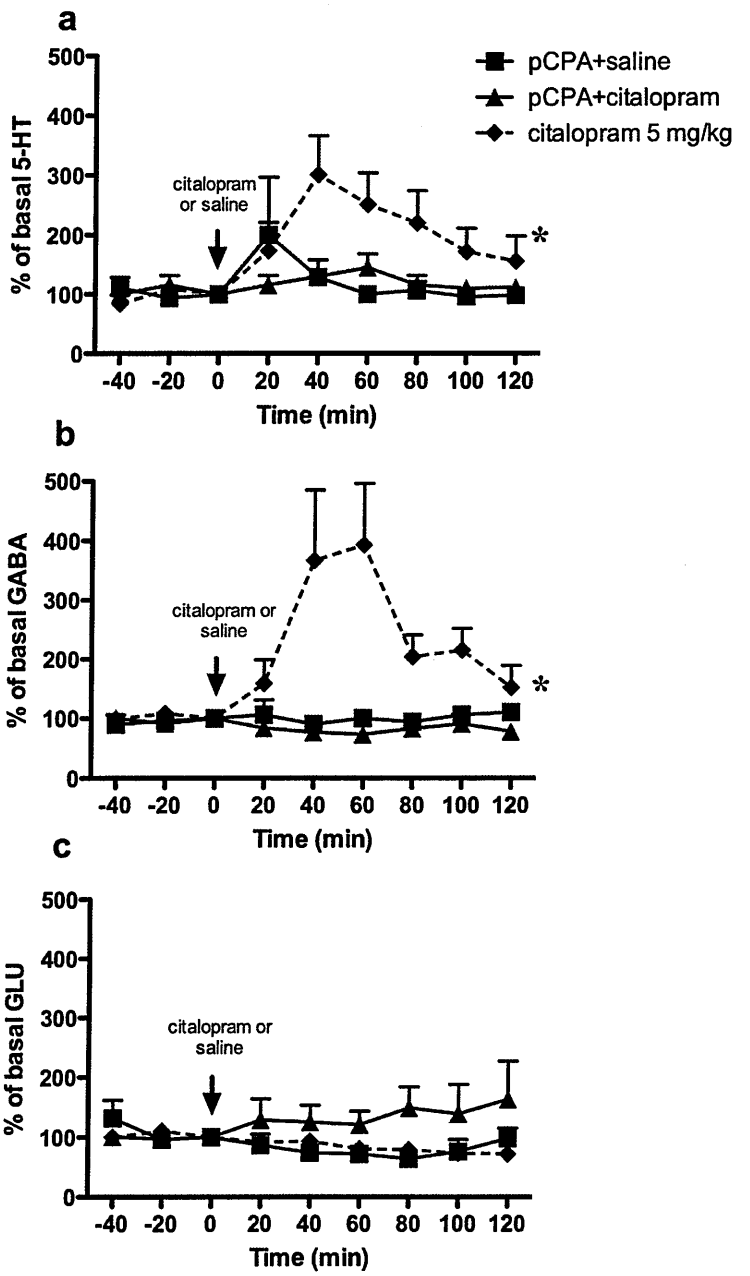


Figure 5.7 Effect of pCPA on extracellular 5-HT (***a***), GABA (***b***) and GLU (***c***) in the DR of DBA/2N mice treated with citalopram.
Arrows indicate the injection of citalopram or saline.
Results are Mean±SEM and are expressed as percentage of basal levels. Mice receiving citalopram alone are the same as in ***Figures 5.6a,c*** and ***e*** (dotted line).
Basal levels of 5-HT in fmol/20 µL were: pCPA+saline 1.2±0.1 (n=7) and pCPA+citalopram 5 mg/kg 1.1±0.0 (n=7).
Basal levels of GABA in pmol/20 µL were: pCPA+saline 0.25±0.02 (n=5) and pCPA+citalopram 5 mg/kg 0.36±0.04 (n=6).
Basal levels of GLU in pmol/20 µL were: pCPA+saline 21.6±4.3 (n=6) and pCPA+citalopram 5 mg/kg 24.0±4.4 (n=6).
*$p < 0.05$ vs. pCPA (Tukey-Kramer's test).

5.3.6 Effect of citalopram on extracellular 5-HT, GABA and GLU in C57BL/6N mice

As shown in ***Figure 5.8***, saline had no effect on extracellular 5-HT, GABA and GLU in the DR.

Citalopram dose-dependently increased extracellular 5-HT. Citalopram 1.25 mg/kg rose extracellular 5-HT to about 240% of basal values but post-hoc comparison shows no significant effect (***Figure 5.8a***). Five and 20 mg/kg citalopram significantly increased extracellular 5-HT [citalopram: $F_{3,22}$= 21.5, p<0.0001; time: $F_{6,132}$= 35.6, p<0.0001; time x citalopram: $F_{18,132}$= 14.2, p<0.0001] by about 500% and 1100% 60 min after the injection reaching about 126 and 150 fmol/20 µL respectively.

Figure 5.8b shows that 5 and 20 mg/kg citalopram significantly increased extracellular GABA, respectively by about 250%, 80 min after citalopram, and 290%, 40 min after citalopram [citalopram: $F_{3,19}$= 3.4, p=0.04; time: $F_{6,114}$= 6.5, p<0.0001; time x citalopram: $F_{18,114}$= 1.7, p=0.05] but post-hoc comparison shows no significant difference between the two groups. At the lower dose (1.25 mg/kg) citalopram had no effect on extracellular GABA.

Citalopram had no effect on extracellular GLU at any dose (***Figure 5.8c***: [citalopram: $F_{3,26}$= 1.3, p>0.05; time: $F_{6,156}$= 1.3, p>0.05; time x citalopram: $F_{18,156}$= 1.1, p>0.05]).

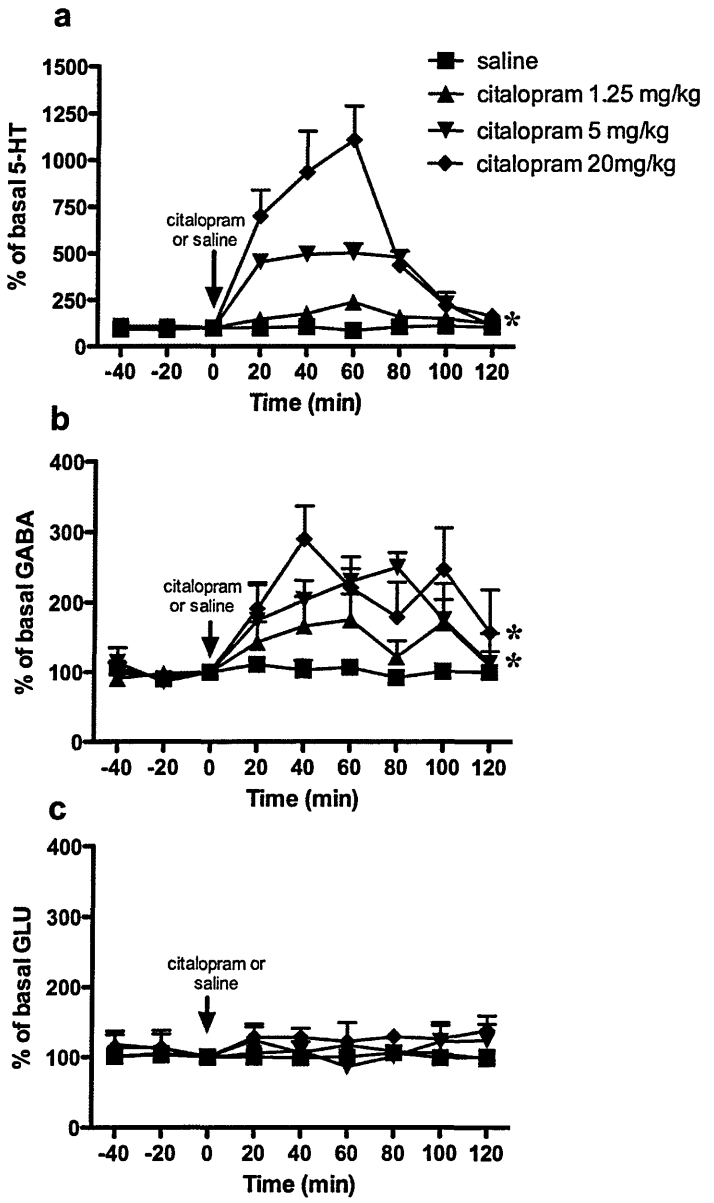


Figure 5.8 Effect of citalopram on extracellular 5-HT (***a***), GABA (***b***) and GLU (***c***) in the DR of C57BL/6N mice.
Arrows indicate the injection of citalopram or saline.
Basal levels of 5-HT in fmol/20 µL were: saline 18.3±2.7 (n=8); citalopram 1.25 mg/kg: 19.1±1.7 (n=6); citalopram 5 mg/kg: 26.7±4.8 (n=6) and citalopram 20 mg/kg: 14.7±1.5 (n=6).
Basal levels of GABA in pmol/20 µL were: saline 1.03±0.16 (n=6); citalopram 1.25 mg/kg: 0.77±0.09 (n=6); citalopram 5 mg/kg: 0.82±0.12 (n=5) and citalopram 20 mg/kg: 0.77±0.12 (n=6).
Basal levels of GLU in pmol/20 µL were: saline 21.7±2.0 (n=9); citalopram 1.25 mg/kg: 22.0±2.3 (n=6); citalopram 5 mg/kg: 26.6±4.3 (n=9) and citalopram 20 mg/kg: 20.6±2.6 (n=6).
*$p<0.05$ citalopram 5 and 20 mg/kg vs. saline (Tukey-Kramer's test).

5.3.7 Blockade of 5-HT_{2C} receptors prevents the ability of citalopram to increase extracellular GABA and enhances the citalopram-induced rise of extracellular 5-HT in DBA/2N mice.

Blockade of 5-HT_{2C} receptors with the selective antagonist SB242084 (1 mg/kg) enhanced the overall effect of citalopram on extracellular 5-HT in the DR of DBA/2N mice from 18.6±1.7 to 48.5±9.1 fmol/20 µL at peak (***Figure 5.9a***).

ANOVA indicated a significant effect of citalopram [$F_{1,21=}$ 10.1, $p<0.001$], the interaction between time and citalopram [$F_{7,147}$= 5.4, $p<0.0001$], between time and SB242084 [$F_{7,147}$= 2.1, $p<0.05$] and between time, citalopram and SB242084 [$F_{7,147}$= 2.3, $p<0.05$]. The effect of SB242084 [$F_{1,21}$= 3.3, $p=0.08$] and the interaction between SB242084 and citalopram [$F_{1,21}$= 3.8, $p=0.06$] were not significant.

SB242084 (1 mg/kg), injected 20 min before 5 mg/kg citalopram, completely abolished the citalopram-induced rise of extracellular GABA (***Figure 5.9b***).

ANOVA indicated a significant effect of citalopram [$F_{1,20}$= 9.8, $p<0.005$], SB242084 [$F_{1,20}$= 12.2, $p<0.005$], their interaction [$F_{1,20}$= 17.9, $p<0.001$], time [$F_{7,140}$= 3.2, $p<0.005$], and the interaction between time, SB242084 and citalopram [$F_{7,140}$= 3.8, $p<0.005$].

SB242084 by itself had no effect on extracellular 5-HT and GABA.

None of the treatments had any effect on extracellular GLU (data not shown).

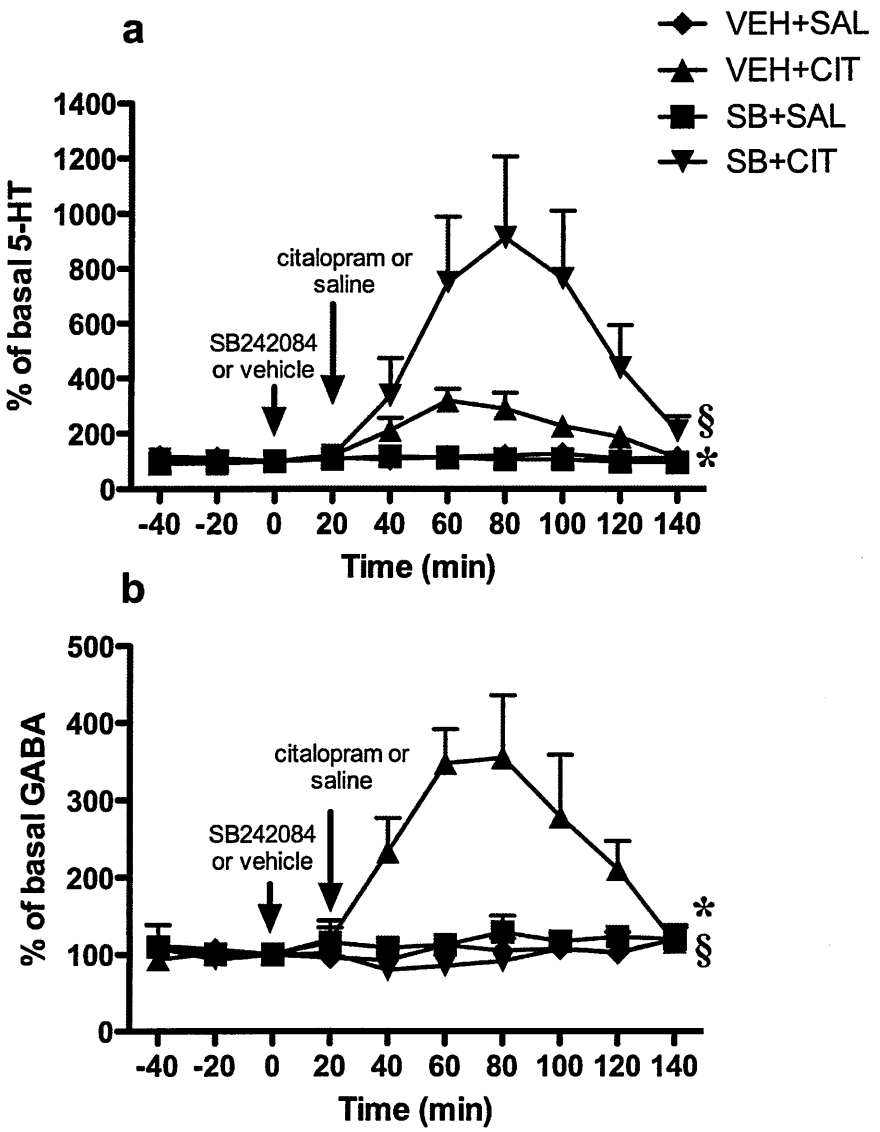


Figure 5.9 Effect of 1 mg/kg SB242084 on citalopram-induced rise of extracellular 5-HT (***a***) and GABA (***b***) in the DR of DBA/2N mice.
The first arrow indicates the injection of vehicle (VEH) or SB242084 (SB) and the second the injection of saline (SAL) or citalopram (CIT).
Results are Mean±SEM and are expressed as percentage of basal levels. Basal levels of 5-HT in fmol/20 µL were: VEH+CIT, 6.0±0.6 (n=7); SB+SAL, 5.4±0.6 (n=6); SB+CIT, 6.8±1.0 (n=7); VEH+SAL, 7.0±0.9 (n=5).
Basal levels of GABA in pmol/20 µL were: VEH+CIT, 0.21±0.02 (n=7); SB+SAL, 0.20±0.03 (n=5); SB+CIT, 0.30±0.06 (n=8); VEH+SAL, 0.36±0.02 (n=4).
*$p<0.05$ VEH+CIT vs. VEH+SAL; §$p<0.05$ SB+CIT vs. VEH+CIT (Tukey-Kramer's test).

5.3.8 Blockade of 5-HT_{2C} receptors in the DR abolishes the effect of citalopram on extracellular GABA and enhances the citalopram-induced increase of extracellular 5-HT in the DR and mPFC.

To examine whether 5-HT_{2C} receptors in the DR were responsible for the effect of SB242084 on citalopram-induced increase of extracellular 5-HT, dual probe experiments were conducted. Two probes were implanted, one in the DR and the other in the mPFC at the coordinates indicated in *section 2.2.2*, and SB242084 was perfused through the probe in the DR, 20 min before systemic administration of citalopram.

The effects of perfusion of SB242084 (0.1 μM) in the DR on citalopram-induced increase of 5-HT in the DR and in the mPFC are shown respectively in ***Figures 5.10a*** and ***b***.

Five mg/kg citalopram increased extracellular 5-HT in the DR by about 340% of basal values. At 0.1 μM SB242084 significantly enhanced the citalopram-induced rise of extracellular 5-HT from 24.8±2.2 to 34.7±3.1 fmol/20 μL at peak (***Figure 5.10a***).

ANOVA indicated a significant effect of citalopram [$F_{1,20}$= 24, $p<0.0001$], time [$F_{7,140}$= 8.3, $p<0.0001$], the interaction between time and citalopram [$F_{7,140}$= 7.2, $p<0.0001$] and the interaction between time, SB242084 and citalopram [$F_{7,140}$= 2.4, $p=0.02$]. The effect of SB242084 [$F_{1,20}$= 3.7, $p=0.07$] and the interaction between SB242084 and citalopram [$F_{7,140}$= 4.3, $p=0.052$] were not significant.

As shown in ***Figure 5.10b***, 5 mg/kg citalopram increased extracellular 5-HT in the mPFC of DBA/2N mice, reaching 6.9±0.4 fmol/20 μL at 60 min (225% of basal value). SB242084, infused into the DR, potentiated the overall effect of citalopram on extracellular 5-HT in the mPFC peaking at

9.7±1.2 fmol/20 µL (530% of basal value), confirming previous results (see *Chapter 4*). ANOVA indicated a significant interaction between SB242084, citalopram and time [$F_{7,105}$= 3.5, p=0.002], a significant effect of citalopram [$F_{1,15}$= 8.2, p=0.01], time [$F_{7,105}$= 9.7, p<0.0001], the interaction between citalopram and SB242084 [$F_{1,15}$= 6.2, p=0.03], and the interaction between citalopram and time [$F_{7,105}$= 9.0, p<0.0001] and between SB242084 and time [$F_{7,105}$= 3.2, p=0.004].

SB242084 and vehicle by itself had no effect on extracellular 5-HT in the DR and in the mPFC.

SB242084 had no significant effects by itself on extracellular GABA in the DR but completely prevented the raise of GABA induced by citalopram (***Figure 5.10c***). ANOVA indicated a significant effect of citalopram [$F_{1,18}$= 10.1, p=0.005], SB242084 [$F_{1,18}$= 4.8, p=0.04], time [$F_{7,126}$= 4.4, p=0.0002] and the interaction between time, SB242084 and citalopram [$F_{7,126}$= 2.8, p=0.009] but no significant interaction between SB242084 and citalopram [$F_{1,18}$= 3.6, p=0.07].

In addition, the interaction between time and citalopram was significant [$F_{7,126}$= 4.6, p=0.0001] as well as between time and SB242084 [$F_{7,126}$= 2.6, p=0.02].

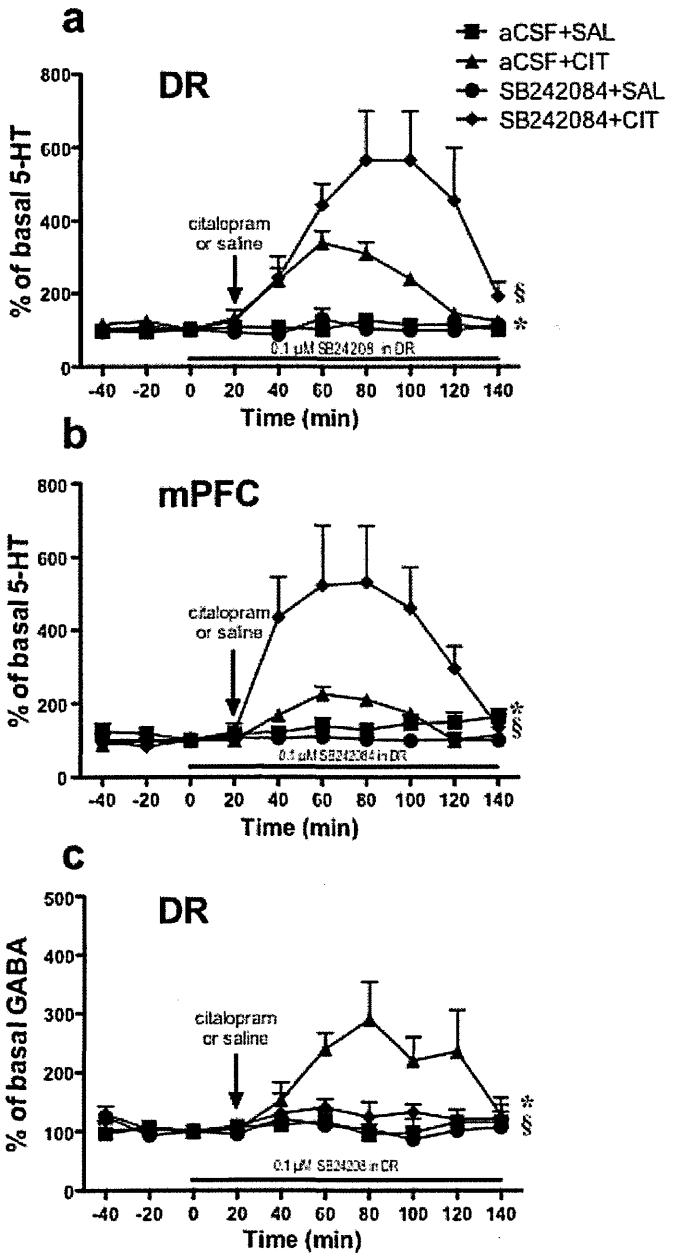


Figure 5.10 Effect of 0.1 µM SB242084 on citalopram-induced rise of extracellular 5-HT in the DR (***a***) and mPFC (***b***) and of extracellular GABA (***c***) of DBA/2N mice.

Horizontal bar indicates the duration of SB242084 (SB) or aCSF infusion through the probe. The arrow indicates the injection of saline (SAL) or citalopram (CIT).
Results are Mean±SEM and are expressed as percentage of basal levels. Basal levels of 5-HT in fmol/20 µL in the DR were: aCSF+CIT, 7.5±0.8 (n=5); SB+SAL, 6.9±0.8 (n=7); SB+CIT, 7.6±1.3 (n=7); aCSF+SAL, 7.7±1.1 (n=5).
Basal levels of 5-HT in fmol/20 µL in the mPFC were: aCSF+CIT, 3.1±0.1 (n=5); SB+SAL, 3.3±0.6 (n=4); SB+CIT, 2.4±0.5 (n=5); aCSF+SAL, 2.8±0.3 (n=5).
Basal levels of GABA in pmol/20 µL were: aCSF+CIT, 0.27±0.03 (n=5); SB+SAL, 0.33±0.07 (n=5); SB+CIT, 0.26±0.05 (n=7); aCSF+SAL, 0.33±0.02 (n=5).
*$p<0.05$ aCSF+CIT vs. aCSF+SAL; §$p<0.05$ SB+CIT vs. aCSF+CIT (Tukey-Kramer test).

5.3.9 Blockade of 5-HT$_{2C}$ receptors has no effect on the ability of citalopram to increase extracellular GABA and on the citalopram-induced rise of extracellular 5-HT in C57BL/6N mice.

Blockade of 5-HT$_{2C}$ receptors with 1 mg/kg SB242084 had no significant effect by itself on extracellular 5-HT in the DR of C57BL/6N mice and did not boost the citalopram-induced rise of extracellular 5-HT (***Figure 5.11a***). ANOVA indicated a significant effect of citalopram [$F_{1,19}$= 93.6, $p<0.0001$] but not of SB242084 [$F_{1,19}$= 0.2, $p=0.7$], SB242084 x citalopram [$F_{1,19}$= 1.8, $p=0.2$] or the interaction between SB242084, citalopram and time [$F_{7,133}$= 1.7, $p=0.1$].

Citalopram (5 mg/kg) raised extracellular GABA in the DR by about 215% at peak and tended to enhance this effect but it was not significant (***Figure 5.11b***). ANOVA indicated a significant effect of citalopram [$F_{1,21}$= 25.9, $p<0.0001$] but not of SB242084 [$F_{1,21}$= 0.5, $p=0.5$] and SB242084 x citalopram [$F_{1,21}$= 1.0, $p=0.3$] whereas the interaction between these factors and time [$F_{7,147}$= 3.3, $p=0.003$] was significant.

SB242084 by itself had no effect on extracellular GABA.

None of the treatment affected extracellular GLU (data not shown).

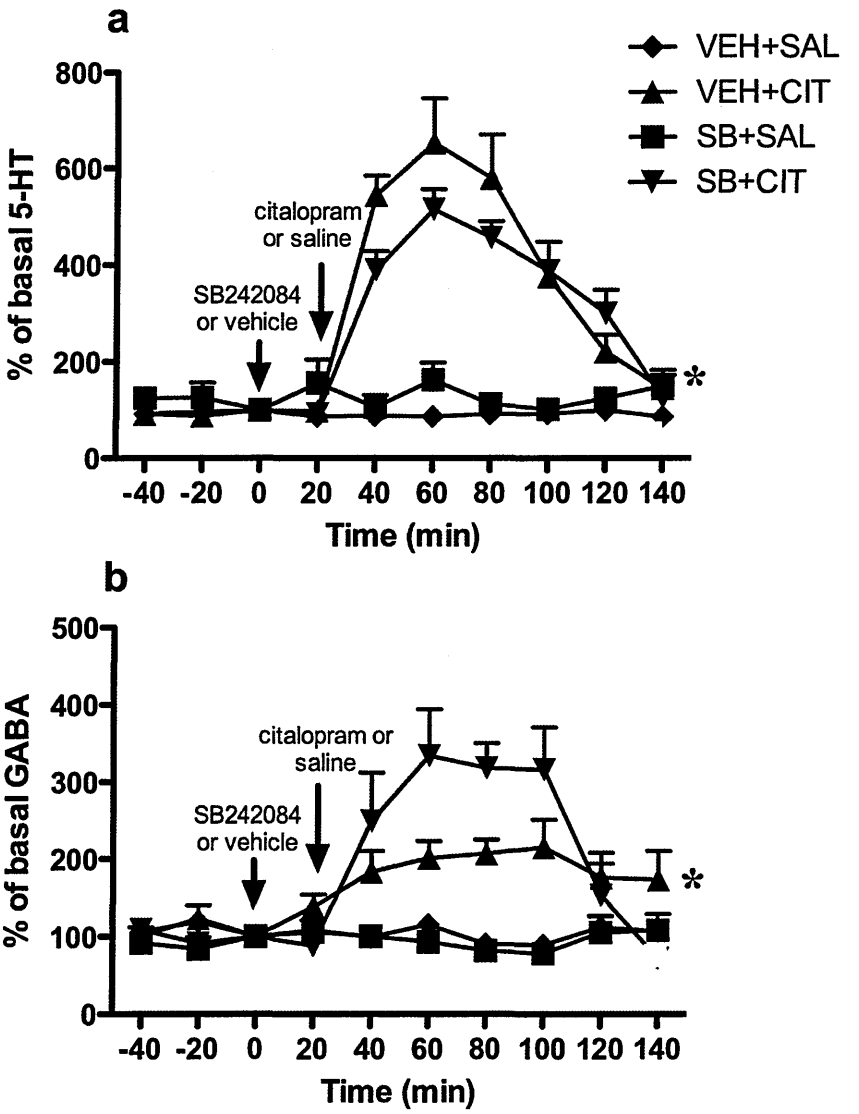


Figure 5.11 Effect of 1 mg/kg SB242084 on citalopram-induced rise of extracellular 5-HT (***a***) and GABA (***b***) in the DR of C57BL/6N mice.

The first arrow indicates the injection of vehicle (VEH) or SB242084 (SB) and the second the injection of saline (SAL) or citalopram (CIT).
Results are Mean±SEM and are expressed as percentage of basal levels. Basal levels of 5-HT in fmol/20 µL were: VEH+CIT, 24.1±3.6 (n=7); SB+SAL, 13.5±3.0 (n=4); SB+CIT, 18.4±1.6 (n=7); VEH+SAL, 14.3±0.7 (n=5).
Basal levels of GABA in pmol/20 µL were: VEH+CIT, 0.87±0.08 (n=9); SB+SAL, 1.01±0.22 (n=5); SB+CIT, 0.74±0.14 (n=6); VEH+SAL, 0.94±0.08 (n=5).
*$p<0.05$ VEH+CIT vs. VEH+SAL (Tukey-Kramer's test).

5.4 Discussion

Two main findings derive from this set of experiments. First, citalopram increases extracellular GABA in the dorsal raphé (DR) of DBA/2N mice and second this increase depends on the rise of extracellular 5-HT and the activation of 5-HT_{2C} receptors located in the DR.

Basal extracellular 5-HT in the DR of DBA/2N was lower than in C57BL/6N mice. This is in line with the results obtained in the mPFC and the DH (see *Chapter 3*), further supporting a general impairment of 5-HT transmission in this strain.

GABA and glutamate are respectively the main inhibitory and excitatory neurotransmitters in the central nervous system. Both neurotransmitters play an important role in the physiology of the brain and are strongly implicated in depression.

We found a reduced extracellular GABA and, albeit slightly, GLU in DBA/2N mice compared to C57BL/6N mice.

Given the reciprocal modulation of serotonergic, GABAergic and glutamatergic systems (Bagdy et al., 2000; Martín-Ruiz et al., 2001; Tao and Auerbach, 2002; Tao and Auerbach, 2003), it is unclear whether low basal 5-HT levels lead to reduced GABAergic and glutamatergic transmission. In fact, it should be noted that, in turn, also serotonergic neurons are directly under the control of GABAergic nerves and are indirectly controlled by glutamatergic projections.

Neuronal origins of 5-HT, GABA and GLU

Neuronal release in the CSF is expected to be sensitive to potassium (K^+) depolarization, sodium channel blockage induced by tetrodotoxin (TTX), removal of calcium (Ca^{2+}) and depletion of presynaptic vesicles by local administration of the selective neurotoxin. For the monoamines it was found that under a wide variety of experimental conditions, dialysate contents of these neurotransmitters arise predominately from exocytotic processes.

A preliminary part of the study was aimed at evaluating the neuronal dependence of extracellular level of 5-HT, GABA and GLU measured by microdialysis under our conditions. Two criteria were applied: K^+ depolarization and Na^+ channel blockade induced by TTX.

It is controversial whether the 5-HT output in the DR can be used as an index of neuronal activity. In the forebrain, dialysate 5-HT is representative of the impulse-dependent axonal release of 5-HT (Carboni and Di Chiara, 1989). In the DR, 5-HT may arise from dendrites but also from the initial segments of the branching axons, since the DR contains a dense network of 5-HT efferent fibers (Steinbusch, 1981). The spontaneous release of 5-HT in the raphé area was shown to be Ca^{2+} dependent (Hery et al., 1982) and was also enhanced after K^+ stimulation, by the Na^+-channel activator veratridine (Adell et al., 2002) and markedly reduced after TTX *in vivo* but not *in vitro* (Hery et al., 1982; Celada et al., 2002). Matos et al. (1996) found that 5-HT release was TTX-independent when the probes were implanted in close apposition but outside the DR. However, when the microdialysis probes were implanted within the DR, this nucleus remained entirely functional and the release of 5-HT could be reliably measured.

In the present study, the dependence of 5-HT release in the DR on TTX and high K^+ has been further confirmed. In any case, a fraction of 5-HT release in the raphé nuclei is resistant to the action of TTX (*present study*; Adell et al., 2005; Bosker et al., 1994; Matos et al., 1996) suggesting the existence of a limited pool of the transmitter in the raphé nuclei that is not released in an impulse-dependent manner (*present study*; Adell et al., 2002).

The neuronal origin of GABA in microdialysis samples is debated as infusion of TTX or Ca^{2+}-free solutions on GABA levels in dialysate produced inconsistent results. Moreover Rea et al. (2005) have suggested that the chromatographic separation of GABA may contribute to discrepancies in the literature. In this work the proper analytical conditions and a validated method were used (see ***Figure 5.2***). The reduction of extracellular GABA observed in DBA/2N and C57BL/6N mice after 1 h TTX is similar to those previously reported in rats (Osborne et al., 1990; Drew and Ungerstedt, 1991; Campbell et al., 1993; Rakovska et al., 1998; Herrera-Marschitz et al., 1996; de Groote and Linthorst, 2007; van der Zeyden et al., 2008). The data confirmed that GABA levels decreased very slowly, reaching only statistical significance after 30 min or more (van der Zeyden et al., 2008). Moreover high K^+ induces an increase of extracellular GABA variable between different studies (de Groote and Linthorst, 2007; Rea et al., 2005).

Most authors agree that basal values of GLU in microdialysis samples do not respond to TTX infusion or omission of Ca^{2+} (*for review see* van der Zeyden et al., 2008) and the presence of a non-exocytotic extracellular pool

of GLU is well established (Herrera-Marschitz et al., 1996; Timmerman and Westerink, 1997). Several authors have suggested that this GLU pool might be derived from astroglial cells by non-vesicular release that is insensitive to TTX and to removal of calcium ions, glial release via the cystine–GLU exchanger that is sodium-dependent or via GAP-junction hemichannels in which Ca^{2+} can facilitate GLU release from adjacent glial cells (*for review see* van der Zeyden et al., 2008).

Infusion of excitatory agents as the K^+-channel blocker, 4-aminopyridine, that prolongs the opening of the K^+ channels, or high concentration of KCl induce an increase in GLU levels in dialysates, which was largely TTX dependent (Peña and Tapia, 2000; van der Zeyden et al., 2008). Therefore synaptic GLU release can be detected by microdialysis following stimulation GLU pathways.

On the contrary, few researchers have reported a decrease in extracellular GLU after pharmacological treatments (Baker et al., 2002; Xi et al., 2002; Xi et al., 2003).

In our experimental condition, K^+ depolarization induced a twofold increase in GABA and GLU. The small increase in GLU induced by K^+ depolarization is, perhaps, due to the fact that the high concentration of KCl in the extracellular space activates the transport into the surrounding tissue (Schousboe et al., 1993; Herrera-Marschitz et al., 1996; van der Zeyden et al., 2008).

From these results, it is interpreted that a large proportion of basal dialysate concentrations of 5-HT and GABA in the DR of DBA/2N and

C57BL/6N mice, but not those of GLU, originate from neuronal terminals and reflect neuronal activity.

Effect of citalopram

Animal and clinical studies suggested, although with conflicting findings, that a deficit in GABAergic activity might be involved in the pathophysiology of mood disorders and in the mechanism of action of antidepressant treatments.

We found that citalopram, 1.25, 5 and 20 mg/kg, dose-dependently raised extracellular 5-HT in the DR of C57BL/6N mice and had significantly less effect in the DR of DBA/2N mice. This confirms the previous results obtained in the mPFC and the DH (*Chapter 3*).

As previously observed in rat and mouse, the increase of extracellular 5-HT induced by SSRIs in the DR is higher than in the forebrain (*Chapter 3*; Gardier et al., 1996; Tao et al., 2000; Invernizzi et al., 1992; Guiard et al., 2004; Guilloux et al., 2006). Moreover, citalopram infusion through the probe (at 1 and 10 µM) had larger effects than after systemic administration, presumably due to a limited activation of autoreceptor (Tao et al., 2000; Piñeyro and Blier, 1999).

Citalopram, with a good matching of doses with the increase of extracellular 5-HT, also raised extracellular GABA in the DR of C57BL/6N and DBA/2N mice.

The raise of GABAergic neurotransmission after SSRIs has been already described in clinical and animal studies. Treatment of depressed patients with SSRIs increases cortical GABA levels as measured by proton magnetic resonance spectroscopy (Sanacora et al., 2002). In healthy subjects, a

single intravenous administration of citalopram (Bhagwagar et al., 2004) increased GABA levels in occipital cortex. This finding suggests that an acute increase in 5-HT neurotransmission can increase cortical GABA activity in humans as it does in animals (Taylor et al., 2003; Gören et al., 2007).

Role of endogenous 5-HT

Interestingly, inhibition of 5-HT synthesis with doses of pCPA, depleting extracellular 5-HT by about 80% in the DR, completely prevented the citalopram-induced rise of extracellular 5-HT and GABA in the DR of DBA/2N mice without affecting basal release of GABA.

This finding is supported by several studies. Serotonergic lesion does not change the spontaneous GABA release in the basolateral amygdala (Lehner et al., 2008). Moreover, depletion of 5-HT with pCPA does not reduce GABA content in raphé nuclei slice (Bagdy et al., 2000) and does not affect GABA levels in rat striatum, however after K^+ extracellular GABA was higher in 5-HT-depleted rats (Di Cara et al., 2003).

Also in human studies it was suggested that lowering brain 5-HT function using tryptophan depletion does not diminish occipital GABA levels in healthy subjects (Selvaraj et al., 2006).

The present study clearly shows a prominent interaction between the serotonergic and the GABAergic system suggesting that in the presence of an SSRI, the extent of 5-HT release is governed by a GABA-mediated feedback control. However, this effect was not apparent under pre-SSRI administration conditions. In fact, basal levels of GABA are not affected by the depletion of 5-HT.

Feedback regulation is an essential aspect of the physiology of central 5-HT neurones (Aghajanian et al., 1978) and earlier *in vivo* electrophysiological experiments indicate that ascending serotonergic pathway causes inhibition of 5-HT cells firing in the DR (Wang and Aghajanian, 1977a).

However feedback control of 5-HT cells is not limited to 5-HT_1 autoreceptors but includes also 5-HT receptors located on postsynaptic targets. Evidence suggests that these postsynaptic feedback mechanisms involve multiple 5-HT receptor subtypes as the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_4 receptors and operate through neural pathways that input to 5-HT neurones (Sharp et al., 2007).

There is morphological and neurochemical evidence for the GABAergic-serotonergic interaction in the raphé nuclei (Harandi et al., 1987; Becquet et al., 1990; Tao et al., 1996).

As indicated by neurochemical and electrophysiological studies, serotonergic neurones are subject to GABAergic inhibitory regulation (Gervasoni et al., 2000; Tao and Auerbach, 2003). In fact, local application of GABA agonists into raphé nuclei inhibits 5-HT cell firing (Gallager and Aghajanian, 1976). Furthermore, release of 5-HT in the DR activates local GABA release that in turns inhibits 5-HT release, indicating the existence of a reciprocal innervation between the two major neuronal cell types in the DR. This effect is mediated through 5-HT released from dendrites and axon varicosities within the raphé nuclei leading to activation of inhibitory somatodendritic 5-HT autoreceptors and through GABA heteroreceptors

(GABA-A and GABA-B) located on serotonergic neurons (Tao et al., 1996; Bagdy et al., 2000).

Role of 5-HT_{2C} receptors in DBA/2N mice

The blockade of 5-HT_{2C} receptors augmented the citalopram-induced increase of extracellular 5-HT in the mPFC (*Chapter 4*; Calcagno et al., 2009a).

Several possible mechanisms could underlie the augmentation of SSRI-induced rise of extracellular 5-HT by 5-HT_{2C} receptor inactivation.

Although an autoreceptor function has not been demonstrated for 5-HT_{2C} receptors, it is notable that these receptors are expressed near the mesencephalic raphé serotonergic neurons (Clemett et al., 2000) and recently, 5-HT_{2C} receptor mRNA was found in GABA neurones in DR (Serrats et al., 2005).

This evidence of a role for 5-HT_{2C} receptors in 5-HT neuron control accords with observations that the non selective 5-HT_2 receptor agonists, DOM, DOB and DOI, inhibited 5-HT cell firing and their effect was attenuated by pre-treatment with the 5-$HT_{2B/C}$ receptor antagonist SB 206553 *in vivo* and in raphé slice preparation (Aghajanian et al., 1970; Garratt et al., 1991; Wright et al., 1990; Boothman et al., 2003; Liu et al., 2000). Consistently, a combination of electrophysiological and *c-fos* immunohistochemical studies show that 5-HT_2 receptor agonists inhibit 5-HT neurones and activate GABA neurones in the DR (Liu et al., 2000; Boothman et al., 2003; Boothman and Sharp, 2005; Boothman et al., 2006b; Singewald and Sharp, 2000). In particular, administration of the GABA-A receptor antagonist picrotoxin restored the inhibition of 5-HT cell firing induced by the 5-HT_{2C}

agonist, WAY161503, suggesting that the activation of GABA neurones may be involved in the inhibitory action of WAY161503 (Boothman et al., 2006b).

Taking together the above findings, it is plausible that the selective 5-HT_{2C} receptor agonist, Ro 60-0175 (Martin et al., 1998), inhibits extracellular 5-HT in the DR of DBA/2N mice by activating GABA release. Furthermore, the selective 5-HT_{2C} antagonist, SB242084, completely reversed the reduction of extracellular 5-HT induced by Ro 60-0175, abolishing the increase of extracellular GABA.

The dose of Ro 60-0175 chosen was those reducing extracellular 5-HT in the DR. Ro 60-0175 at lower dose had not effect on extracellular 5-HT confirming the results obtained in the rat mPFC (Calcagno et al., 2009b). The dose of SB242084 used was that enhancing the effect of citalopram on extracellular 5-HT and restoring its effect in the FST (*Chapter 4*).

Interestingly, DR 5-HT neurones were inhibited by WAY161503 (Boothman et al., 2006b) and Ro 60-0175 (*present study*) as well as the selective 5-HT_{1A} agonist, 8-OH-DPAT. This finding shows that 5-HT neurones are sensitive to feedback control by both 5-HT_{2C} and 5-HT_{1A} receptors (Hajós et al., 1999). Therefore they are subjected to 5-HT feedback control at several levels.

Our data show that the blockade of 5-HT_{2C} receptors enhances the citalopram-induced raise of extracellular 5-HT abolishing the increase of extracellular GABA in the DR of DBA/2N mice. In fact, the co-administration of SB242084 with citalopram prevents the inhibitory effect of GABA

neurotransmission on 5-HT release, allowing a further increase of extracellular 5-HT. Arguably, a diminished GABAergic tone due to the 5-HT_{2C} receptor blockade could reduce the amount of 5-HT-mediated excitation of raphé GABA neurons and may contribute to the increased effect of SSRIs.

The same effect was achieved also with local infusion of SB242084 in the DR suggesting a prominent role of DR 5-HT_{2C} receptors. It is very interesting that SB242084, perfused in the DR, potentiated the citalopram-induced raise of extracellular 5-HT in the DR and in the mPFC. This latter increase was similar to those obtained with the combination of systemic SB242084 and citalopram on extracellular 5-HT in the mPFC, corresponding to a reinstated effect on immobility time in the FST in DBA/2N mice (*Chapter 4*).

However, 5-HT_{2C} receptors are abundant in other brain regions and the present data do not exclude the additional possibility that 5-HT_{2C} receptors also play a role in the modulation of DR afferents from more distant regions such as the lateral habenula, hippocampus or PFC (Wright et al., 1995; Cremers et al., 2007; Sharp et al., 2007). A combination of electrophysiological and neuroanatomical data suggests that the DR GLU projections, respectively from the mPFC and the lateral habenula (Varga et al., 2001; Hajós et al., 1998; Jankowski and Sesack, 2004), target DR GABA neurones to inhibit 5-HT cell firing, as well as a direct inhibitory habenula-raphé GABA projection (Ferraro et al., 1996; Varga et al., 2003).

Moreover, the 5-HT_{2C} receptors are not only located as heteroceptors on GABA-ergic neurons but may also be located on glutamatergic and dopaminergic neurons (*see reviews* Di Giovanni et al., 2006; Alex and

Pehek, 2007; Fink and Göthert, 2007). In addition they are also involved in the control of noradrenergic neurons through GABAergic interneurones. Theoretically, all these systems might be involved in the mechanism through which 5-HT_{2C} antagonists augment the effects of SSRIs on 5-HT levels in the brain.

Despite the robust neurochemical effect on extracellular 5-HT when pharmacological and genetic inactivation of 5-HT_{2C} receptors is combined with SSRIs (*Chapter 4*; Cremers et al., 2004; Boothman et al., 2006a), 5-HT_{2C} receptor antagonism alone has no significant effects on extracellular 5-HT and GABA. This suggests that 5-HT_{2C} feedback system does not appear to be "tonically active" and that these receptors may contribute to a negative feedback mechanism recruited only under conditions of elevated serotonergic tone.

Role of 5-HT_{2C} receptors in C57BL/6N mice

Interestingly, SB242084 had no effect on citalopram-induced increase of extracellular 5-HT and GABA in the DR of C57BL/6N mice. A possible explanation is that the 5-HT_{2C} receptors have reduced constitutive and agonist-stimulated activity in C57BL/6 mice (Englander et al., 2005) compared to BALB/c (Englander et al., 2005) and DBA/2J mice (Hackler et al., 2006), in which the majority of 5-HT_{2C} mRNA is non-edited and encodes receptors with the highest constitutive activity and the highest agonist affinity and potency. The 5-HT_{2C} pre-mRNA editing is likely to be an adaptive response to the low-baseline forebrain 5-HT levels in BALB/c and DBA/2J mice. In fact, acute stress (as the FST) and chronic treatment with

the SSRI, fluoxetine, did not induce significant changes in 5-HT_{2C} pre-mRNA editing in C57BL/6 mice. In contrast, exposure of BALB/c mice to acute stress and chronic treatment with fluoxetine elicit an increase in 5-HT_{2C} pre-mRNA editing leading to receptors with reduced function (Englander et al., 2005). These changes in 5-HT_{2C} pre-mRNA editing resemble those detected previously in the PFC of depressed patients (Gurevich et al., 2002b). In addition, depletion of 5-HT increased the expression of 5-HT_{2C} mRNA isoforms encoding receptors with higher sensitivity to 5-HT (Gurevich et al., 2002a), confirming that editing is regulated by endogenous 5-HT.

Therefore the failure of SB242084 to enhance the citalopram-induced rise of extracellular 5-HT in the DR of C57BL/6N mice might be due to a reduced constitutive activity of 5-HT_{2C} receptors.

Role of Glutamate

Glutamatergic axon terminals make synaptic connection with GABAergic neurons in the raphé nuclei (Jankowski and Sesack, 2004) where GLU can indirectly inhibit 5-HT release (Celada et al., 2001; Varga et al., 2001; Martín-Ruiz et al., 2001).

SSRIs, increasing extracellular 5-HT in forebrain regions, might activate both 5-HT_{2A} and 5-HT_{2C} receptors on glutamatergic neurons. The application of SB242084 would block 5-HT_{2C} receptor-mediated regulation of GLU release, leaving the response to be mediated by 5-HT_{2A} receptors, which have been shown to be located on glutamatergic neurons and therefore increase extracellular GLU (Martín-Ruiz et al., 2001). Furthermore, GABA interneurons in the raphé nuclei are the primary target for the cortico-raphé glutamatergic neurons and their stimulation by GLU leads to inhibition of

serotonergic cells (Hajós et al., 1998). Therefore this pathway may play a role in long-loop feedback inhibition of serotonergic neurons.

However, the glutamatergic AMPA/kainate antagonist, DNQX, did not attenuate the augmentation observed with SB242084 on citalopram-induced raise of extracellular 5-HT (Cremers et al., 2007).

In addition, evaluation of the effects of citalopram with and without SB242084 on GLU levels did not show any effects (*present study*).

In summary, citalopram raised extracellular GABA in the DR and, in DBA/2N mice, this increase depends on the rise of extracellular 5-HT and on the activation of 5-HT_{2C} receptors.

Thus, current data support a model of 5-HT-feedback control in which 5-HT_{2C} receptors activate DR GABA neurones to inhibit 5-HT neurotransmission.

Therefore the blockade of the 5-HT_{2C} receptors and of the GABAergic activity in the DR might be a useful strategy to reinstate the antidepressant effects of SSRIs in treatment resistant depressed patients.

Chapter 6

EFFECT OF CHRONIC CITALOPRAM IN THE FST AND ON BDNF LEVELS

6.1 Introduction

Although SSRIs produce a rapid blockade of the 5-HT transporter increasing brain extracellular 5-HT in animals acutely following administration (Fuller, 1994; Stahl, 1998), the onset of their therapeutic effect takes several weeks. This paradox has not been solved yet.

It was suggested that the sustained elevated 5-HT levels following chronic treatment with SSRIs induces adaptive changes, such as the gradual loss of responsiveness of 5-HT autoreceptors (*for review see* Blier, 2003). The duration of chronic treatment with antidepressants needed for the desensitisation of 5-HT_{1A} autoreceptors was similar to the latency for the onset of antidepressants in patients (2-4 weeks).

As discussed in *sections 1.5.2*, these findings had led to the modified amine theory, suggesting that the acute increase in the levels of the monoamines at the synapse may be only an early step in a potentially complex cascade of events that ultimately results in antidepressant activity (Piñeyro and Blier, 1999; Nestler et al., 2002).

Recent studies have identified modifications of intracellular signalling proteins and target genes that could contribute to antidepressant-like activity of SSRIs (e.g. increases in neurogenesis and brain derived neurotrophic factor, BDNF), and may explain, at least in part, their long delay of action (Malberg and Blendy, 2005; Blier, 2003).

As discussed in *section 1.6*, studies of neurotrophic factors, particularly BDNF, have been of particular interest and have lead to the formulation of the neurotrophic hypothesis of depression, which proposes that reduced

brain BDNF levels predispose to depression, whereas increases in brain BDNF levels produce an antidepressant action (Duman, 2004b, Castrén et al., 2007).

BDNF is one member of the neurotrophin family of growth factors that is widely expressed throughout the mammalian brain (Thoenen, 1991). BDNF promotes the growth and development of immature neurons and enhances the survival, differentiation and maintenance of neurons in peripheral and central nervous development (Lindsay, 1994; Ventimiglia et al., 1995; Lindvall et al., 1994). It influences axonal growth and connectivity and participates in local responses to various types of neuronal insults or stress (Nitta et al., 1999). On the contrary, decreased levels of BDNF may contribute to the atrophy of certain limbic structures, including the hippocampus and prefrontal cortex that has been observed in depressed patients.

Recent evidence linked the action of the neurotrophin BDNF and its receptor, the tropomyosin-related kinase B (TrkB), a protein tyrosine kinase receptor, to the action of antidepressant drugs (Saarelainen et al., 2003; Kozisek et al., 2008). In fact, mice overexpressing the truncated TrkB receptor were resistant to the effects of antidepressants in the FST.

Several reports show that serum BDNF, which is possibly related to BDNF levels in the brain (Pan et al., 1998; Karege et al., 2002b), is significantly decreased in depressed patients (Karege et al., 2002; Shimizu et al., 2003). In addition, antidepressant treatments can reverse this effect (Aydemir et al., 2005; Gervasoni et al., 2005; Gonul et al., 2005; Huang et al., 2008).

It was recently shown that BDNF plasma levels were increased significantly in depressed patients receiving ECT showing a significant improvement in depressive symptoms (Marano et al., 2007).

Moreover, postmortem analysis of the hippocampus demonstrates that the expression of BDNF is decreased in depressed suicide patients and increased in patients receiving antidepressant medication at the time of death (Chen et al., 2001; Dwivedi et al., 2003; Karege et al., 2005).

Preclinical studies showed differential alterations of BDNF levels in candidate brain regions mediating depressive behaviour. Various classes of antidepressant drugs, including monoamine oxidase inhibitors, SSRIs, noradrenaline reuptake inhibitors and tricyclic antidepressants, increase both mRNA and protein levels of BDNF in various areas of the rat brain to a different extent depending on the brain region (Russo-Neustadt et al., 2001; Nibuya et al., 1995; Duman et al., 1997; Duman et al., 2000; Coppell et al., 2003; Dias et al., 2003; De Foubert et al., 2004). Importantly, the ability of these drugs to increase BDNF is dependent on chronic administration, suggesting that their mood-enhancing effects may be functionally related to chronic changes in neurotrophic activity.

Moreover, chronic intracerebral BDNF infusion induces antidepressant-like activity in animal models of depression (Siuciak et al., 1997; Shirayama et al., 2002). Infusions of BDNF directly into the midbrain, the periaqueductal gray and raphé nuclei (Siuciak et al., 1997), lateral ventricles (Hoshaw et al., 2005) or the hippocampus (Shirayama et al., 2002) decreases immobility time in the FST similar to antidepressants. Furthermore, expression of BDNF mRNA is decreased in the hippocampus of mice exposed

to social defeat stress, an animal model of depression mimicking many symptoms of human depression, and this effect is reversed by chronic antidepressant administration (Tsankova et al., 2006).

Taken together, these findings support the possibility that increased expression of BDNF contributes to the neural adaptations that underlie the action of chronic antidepressant treatment.

However, reduced expression of BDNF in the nucleus accumbens (NAc) and ventral tegmental area (VTA) is also associated to antidepressant-like effects in rodents. The NAc-VTA pathway is an important circuit mediating aversive and rewarding responses to emotional stimuli, and, as a result, could mediate the anhedonia, anxiety and reduced motivation, some important symptoms of human depression.

BDNF infused into VTA exerts a depressive-like response in the FST, whereas blockade of BDNF action in the NAc, through viral-mediated overexpression of a dominant negative mutant of TrkB, causes an antidepressant-like effect in the same test (Eisch et al., 2003) and decreased expression of BDNF, through a local deletion of the gene encoding BDNF in the VTA neurons, produces antidepressant-like behavioural effects in a social defeat stress paradigm (Berton et al., 2006).

Therefore, increased expression of BDNF in the hippocampus has antidepressant activity (Shirayama et al., 2002), whereas increased BDNF expression in the VTA or NAc results in a prodepressive state (Eisch et al., 2003).

Interactions between BDNF and 5-HT further suggest the role of this neurotrophin in depression and antidepressant response (Mattson et al., 2004). Intracerebral infusion of BDNF stimulates 5-HT turnover, synthesis and sprouting of 5-HT axons (Mamounas et al., 1995; Mamounas et al., 2000; Siuciak et al., 1996; Siuciak et al., 1998). Heterozygous BDNF knockout mice develop 5-HT deficits with age (Lyons et al., 1999) and are unresponsive to antidepressants in the FST (Saarelainen et al., 2003).

Surprisingly, only few data are available on the effect of chronic SSRIs in the FST in mice, likely because a single dose of SSRIs is sufficient to elicit a reduction of immobility time in this species (*Chapter 2*). Nevertheless, Dulawa et al. (2004) showed that chronic (24 days) but not subchronic (4 days) treatment with a SSRI, fluoxetine, reduced the immobility time in the FST in BALB/c mice.

Furthermore, numerous studies on the relationship between BDNF and chronic SSRIs treatment have focused on assessing the modulation of BDNF at the level of transcription, but only few studies addressed the effects of SSRIs on BDNF protein levels.

Therefore, to gain information on the role of BDNF in the response to long-term treatment with SSRIs, the immobility time in the FST and the concentration of the protein in various brain regions were measured in C57BL/6J and DBA/2J mice, given citalopram for 14 days.

To establish if chronic citalopram schedule effectively induced adaptive changes in 5-HT transmission, as previously shown in rats (Invernizzi et al., 1994), a preliminary study was aimed at assessing the sensitivity of 5-HT_{1A}

autoreceptors controlling 5-HT release after treatment with chronic citalopram in mice. It is known that in rats given citalopram for 2 weeks, somatodendritic 5-HT_{1A} autoreceptors, controlling 5-HT release in the mPFC, are desensitized (Invernizzi et al., 1994). No such data are available for mice.

Changes in the sensitivity of 5-HT_{1A} autoreceptors were assessed examining the effect of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, on extracellular 5-HT in the mPFC of DBA/2J and C57BL/6J mice, given citalopram for two weeks.

6.2 Methods

Microdialysis and behavioural studies were carried out as described in *Chapter 2*. The brain areas dissection was performed as described in *section 3.2.1*.

6.2.1 Extraction procedure for BDNF

Brain areas were frozen, weighed and stored at -80 °C. BDNF was quantified with ELISA Immunoassay, according to Szapacs et al. (2004). Tissue samples were removed from the freezer and homogenized by sonication (as described in *section 3.2.1*) in 200 volume of lysis buffer, prepared as follows: 100 mM PIPES (pH 7), 500 mM NaCl, 0.2% Triton X-100, 0.1% NaN_3, 2% BSA, 2 mM EDTA·Na_2·$2H_2O$, 200 µM PMSF (frozen in isopropanol), 10 µM leupeptin (frozen in deionized water), 0.3 µM aprotinin (frozen in 0.01 M HEPES (pH 8) and 1 µM pepstatin (frozen in DMSO). Then, sample were diluted in lysis buffer and resonicated.

A preliminary experiment was address at identifying the correct sample dilution. I found that frontal cortex (FCX) and hippocampus should be diluted 1:20 in lysis buffer, whereas 1:10 dilution was optimal for NAc and striatum. Using these dilutions, BDNF protein levels fall in the range of linearity of the standard curve and its recovery from tissue range from 40 to 70%.

After diluting, samples were centrifuged at 16,000 x g for 30 min at 4°C.

Finally, supernatants were removed and frozen at -80 °C until analysis.

6.2.2 BDNF enzyme-linked immunoassay

The Promega BDNF Emax ImmunoAssay System was employed to measure the amount of BDNF in each sample (Promega Co., Madison, WI, USA).

Each well of a 96-well polystyrene plate was incubated overnight at 4°C with 100 µL anti-BDNF monoclonal antibody (mAb) diluted 1:1000 in carbonate coating buffer (25 mM sodium bicarbonate and 25 mM sodium carbonate, pH 9.7). Unadsorbed mAb was removed and plates were washed once with TBST washing buffer (20 mM Tris–HCl at pH 7.6, 150 mM NaCl and 0.05% v/v Tween 20).

Just prior to blocking, tissue extracts were removed from the freezer and allowed to thaw at room temperature. Plates were blocked using 200 µL Promega 1X Block and Sample buffer (used to block non-specific binding) followed by incubation for 1 h at room temperature. Plates were then washed once using TBST washing buffer.

One hundred µL of each sample or standard (50, 25, 12.5, 6.25, 3.13, 1.56, 0 pg/100 µL) were added in duplicate to the plates. Plates were incubated for 2 h with shaking (400±100 rpm) at room temperature. Plates were then washed five times with TBST washing buffer.

Anti-human BDNF polyclonal antibody (pAb; 100 µL diluted 1:500 in 1X Block and Sample) was added to each well and plates were incubated for 2 h with shaking (400±100 rpm) at room temperature. Plates were washed again five times using TBST washing buffer.

Anti-IgY horseradish peroxidase conjugate (100 µL diluted 1:200 in 1X Block and Sample) was then added to each well and plates were incubated

for 1 h with shaking (400±100 rpm) at room temperature. Plates were emptied again and washed using TBST washing buffer.

Finally, plates were developed using 100 µL Promega TMB One Solution and the reaction was stopped using 100 µL HCl 1 N. Absorbance was measured at 450 nm. BDNF was quantified based on the calibration curve (***Figure 6.1***).

To further validate the method, some samples were split and spiked with 25 pg/100 µL BDNF to determine percent recovery of BDNF in the ELISA assay, calculated as the increase in optical density signal relative to the signal generated by 25 pg/100 µL of BDNF in lysis buffer alone.

Furthermore, omitting the pAb from samples and standards yielded optical density signals not different from blank (data not shown).

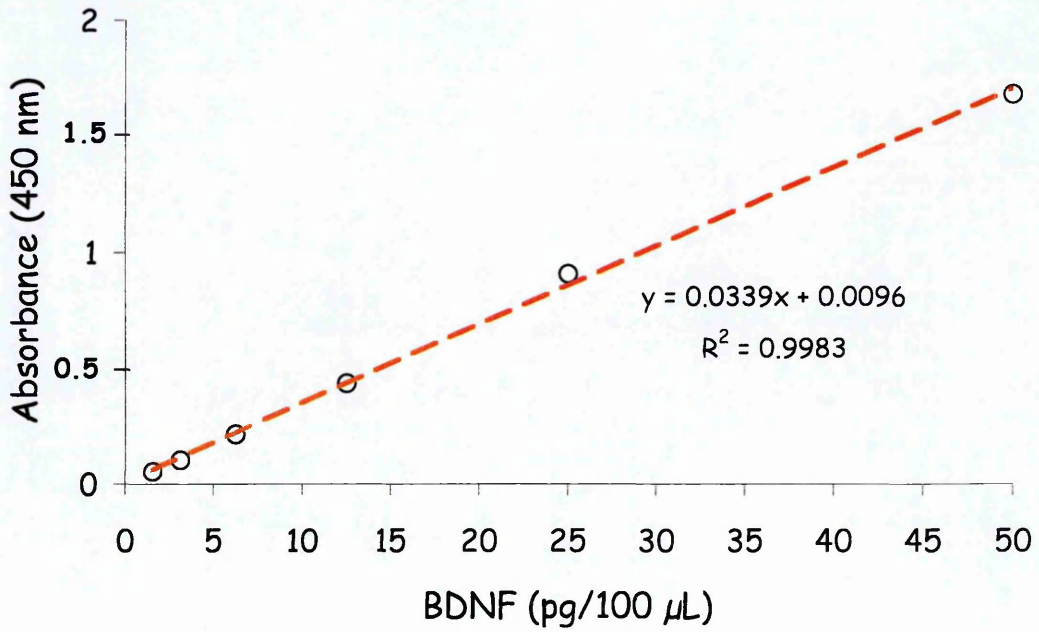


***Figure 6.1** example of BDNF calibration curve*

6.2.3 Drug treatments

Citalopram hydrobromide (Tocris Cookson, Bristol, UK) and the 5-HT_{1A} receptor agonist, (±)-8-hydroxy-2-(di-n-propylamino) tetralin HBr (8-OH-DPAT; Research Biochemical International, MA, USA), were dissolved in saline (NaCl 0.9%; 10 mL/kg) and injected respectively i.p. and s.c. at the doses indicated.

Citalopram was given at 10 mg/kg for 2 weeks, twice a day (h 8:00; 20:00). The 14th day, mice received citalopram only once at 8:00 a.m. Microdialysis, behavioural studies and BDNF assay were performed on separate groups of mice, 24 h after the last dose of chronic citalopram.

The schedule of the experiments is shown in ***Figure 6.2***.

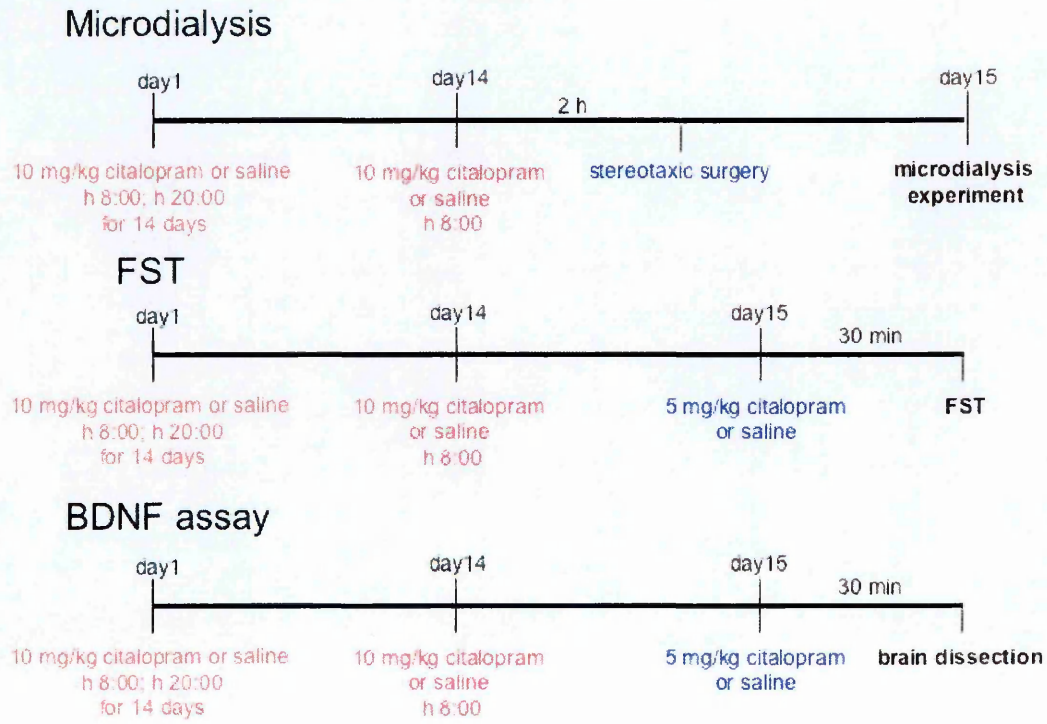


***Figure 6.2** Experimental protocols*

6.2.4 Data analysis

Extracellular levels of 5-HT, not corrected for *in vitro* recovery of the probe, were expressed as fmol/20 µL. Basal values of 5-HT in different experiments and in different strains of mouse were compared by one-way analysis of variance (ANOVA) or Student's t-test. All time-course data were analyzed by ANOVA for repeated measures with treatments as between-subjects factor and time as within-subjects factor. *Post-hoc* comparisons between pre- and post-injection values and comparisons between treatments were done with Tukey-Kramer's test.

The effects of chronic citalopram in the FST and on BDNF levels were analyzed by two-way ANOVA followed by Tukey-Kramer's test.

Only for the NAc, the effect of citalopram on BDNF levels in DBA/2J and C57BL/6J mice was compared by three-way ANOVA with strain, chronic and acute citalopram as main factors.

BDNF protein levels, uncorrected for the percentage of recovery, were expressed as pg/mg tissue. Values of BDNF in different brain areas of DBA/2J and C57BL/6J mice, receiving chronic saline and the challenge of saline, were compared by Student's t-test.

6.3 Results

6.3.1 Basal level of extracellular 5-HT

Mean (±SEM) basal extracellular 5-HT in the mPFC of DBA/2J and C57BL/6J mice was respectively 2.3±0.1 (n=19) and 3.3±0.2 (n=17) fmol/20 µL. No significant differences were found across different experiments (DBA/2J: [$F_{2,16}$= 0.6, p>0.05]; C57BL/6J: [$F_{2,14}$= 3.1, p>0.05]). Basal extracellular 5-HT in the mPFC of DBA/2J mice was significantly lower than in C57BL/6J mice ([t_{34}= 5, p<0.0001]).

6.3.2 Sensitivity of 5-HT_{1A} receptors controlling 5-HT release in the mPFC of DBA/2J and C57BL/6J mice after chronic citalopram

8-OH-DPAT, at 0.1 and 0.2 mg/kg, significantly reduced extracellular 5-HT in the mPFC of DBA/2J mice by about 35-40%, and in C57BL/6J mice by about 50% (***Figure 6.3***, DBA/2J: [$F_{10,40}$ = 4.7, p<0.001]; C57BL/6J: [$F_{10,30}$ = 7.3, p<0.0001]).

8-OH-DPAT at 0.1 mg/kg, maximally reduced extracellular 5-HT. No further reduction was observed at 0.2 mg/kg in both strains. Lower dose of 8-OH-DPAT (0.05 mg/kg) had no significant effect on extracellular 5-HT in both strains (data not shown).

In DBA/2J and C57BL/6J mice, chronically treated with citalopram, 0.1 mg/kg 8-OH-DPAT had no significant effect on extracellular 5-HT (***Figure 6.4***) while it significantly reduced extracellular 5-HT by about 35 and 40% respectively in DBA/2J and C57BL/6J mice receiving saline for 2 weeks.

ANOVA showed a significant effect of treatment [$F_{1,12}$ = 16.6, p<0.001], time [$F_{6,72}$ = 5.2, p<0.001] and time x treatment interaction [$F_{6,72}$ = 3.5,

$p<0.05$] for DBA/2J mice and a significant effect of time [$F_{6,60}$ = 6.6, $p<0.0001$] for C57BL/6J mice but not of treatment [$F_{1,10}$ = 3.8, $p>0.05$] and time x treatment interaction [$F_{6,60}$ = 1.1, $p>0.05$].

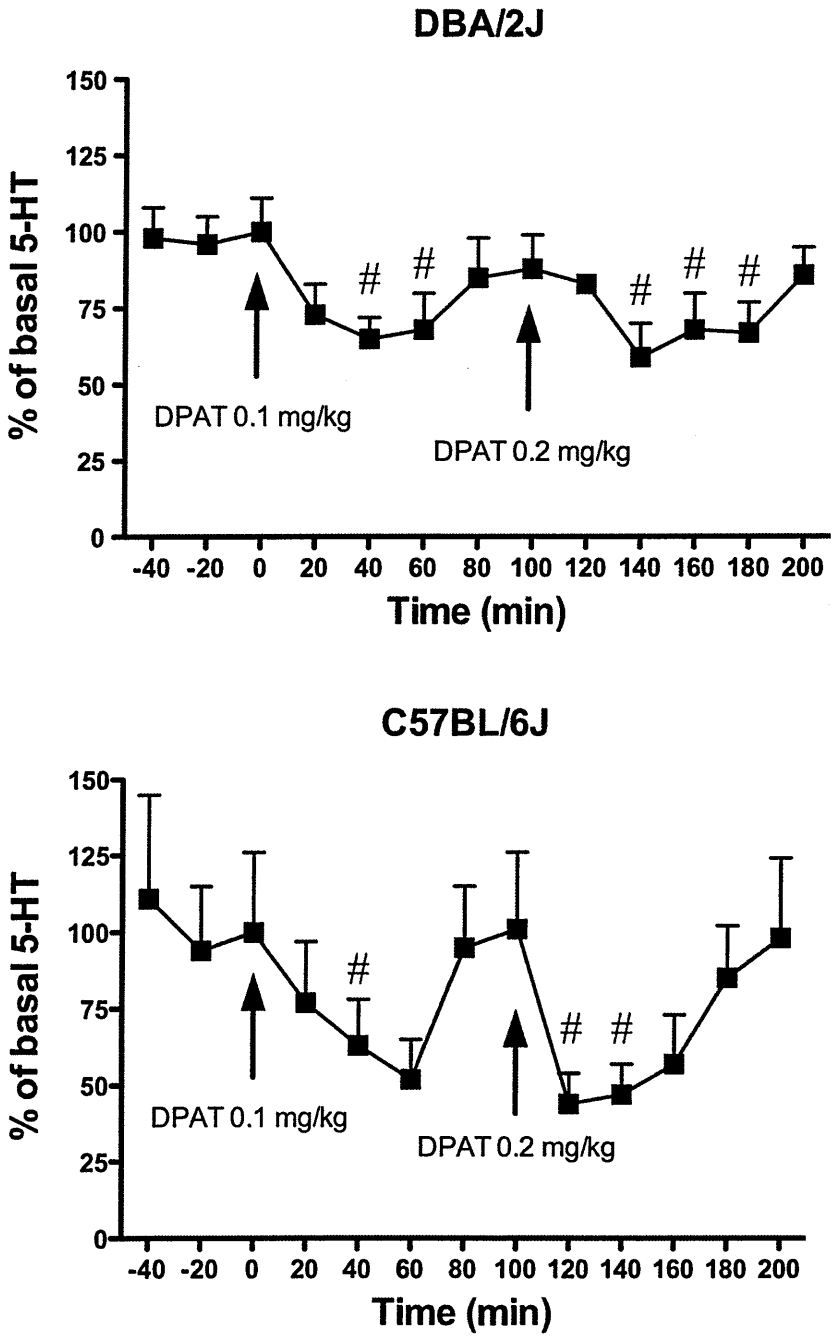


Figure 6.3 Effect of 8-OH-DPAT on extracellular 5-HT in the mPFC of DBA/2J and C57BL/6J mice
Results are Mean±SEM and are expressed as percentage of basal values. Basal values of 5-HT were 2.6±0.3 fmol/20 µL (n=5) for DBA/2J mice and 3.2±0.8 fmol/20 µL (n=4) for C57BL/6J mice.
Arrows indicate the injection of 8-OH-DPAT (DPAT), respectively 0.1 and 0.2 mg/kg.
#$p<0.05$ vs. basal values (Tukey-Kramer's test).

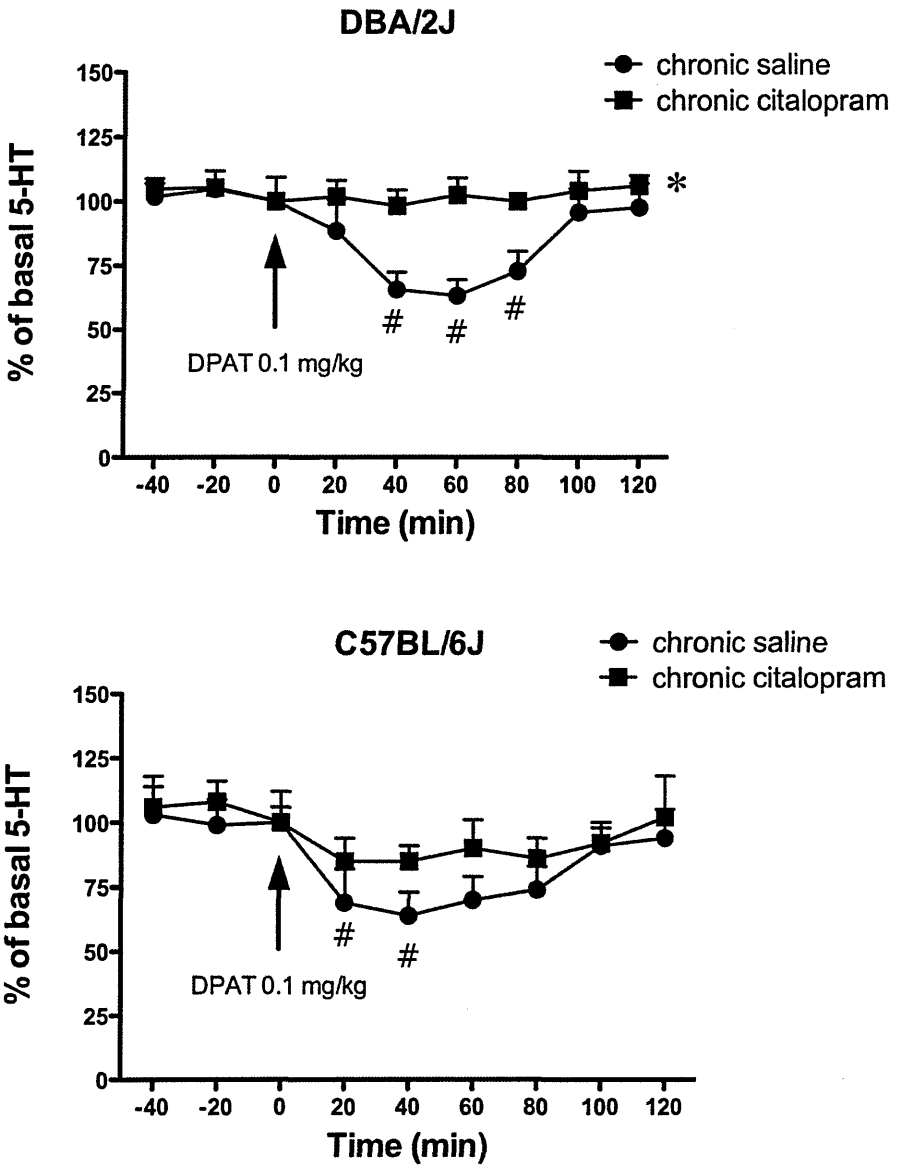


Figure 6.4 Effect of 8-OH-DPAT on extracellular 5-HT in the mPFC of DBA/2J and C57BL/6J mice treated with citalopram 10 mg/kg, twice a day for 14 days.
Results are Mean±SEM and are expressed as percentage of basal values.
Basal levels of 5-HT for DBA/2J mice were: saline, 2.4±0.2 fmol/20 µL (n=7) and citalopram, 2.4±0.1 fmol/20 µL (n=7).
Basal levels of 5-HT for C57BL/6J mice were: saline, 3.8±0.3 fmol/20 µL (n=6) and citalopram, 3.0±0.2 fmol/20 µL (n=6).
Arrow indicates the injection of 8-OH-DPAT (DPAT).
*$p < 0.05$ saline vs. citalopram (Tukey-Kramer's test).
#$p<0.05$ vs. basal values (Tukey-Kramer's test).

6.3.3 Effect of chronic citalopram on immobility time in the FST

A challenge dose of citalopram (5 mg/kg) significantly reduced immobility time in C57BL/6J mice given chronic saline [$F_{1,25}$= 7.4, $p<0.05$] but had no effect in DBA/2J mice (***Figure 6.5***).

Chronic citalopram reduced immobility time in C57BL/6J mice [$F_{1,25}$= 8.9, $p=0.006$], 24 h after the last dose of the chronic schedule and an acute challenge had no further effect.

No significant changes of immobility time were found in DBA/2J mice given citalopram for two weeks. Although an acute dose of citalopram tended to reduce immobility time in DBA/2J mice given the drug for two weeks, the effect was no significant [$F_{1,27}$= 3.4, $p>0.05$].

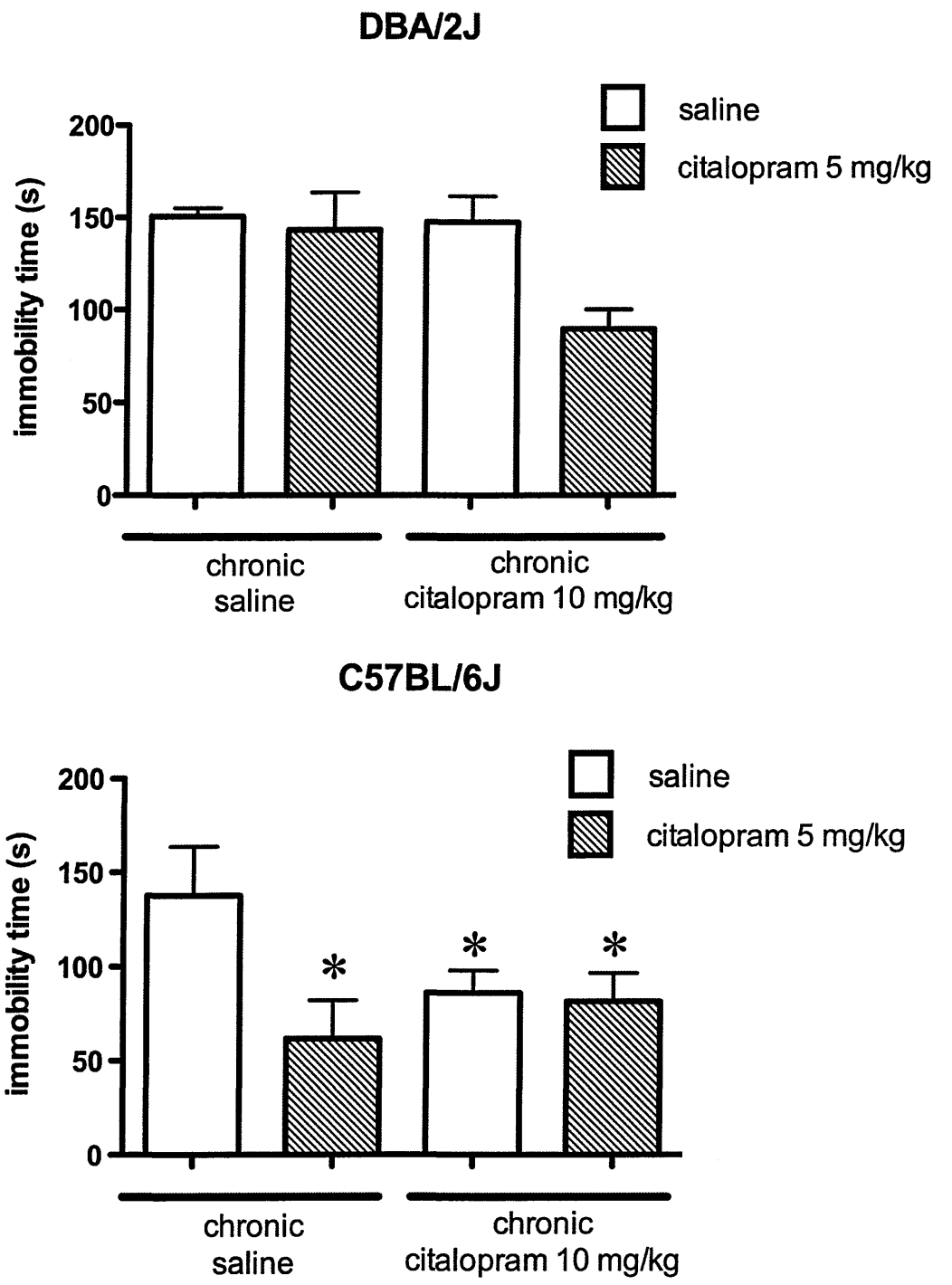


Figure 6.5 Effect of two weeks citalopram or saline on immobility time in the FST in DBA/2J and C57BL/6J mice given 5 mg/kg citalopram or saline 30 min before the test.
Mean ± SEM of 6-8 mice per group.
*$p<0.05$ vs. saline+saline (Tukey-Kramer test).

6.3.4 Effect of chronic citalopram on BDNF levels in several brain regions

Levels of BDNF protein in pg/mg tissue, in mice given chronic saline and a challenge of saline, were: FCX, DBA/2J 119±9 and C57BL/6J 144±11; hippocampus, DBA/2J 325±37 and C57BL/6J 272±21; NAc, DBA/2J 206±34 and C57BL/6J 256±34; striatum, DBA/2J 134±14 and C57BL/6J 155±21.

There were no significant differences in the levels of BDNF between DBA/2J and C57BL/6J mice in any of the brain regions examined (FCX: t_{14}= 1.7, p>0.05; hippocampus: t_{14}= 1.3, p>0.05; NAc: t_{14}= 1.1, p>0.05; striatum: t_{14}= 0.8, p>0.05).

Spiking the tissue with a known amount of BDNF yielded 49, 69, 65 and 40% recovery from cortical, hippocampal, accumbens and striatal samples.

Figure 6.6 shows the effect of chronic treatment with citalopram on BDNF protein levels in the FCX of DBA/2J and C57BL/6J mice.

ANOVA showed a significant effect of chronic citalopram in DBA/2J mice [$F_{1,27}$= 10.8, p=0.003] but not of acute citalopram [$F_{1,27}$= 3.9, p>0.05] and the interaction between chronic and acute citalopram [$F_{1,27}$= 0.2, p>0.05].

None of the treatments had significant effects on BDNF levels in the FCX of C57BL/6J mice (chronic citalopram [$F_{1,27}$= 1.6, p>0.05], citalopram 5 mg/kg [$F_{1,27}$= 0.5, p>0.05] and their interaction [$F_{1,27}$= 2, p>0.05]).

As shown in ***Figure 6.7*** neither chronic citalopram nor a single dose of citalopram modified BDNF levels in the hippocampus of DBA/2J and C57BL/6J mice (DBA/2J: chronic citalopram [$F_{1,27}$= 0.5, p>0.05], acute citalopram [$F_{1,27}$= 1.3, p>0.05] and their interaction [$F_{1,27}$= 0.5, p>0.05]; C57BL/6J: chronic citalopram [$F_{1,27}$= 0.7, p>0.05], citalopram 5 mg/kg [$F_{1,27}$= 2.7, p>0.05] and their interaction [$F_{1,27}$= 0.2, p>0.05]).

The effect of chronic treatment with citalopram on BDNF levels in the NAc of DBA/2J and C57BL/6J mice is shown in ***Figure 6.8***.

Treatment with citalopram for 2 weeks induced a significant increase (about 60%) of BDNF levels in DBA/2J mice [$F_{1,26}$= 8.1, p=0.008]. A challenge dose of 5 mg/kg citalopram had no further effects on BDNF levels in the NAc of DBA/2J mice given chronic saline or citalopram (acute citalopram [$F_{1,26}$= 0.3, p>0.05]; interaction between chronic and acute citalopram [$F_{1,26}$= 0.02, p>0.05]).

Two-way ANOVA showed a significant interaction between chronic and acute citalopram in the NAc of C57BL/6J mice [$F_{1,26}$= 5.9, p=0.02]. This likely reflects the fact that an acute challenge with citalopram slightly decreases BDNF levels in mice given chronic saline, while having the opposite effect in those receiving the drug for two weeks. These opposite changes and the lowering of BDNF observed 24 h the last dose of the chronic schedule with citalopram, account for the lack of significant effects of acute [$F_{1,26}$= 0.04, p>0.05] and chronic citalopram [$F_{1,26}$= 0.2, p>0.05].

Overall, the effect of chronic treatment on BDNF levels in the NAc of DBA/2J and C57BL/6J mice was significantly different [strain x chronic citalopram: $F_{1,52}$= 3.9, p=0.05]. No such differences were found in other brain regions (FCX: [$F_{1,54}$= 2.1, p>0.05]; hippocampus: [$F_{1,54}$= 0.0004, p>0.05]; striatum: [$F_{1,52}$= 0.005, p>0.05]).

Chronic citalopram, a single dose of citalopram or their combination had no effect on BDNF levels in the striatum of DBA/2J and C57BL/6J mice (***Figure 6.9***; DBA/2J: chronic citalopram [$F_{1,25}$= 0.2, p>0.05], acute citalopram [$F_{1,25}$= 0.01, p>0.05] and their interaction [$F_{1,25}$= 1.7, p>0.05];

C57BL/6J: chronic citalopram [$F_{1,27}$= 0.2, p>0.05], citalopram 5 mg/kg [$F_{1,27}$= 0.8, p>0.05] and their interaction [$F_{1,27}$= 0.07, p>0.05]).

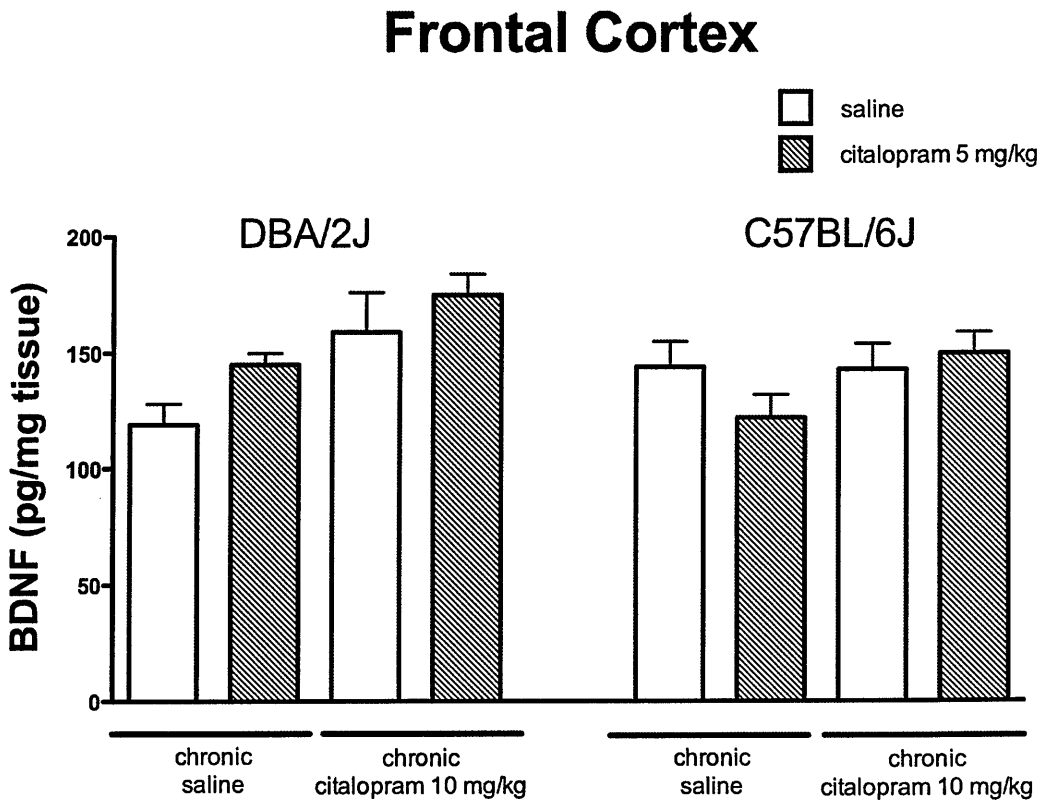


Figure 6.6 Effect of two weeks citalopram or saline on BDNF levels in the FCX of DBA/2J and C57BL/6J mice given an acute challenge dose of citalopram or saline 30 min before sacrifice.
Mean ± SEM of 7-8 mice per group.

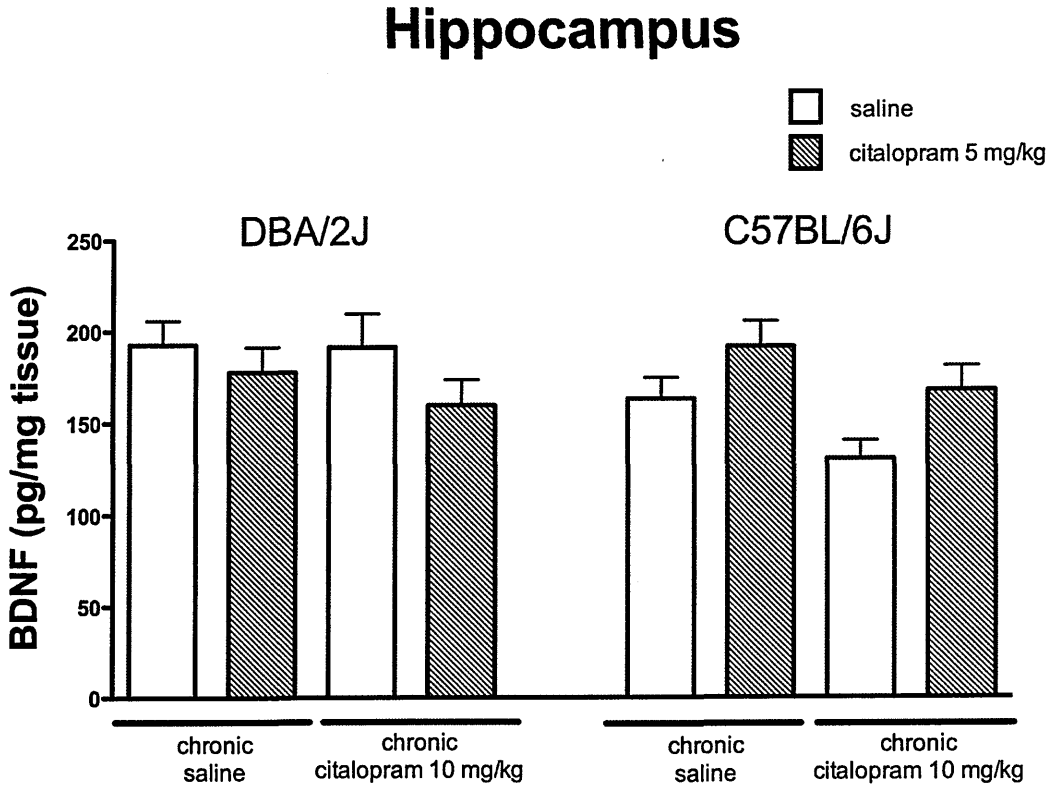


Figure 6.7 Effect of chronic citalopram or saline on BDNF levels in the hippocampus of DBA/2J and C57BL/6J mice.
Mean ± SEM of 7-8 mice per group.

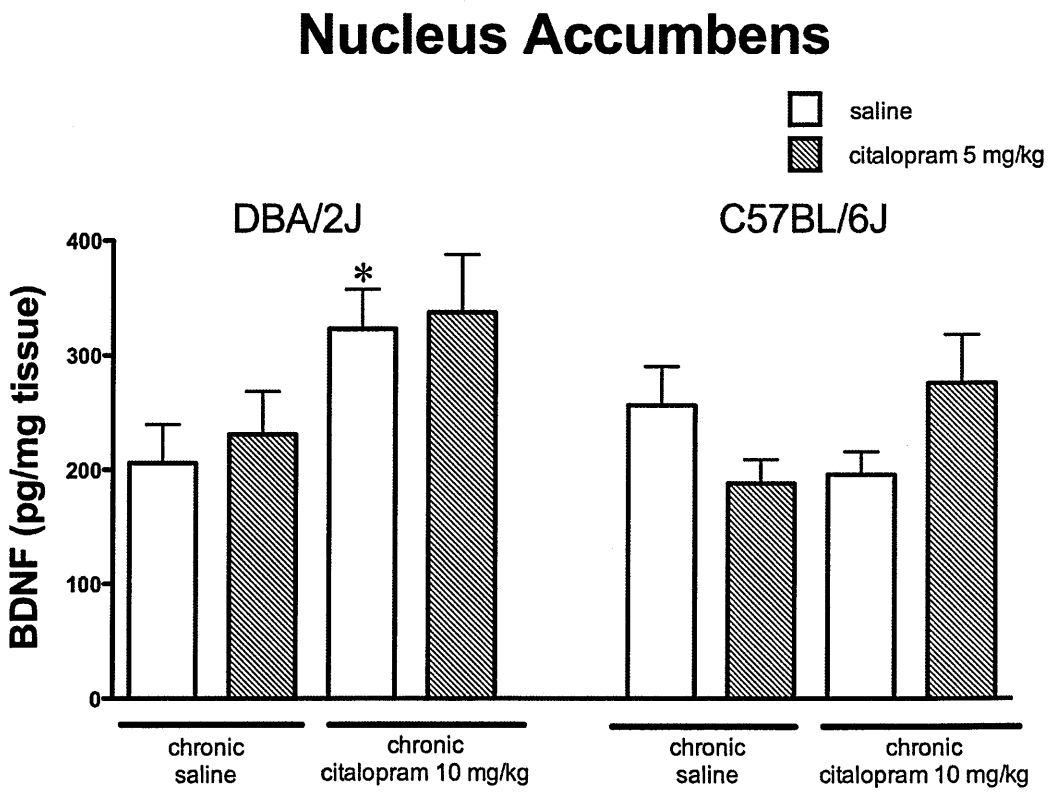


Figure 6.8 Effect of two weeks citalopram or saline on BDNF levels in the Nucleus Accumbens of DBA/2J and C57BL/6J mice.
Mean ± SEM of 7-8 mice per group.
*$p<0.05$ vs. saline+saline (Tukey-Kramer test).

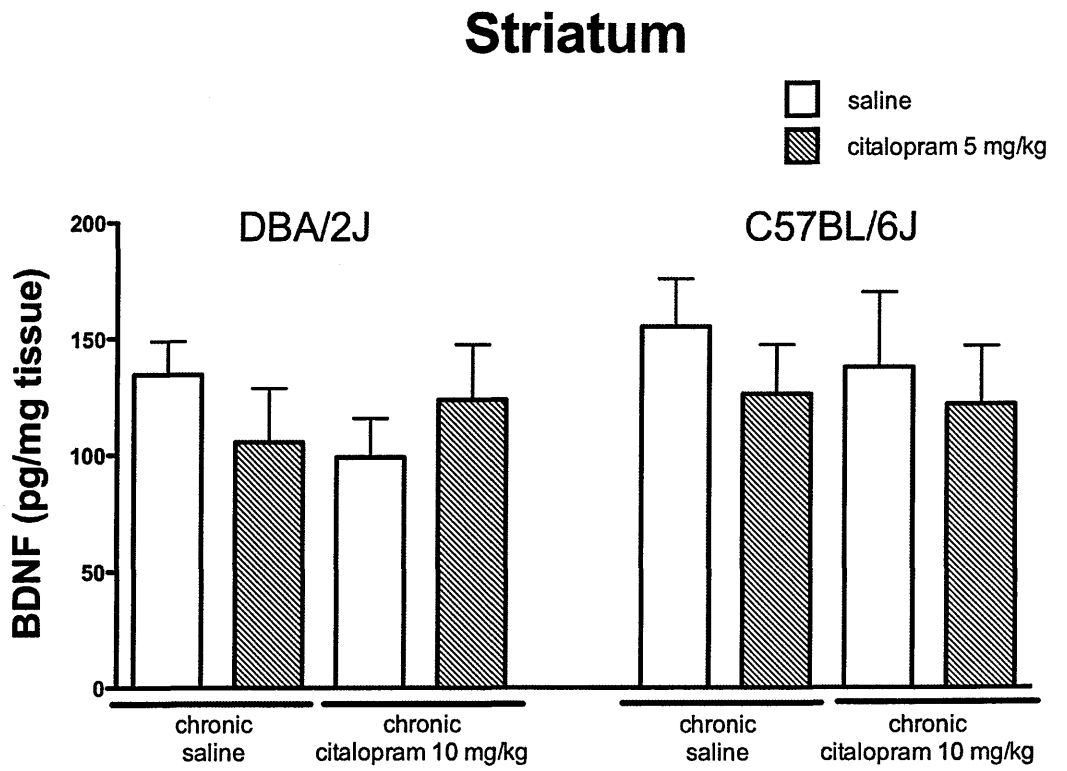


Figure 6.9 Effect of chronic citalopram or saline on BDNF levels in the striatum of DBA/2J and C57BL/6J mice.
Mean ± SEM of 7-8 mice per group.

6.4 Discussion

This part of the study investigated the effects of chronic SSRI on immobility time in the FST and on BDNF protein levels in DBA/2J and C57BL/6J mice, two strains differing in serotonergic neurotransmission and responsivity to SSRIs in the FST (Cervo et al., 2005; *Chapter 3*).

The major findings of this set of experiments are that chronic citalopram for two weeks had no effect on immobility time in DBA/2J mice, indicating that this strain of mice remains "non responder" to SSRI even after repeated treatment.

Chronic citalopram was associated with an increase of BDNF protein levels in the NAc of "non responder" DBA/2J mice while BDNF was not changed or slightly reduced in the same brain region of "responder" C57BL/6J mice.

These findings suggest a possible relationship between the response to chronic citalopram in the FST and modification of BDNF in the NAc.

Effect of chronic citalopram in the FST

Chronic citalopram reduced immobility time in C57BL/6J mice whereas it had no effect in DBA/2J mice, resembling the effect of acute citalopram.

This indicates that no tolerance developed to the ability of citalopram to reduce immobility time in "responder" mice while confirming the failure of citalopram to reduce the immobility time in DBA/2J mice. This suggests that the lack of effect of citalopram in DBA/2J mice was not simply due to the insufficient duration of the treatment, but likely reflects the fact that the neurobiological substrates responsible for the response in the FST are differently affected by citalopram in C57BL/6J and DBA/2J mice. However, it

cannot be excluded that longer treatment with citalopram may result in an antidepressant-like effect in the FST. BALB/c mice that do not respond to 4-days repeated fluoxetine showed a reduction of immobility time after 24-days of repeated treatment (Dulawa et al., 2004) confirming that different strains of mice have different sensitivity to the effects of antidepressants and possibly in the ability to develop the adaptive changes in neurotransmission that ultimately lead to the antidepressant effect.

The lack of effect of citalopram in DBA/2J mice is unlikely due to the inability of developing adaptive changes in 5-HT neurotransmission. In fact, the present results clearly show that the inhibitory effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT on 5-HT release in the mPFC was abolished after two weeks of repeated citalopram. This finding confirms previous studies, mainly in rats, showing that chronic SSRIs desensitize 5-HT_{1A} autoreceptors (Blier, 2003; Artigas et al., 1996; Invernizzi et al., 1994; Hervás et al., 2001) in the raphé and in turn enhance 5-HT neurotransmission in terminal regions (Invernizzi et al., 1994; Artigas et al., 1996) possibly leading to an improved therapeutic effect (Pérez et al., 1997). Thus, it is clear that the desensitization of 5-HT_{1A} receptors controlling 5-HT release is not sufficient to elicit an antidepressant-like effect of citalopram in DBA/2J mice. Therefore, other mechanisms should be involved in the lack of response to citalopram in this strain.

Interestingly, immobility time in C57BL/6J mice is still reduced 24 h after the last dose of the chronic schedule with citalopram. Because of the short half-life of the drug (1-2 h), 24 h after the last dose the levels of citalopram

in the rat (Invernizzi et al., 1994) and mouse (*Caccia et al., unpublished results*) brain, were below the limit of quantification. This clearly indicates that, after chronic treatment, the presence of citalopram is not required to elicit the reduction of immobility time and further suggests that adaptive changes developing during the course of the chronic treatment sustain this effect.

The present results suggest that changes in BDNF levels in the NAc might be involved because of selective increase in BDNF in the NAc of DBA/2J mice given chronic citalopram.

Effect of chronic citalopram on BDNF

In the present study, chronic citalopram increased BDNF in the NAc of DBA/2J mice, "non responder" to citalopram in the FST, whereas had no effect in C57BL/6J "responder" mice.

There are several reports on increased BDNF being depressogenic (Krishnan and Nestler, 2008). Although BDNF might exert antidepressant-like effects at the level of hippocampus, its action might be opposite in other neural circuits (Berton and Nestler, 2006).

NAc BDNF levels and depressive-like behaviour are increased in the mouse following social defeat stress (Berton et al., 2006) and BDNF infusions into the VTA increase depressive-like behaviour in the rat FST (Eisch et al., 2003). On the contrary, suppression of BDNF receptor TrkB expression within the VTA is associated to an antidepressive-like behaviour in the rat FST (Eisch et al., 2003).

It is noteworthy that withdrawal after chronic exposure to drugs of abuse,

such as cocaine (Grimm et al., 2003; Filip et al., 2006; *for review see* Shirayama and Chaki, 2006), up-regulated BDNF mRNA and protein levels in the rat NAc. Moreover this BDNF increase might correlate with depressive-like phenotype in the FST (Filip et al., 2006).

This further suggest that enhanced BDNF signaling in the VTA–NAc pathway is "depressive", whereas blunted BDNF signaling in the VTA–NAc pathway is "antidepressive".

The results obtained in the NAc suggest a role for BDNF in the VTA–NAc pathway that is opposite of those proposed in the hippocampus and other brain regions. The data show that citalopram had no effect on BDNF levels in the hippocampus and frontal cortex in both strains.

It has been shown that chronic but not acute administration of several different antidepressant drugs, not only SSRIs, including tranylcypromine, desipramine and mianserin, significantly increased BDNF mRNA in hippocampus. In contrast, chronic administration of non-antidepressant psychotropic drugs, including morphine, cocaine or haloperidol, did not modify levels of BDNF mRNA in hippocampus and cortex (Nibuya et al., 1995).

However, the effects of antidepressant drug treatment on BDNF gene expression are highly variable and may be influenced by several factors, including detection method, age of animal, strain, class of antidepressant drug, dose, dosing interval, route of administration, time after the last dose before sacrifice and length of treatment (Malberg and Blendy, 2005).

Two studies found a 20-50% increase in BDNF mRNA in the hippocampus of rats respectively 3 h after the last dose of sertraline administered for 3 weeks (Nibuya et al., 1995) and 18 h after the last dose of fluoxetine given for 10 days (Nibuya et al., 1996). Also Martinez-Turrillas et al. (2005) reported a BDNF mRNA levels increase in the CA1, CA3 and the dentate gyrus of the rat hippocampus following chronic paroxetine. Three weeks of fluoxetine up-regulated rat BDNF mRNA in the VTA, frontal cortex and NAc, whereas no changes were detected in the substantia nigra and striatum (Molteni et al., 2006).

However, other studies have demonstrated a 30% decrease and a 30% increase in BDNF mRNA expression in the dentate gyrus (but no effect in any other brain regions) respectively 4 h or 24 h after the last dose of fluoxetine administered for two weeks (Coppell et al., 2003). Furthermore, a recent study found no changes in BDNF mRNA in rats when fluoxetine was administered for 21 days and the animals were sacrificed 2 h after the last dose of drug (Dias et al., 2003). Other studies demonstrated no differences in the levels of BDNF mRNA in the frontal cortex or hippocampus of mice and rats administered 21 days of fluoxetine (Conti et al., 2002; Larsen et al., 2008)

Miró et al. (2002) found that 14 days of chronic fluoxetine treatment downregulated BDNF expression in the rat hippocampus. It is also possible that the BDNF downregulation reported by Miró may be due to an insufficient dosing period, as De Foubert (2004) reports an increase in the hippocampus and in the parietal cortex only following fluoxetine given for 21 days.

Thus, the proposed role of hippocampal BDNF in the antidepressant action has by no means received universal support (*see* Kozisek et al., 2008 *for review*).

Even if several studies reported an effect of chronic SSRIs treatments on BDNF mRNA levels, few studies have been conducted on BDNF protein levels.

The results obtained after two weeks treatment with citalopram in DBA/2J and C57BL/6J mice are in agreement with previous studies reporting no effect of chronic fluoxetine on BDNF protein levels in the hippocampus and frontal cortex (De Foubert et al., 2004; Altar et al., 2003). However, other studies found decreased BDNF protein in the frontal cortex and the hippocampus after chronic escitalopram (Jacobsen et al., 2004) or a BDNF increase in frontal cortex after chronic fluoxetine and no effect in the hippocampus (Balu et al., 2008).

Although 5-HT controls BDNF levels (Zetterström et al., 1999; Zhou et al., 2008), the results did not find an association between the constitutive reduction of brain 5-HT neurotransmission of DBA/2J mice compared to C57BL/6J mice (*Chapter 3*) and basal BDNF protein levels.

Methodological considerations

The selective loss of BDNF in the dentate gyrus, but not in the CA1 region of the hippocampus, attenuated the action of antidepressants in the FST (Adachi et al., 2008). In the present study, the BDNF protein was measured in the whole hippocampus. Thus, we cannot exclude that changes in BDNF in discrete regions of the hippocampus, not picked up in our study but

relevant for the antidepressant-like effects of BDNF, may have occurred.

A large proportion of neuronal BDNF is secreted in the pro-form (proBDNF), which is subsequently converted to mature BDNF (mBDNF) by extracellular proteases (*for review see* Martinowich et al., 2007). Pro-neurotrophins were previously considered predominantly inactive. Using specific antibodies, it was shown that proBDNF is widely and abundantly expressed throughout the adult brain and bind preferentially the p75 low-affinity neurotrophin receptor (p75NTR) whereas mBDNF exerts its influences by signaling preferentially through Trk-B receptors (*see review* Martinowich et al., 2007; Yang et al., 2009).

In addition, proBDNF and mBDNF can elicit different and often opposing effects (Volosin et al., 2006) and, currently, the ELISA immunoassay is not able to distinguish between the two.

In summary, the data obtained show clear strains differences in the effect of chronic citalopram on immobility time between C57BL/6J and DBA/2J mice. This confirms the difference observed after acute administration.

Even if it is important to confirm these findings with other SSRIs, changes in BDNF levels in the NAc might be related to the lack of antidepressant-like effect of citalopram in DBA/2J mice and possibly to the permanence of reduced immobility time in C57BL/6J mice even in the absence of measurable brain levels of the drug.

Chapter 7

SUMMARY AND CONCLUSIONS

Two main observations made in recent years form the basis of this study: the identification of a second isoform of tryptophan hydroxylase (TPH), the enzyme responsible for the synthesis of serotonin (5-HT), named TPH-2, and exclusively located in the brain (Walther et al., 2003); and the discovery that a spontaneous mutation of TPH-2 (C1473G), occurring in DBA/2 and BALB/c mice, determines a reduced synthesis of brain 5-HT (Zhang et al., 2004).

Based on these findings, we found that mice carrying the 1473G allele of TPH-2 did not respond to citalopram in the forced swimming test (FST), a well-validated procedure to assess the antidepressant potential of compounds (Cervo et al., 2005). The discovery of a rare functional polymorphism of TPH-2 in depressed patients associated to poor response to SSRIs further boosted interest in the role of TPH-2 in antidepressant response (Zhang et al., 2005).

This prompted us to hypothesize that impairment of 5-HT neurotransmission plays a major role in the response to antidepressant drugs and may be a potential target for improving the antidepressant effect of SSRIs in subjects not responding to the drug alone.

Thus, the results presented in this dissertation show that DBA/2 and BALB/c mice do not respond to paroxetine in the FST (*Chapter 3*), confirming the previous results obtained with citalopram. In addition, we showed that strain differences in the response to SSRIs was not limited to the acute administration of the drug, as currently assessed in FST studies in mice, but was maintained after repeated administration (*Chapter 6*).

The present results provide convincing evidence that the extracellular concentrations of 5-HT are reduced in the mPFC, DH and DR of DBA/2 and BALB/c "non responder" mice, both under basal conditions and in response to citalopram (*Chapter 3*). We also showed that the reduction of extracellular 5-HT probably reflected a reduced release of the neurotransmitter as no changes of 5-HT reuptake in synaptosomes, obtained from the same brain regions, or the potency of citalopram to inhibit [^{3}H]5-HT uptake were not changed.

Therefore, these results indicate that impaired synthesis and release of 5-HT in key brain regions of DBA/2 and BALB/c "non responder" mice as compared to the SSRI-sensitive C57BL/6 mice could be an underlying factor in the lack of response to SSRIs. In addition, they suggest that the enhancement of serotonergic neurotransmission could restore the response to SSRIs in subjects non-responder to the drugs alone.

Albeit strains of mice carrying the mutated isoform of TPH-2 could model "resistance" to SSRIs dependent on impaired 5-HT transmission, we cannot exclude that other genetic differences across strains may contribute to the lack of response to SSRIs in the FST. To further support our interpretation we showed that the 5-HT synthesis inhibitor, pCPA, prevented the anti-immobility effect of SSRIs in "responder" mice whereas the 5-HT precursors tryptophan rescued the effect of SSRIs in "non responder" mice. Studies on the effects of SSRIs in genetically modified mice such as TPH-2 knockout mice (Savelieva et al., 2008; Gutknecht et al., 2008), knockin mice with functional mutations of TPH-2 (Beaulieu et al., 2008) or congenic mice

carrying either the 1473C or 1473G allele of TPH-2 (Tenner et al., 2008) are needed to further support the validity of our proposal.

In the second part of the thesis (*Chapter 4* and *5*), pharmacological strategies aimed at improving the effect of SSRIs in mice "non responders" to citalopram alone were assessed, verifying whether intervention aimed at enhancing the effect on extracellular 5-HT restored the antidepressant-like effect in the FST.

The results (summarized in ***Tables 7.1***) show that enhancing the effect of SSRIs on extracellular 5-HT in the mPFC, but not in the DH, through the selective blockade of 5-HT_{1A} and 5-HT_{2C} receptors, restored the antidepressant-like response in the FST in "non responders" mice (*Chapter 4*). The selective 5-HT_{1A} and 5-HT_{2C} receptors antagonists used (respectively WAY100635 and SB242084) belong to different chemical classes and act on different inhibitory feedback mechanisms (see *Chapter 4* and *5* and ***Figure 5.1***). This increases the likelihood that the behavioural response observed in the FST depends on the shared ability of the two 5-HT receptor antagonists to raise extracellular 5-HT. The fact that both 5-HT receptor antagonists enhanced citalopram-induced rise of extracellular 5-HT in the mPFC but not in the DH suggests a preferential involvement of 5-HT innervations arising from the DR in the antidepressant response.

***Table 7.1** Effect of augmentation strategies on extracellular 5-HT and immobility time in the FST in DBA/2 mice*

	EXTRACELLULAR 5-HT			FST
	mPFC	DH	DR	IMMOBILTY TIME
EFFECT OF CITALOPRAM ALONE	↗	↗	↗	0
EFFECT OF TRYPTOPHAN+ CITALOPRAM	=	=	n.d.	↙
EFFECT OF WAY100635+ CITALOPRAM	↗↗	=	n.d.	↙
EFFECT OF SB242084+ CITALOPRAM	↗↗	=	↗↗	↙

n.d. not determined; 0 no effect; = same effect as citalopram alone

↗ increase; ↗↗ augmentation

Inasmuch as the anti-immobility effect in the FST in mice is predictive of antidepressant action in humans (see *Chapter 2*), these results suggest that pharmacological strategies aimed at increasing 5-HT neurotransmission may improve the response in depressed patients refractory to SSRIs. Clinical studies aimed at assessing the efficacy and safety of 5-HT_{1A} and 5-HT_{2C} receptor antagonists in combination with SSRIs are needed to confirm the validity of this hypothesis. Available studies show that β-adrenoceptor/5-$HT_{1A/1B}$ receptor antagonist, pindolol, accelerates the antidepressant response in depressed patients but did not improve the effectiveness of

SSRIs in treatment-resistant depressed patients (Artigas et al., 2001). This suggests that the blockade of 5-HT_{1A} receptors might not be sufficient to improve the effect of SSRIs in treatment-resistant depression. However, the occupation of brain 5-HT_{1A} receptors after pindolol administration to depressed patients is very poor (*for see review* Artigas et al., 2001). Thus, it remains to be proved if 5-HT_{1A} receptor antagonists more potent and selective than pindolol, providing a better inhibition of 5-HT_{1A} receptor-mediated inhibitory feedback, may improve the efficacy of SSRIs.

The fact that olanzapine and other atypical antipsychotics, sharing the ability to block 5-HT_{2C} receptors (*for review see* Meltzer et al., 2003) potentiated the effect of fluoxetine and accelerated therapeutic efficacy in resistant depression (Shelton et al., 2001; Corya et al., 2003; *for review see* Millan, 2006) supports the importance of 5-HT_{2C} receptors blockade in antidepressant effect. However, there is a need of more selective drugs to evaluate the potential advantages of the blockade of 5-HT_{2C}-mediated negative feedback in depressed patients.

An important aspect of our study was to assess the role of GABA of the DR in the effect of the 5-HT_{2C} receptor antagonist SB242084 on extracellular 5-HT (*Chapter 5*).

We found that citalopram increased extracellular GABA in the DR of DBA/2N mice and this effect depends on the rise of extracellular 5-HT (prevented by pCPA). The blockade of 5-HT_{2C} receptors, abolishing the increase of extracellular GABA in the DR, suppressed the GABA-mediated inhibitory feedback and in turn enhances the citalopram-induced rise of extracellular 5-HT in the raphé and mPFC of DBA/2 mice. The same effect

was achieved after local infusion of SB242084 in the DR suggesting a prominent role of this nucleus in the mechanism by which 5-HT_{2C} receptors enhances the effects of SSRIs on extracellular 5-HT and in the FST.

These results suggest that the blockade of 5-HT_{2C} receptors, particularly in the DR, might be a useful strategy to reinstate the antidepressant effects of SSRIs in treatment-resistant depression. In addition, blockade of 5-HT_{2C} receptors might attenuate the acute anxiogenic effect of SSRIs at the onset of therapy (*for review see* Millan, 2006).

Besides, the results acquired in *Chapter 5* suggest that the inhibition of GABA-mediated negative feedback controlling 5-HT release may be a potential target for improving the response to SSRIs. In support of this possibility, selective antagonists at GABA-B receptors display antidepressant properties (see *Chapter 5*). However, the possibility of anxiogenic effects occurring at the onset of treatment with GABA-B antagonists, as suggested by the anxiogenic phenotype of mice lacking GABA-B1 or GABA-B2 receptors and by pharmacological studies with GABA-B antagonists (*for review see* Millan, 2006) should be carefully considered. An alternative approach might be the GABA-B positive allosteric modulators (Frankowska et al., 2007).

A further observation concerns the effect of the 5-HT precursor tryptophan. Even if it restored the response to citalopram in mice "non responder" to SSRIs alone (Cervo et al., 2005; *Chapter 3*), it had no effect on citalopram-induced rise of extracellular 5-HT. This raises the interesting possibility that non-serotonergic mechanisms may be involved in the

mechanism by which tryptophan improves the antidepressant-like effect of SSRIs (*Chapter 4*).

Tryptophan is metabolized to melatonin and kynurenines. Melatonin, melatonin receptor agonists and certain kynurenines with NMDA receptor antagonist activity have antidepressant-like effects in rodents (*Chapter 4*). We rule out the contribution of melatoninergic mechanisms to tryptophan effects (*Chapter 4*), but we cannot exclude that kynurenines might play a role in tryptophan effect.

As previously discussed, the increase of 5-HT induced by SSRIs may be only an early step in a cascade of events that lead to the antidepressant response. It is likely that plastic changes occurring after long-term treatment with SSRIs are ultimately responsible for the therapeutic effect. The brain-derived neurotrophic factor (BDNF) has been involved in neuroplasticity and in the long-term effects of antidepressant drugs. Thus, in the last part of the study (*Chapter 6*), we provide preliminary evidence that chronic SSRIs determine opposite changes in BDNF protein levels in the nucleus accumbens of C57BL/6 and DBA/2 mice.

In conclusion, the present results provide functional evidence that the genotype-dependent regulation of 5-HT synthesis is an important factor in the antidepressant-like action of SSRIs and suggest that pharmacological strategies aimed at enhancing 5-HT transmission have a potential for improving the response in treatment-resistant patients. Interstrain comparisons are an important tool for a clearer understanding of the mechanisms underlying the response to SSRIs.

Chapter 8

REFERENCES

Abellán MT, Adell A, Honrubia MA, Mengod G, Artigas F (2000)
GABAB-RI receptors in serotonergic neurons: effects of baclofen on 5-HT output in rat brain. Neuroreport 11:941-945

Abi-Saab WM, Bubser M, Roth RH, Deutch AY (1999)
5-HT2 receptor regulation of extracellular GABA levels in the prefrontal cortex. Neuropsychopharmacology 20:92-96

Adachi M, Barrot M, Autry AE, Theobald D, Monteggia LM (2008)
Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. Biol Psychiatry 63:642-649

Adell A, Celada P, Artigas F (2001)
The role of 5-HT1B receptors in the regulation of serotonin cell firing and release in the rat brain. J Neurochem 79:172-182

Adell A, Celada P, Abellán MT, Artigas F (2002)
Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. Brain Res Brain Res Rev 39:154-180

Adell A, Castro E, Celada P, Bortolozzi A, Pazos A, Artigas F (2005)
Strategies for producing faster acting antidepressants. Drug Discov Today 10:578-585

Aghajanian GK, Foote WE, Sheard MH (1970)
Action of psychotogenic drugs on single midbrain raphe neurons. J Pharmacol Exp Ther 171:178-187

Aghajanian GK, Wang RY, Baraban J (1978)
Serotonergic and non-serotonergic neurons of the dorsal raphe: reciprocal changes in firing induced by peripheral nerve stimulation. Brain Res 153:169-175

Aghajanian GK, Lakoski JM (1984)
Hyperpolarization of serotonergic neurons by serotonin and LSD: studies in brain slices showing increased K+ conductance. Brain Res 305:181-185

Alex KD, Pehek EA (2007)
Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacol Ther 113:296-320

Altar CA, Whitehead RE, Chen R, Wörtwein G, Madsen TM (2003)
Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. Biol Psychiatry 54:703-709

American Psychiatric Association. (1994)
Diagnostic and Statistical Manual of Mental Disorders 4th edn. American Psychiatric Press

Angelucci F, Brenè S, Mathé AA (2005)
BDNF in schizophrenia, depression and corresponding animal models. Mol Psychiatry 10:345-352

Arai I, Tsuyuki Y, Shiomoto H, Satoh M, Otomo S (2000)
Decreased body temperature dependent appearance of behavioral despair in the forced swimming test in mice. Pharmacol Res 42:171-176

Arango V, Huang YY, Underwood MD, Mann JJ (2003)
Genetics of the serotonergic system in suicidal behavior.
J Psychiatr Res 37:375-386

Arias B, Catalán R, Gastó C, Gutiérrez B, Fañanás L (2005)
Evidence for a combined genetic effect of the 5-HT(1A) receptor and serotonin transporter genes in the clinical outcome of major depressive patients treated with citalopram. J Psychopharmacol 19:166-172

Artigas F, Perez V, Alvarez E (1994)
Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. Arch Gen Psychiatry 51:248-251

Artigas F, Romero L, de Montigny C, Blier P (1996)
Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. Trends Neurosci 19:378-383

Artigas F, Celada P, Laruelle M, Adell A (2001)
How does pindolol improve antidepressant action?
Trends Pharmacol Sci 22:224-228

Axelrod J, Weissbach H (1961)
Purification and properties of hydroxyindole-O-methyl transferase.
J Biol Chem 236:211-213

Aydemir O, Deveci A, Taneli F (2005)
The effect of chronic antidepressant treatment on serum brain-derived neurotrophic factor levels in depressed patients: a preliminary study. Prog Neuropsychopharmacol Biol Psychiatry 29:261-265

Azmitia EC, Segal M (1978)
An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 179:641-667

Bagdy E, Kiraly I, Harsing LG (2000)
Reciprocal innervation between serotonergic and GABAergic neurons in raphe nuclei of the rat. Neurochem Res 25:1465-1473

Bai F, Li X, Clay M, Lindstrom T, Skolnick P (2001)
Intra- and interstrain differences in models of "behavioral despair". Pharmacol Biochem Behav 70:187-192

Baker DA, Xi ZX, Shen H, Swanson CJ, Kalivas PW (2002)
The origin and neuronal function of in vivo nonsynaptic glutamate.
J Neurosci 22:9134-9141

Balu DT, Hoshaw BA, Malberg JE, Rosenzweig-Lipson S, Schechter LE, Lucki I (2008)
Differential regulation of central BDNF protein levels by antidepressant and non-antidepressant drug treatments. Brain Res 1211:37-43

Barnes NM, Sharp T (1999)
A review of central 5-HT receptors and their function. Neuropharmacology 38:1083-1152

Beaulieu JM, Zhang X, Rodriguiz RM, Sotnikova TD, Cools MJ, Wetsel WC, Gainetdinov RR, Caron MG (2008)
Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. Proc Natl Acad Sci U S A 105:1333-1338

Beck SG, Pan YZ, Akanwa AC, Kirby LG (2004)
Median and dorsal raphe neurons are not electrophysiologically identical. J Neurophysiol 91:994-1005

Becquet D, Faudon M, Hery F (1990)
The role of serotonin release and autoreceptors in the dorsalis raphe nucleus in the control of serotonin release in the cat caudate nucleus. Neuroscience 39:639-647

Bel N, Artigas F (1996)
Reduction of serotonergic function in rat brain by tryptophan depletion: effects in control and fluvoxamine-treated rats. J Neurochem 67:669-676

Bell CJ, Hood SD, Nutt DJ (2005)
Acute tryptophan depletion. Part II: clinical effects and implications. Aust N Z J Psychiatry 39:565-574

Belmaker RH, Agam G (2008)
Major depressive disorder. N Engl J Med 358:55-68

Benveniste H (1989)
Brain microdialysis. J Neurochem 52:1667-1679

Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ (2006)
Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311:864-868

Berton O, Nestler EJ (2006)
New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7:137-151

Bhagwagar Z, Wylezinska M, Taylor M, Jezzard P, Matthews PM, Cowen PJ (2004)
Increased brain GABA concentrations following acute administration of a selective serotonin reuptake inhibitor. Am J Psychiatry 161:368-370

Blakely RD (2005)
Overview: a rare opportunity or just one less reason to be depressed. Neuron 48:701-2; author reply 705

Blier P, de Montigny C (1994)
Current advances and trends in the treatment of depression. Trends Pharmacol Sci 15:220-226

Blier P, de Montigny C (1998)
Possible serotonergic mechanisms underlying the antidepressant and anti-obsessive-compulsive disorder responses. Biol Psychiatry 44:313-323

Blier P (2003)
The pharmacology of putative early-onset antidepressant strategies. Eur Neuropsychopharmacol 13:57-66

Blier P (2008)
Do antidepressants really work? J Psychiatry Neurosci 33:89-90

Bolonna AA, Arranz MJ, Mancama D, Kerwin RW (2004)
Pharmacogenomics--can genetics help in the care of psychiatric patients? Int Rev Psychiatry 16:311-319

Bonaventure P, Kelly L, Aluisio L, Shelton J, Lord B, Galici R, Miller K, Atack J, Lovenberg TW, Dugovic C (2007)
Selective blockade of 5-hydroxytryptamine (5-HT)7 receptors enhances 5-HT transmission, antidepressant-like behavior, and rapid eye movement sleep suppression induced by citalopram in rodents. J Pharmacol Exp Ther 321:690-698

Boothman LJ, Allers KA, Rasmussen K, Sharp T (2003)
Evidence that central 5-HT2A and 5-HT2B/C receptors regulate 5-HT cell firing in the dorsal raphe nucleus of the anaesthetised rat. Br J Pharmacol 139:998-1004

Boothman LJ, Sharp T (2005)
A role for midbrain raphe gamma aminobutyric acid neurons in 5-hydroxytryptamine feedback control. Neuroreport 16:891-896

Boothman LJ, Mitchell SN, Sharp T (2006**a**)
Investigation of the SSRI augmentation properties of 5-HT(2) receptor antagonists using in vivo microdialysis. Neuropharmacology 50:726-732

Boothman L, Raley J, Denk F, Hirani E, Sharp T (2006**b**)
In vivo evidence that 5-HT(2C) receptors inhibit 5-HT neuronal activity via a GABAergic mechanism. Br J Pharmacol 149:861-869

Borsini F (1995)
Role of the serotonergic system in the forced swimming test. Neurosci Biobehav Rev 19:377-395

Bortolozzi A, Artigas F (2003)
Control of 5-hydroxytryptamine release in the dorsal raphe nucleus by the noradrenergic system in rat brain. Role of alpha-adrenoceptors. Neuropsychopharmacology 28:421-434

Bortolozzi A, Amargós-Bosch M, Toth M, Artigas F, Adell A (2004)
In vivo efflux of serotonin in the dorsal raphe nucleus of 5-HT1A receptor knockout mice. J Neurochem 88:1373-1379

Bosker F, Klompmakers A, Westenberg H (1994)
Extracellular 5-hydroxytryptamine in median raphe nucleus of the conscious rat is decreased by nanomolar concentrations of 8-hydroxy-2-(di-n-propylamino) tetralin and is sensitive to tetrodotoxin. J Neurochem 63:2165-2171

Bourin M, Mocaër E, Porsolt R (2004)
Antidepressant-like activity of S 20098 (agomelatine) in the forced swimming test in rodents: involvement of melatonin and serotonin receptors. J Psychiatry Neurosci 29:126-133

Bradford MM (1976)
A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254

Brambilla P, Perez J, Barale F, Schettini G, Soares JC (2003)
GABAergic dysfunction in mood disorders. Mol Psychiatry 8:721-37, 715

Bryant CD, Zhang NN, Sokoloff G, Fanselow MS, Ennes HS, Palmer AA, McRoberts JA (2008)
Behavioral differences among C57BL/6 substrains: implications for transgenic and knockout studies. J Neurogenet. 22(4):315-31

Calcagno E, Canetta A, Guzzetti S, Cervo L, Invernizzi RW (2007)
Strain differences in basal and post-citalopram extracellular 5-HT in the mouse medial prefrontal cortex and dorsal hippocampus: relation with tryptophan hydroxylase-2 activity. J Neurochem 103:1111-1120

Calcagno E, Guzzetti S, Canetta A, Fracasso C, Caccia S, Cervo L, Invernizzi RW (2009**a**)
Enhancement of cortical extracellular 5-HT by 5-HT1A and 5-HT2C receptor blockade restores the antidepressant-like effect of citalopram in non-responder mice. Int J Neuropsychopharmacol :1-11

Calcagno E, Carli M, Baviera M, Invernizzi RW (2009**b**)
Endogenous serotonin and serotonin2C receptors are involved in the ability of M100907 to suppress cortical glutamate release induced by NMDA receptor blockade. J Neurochem 108:521-532

Campbell K, Kalén P, Lundberg C, Wictorin K, Rosengren E, Björklund A (1993)
Extracellular gamma-aminobutyric acid levels in the rat caudate-putamen: monitoring the neuronal and glial contribution by intracerebral microdialysis. Brain Res 614:241-250

Canli T, Lesch KP (2007)
Long story short: the serotonin transporter in emotion regulation and social cognition. Nat Neurosci 10:1103-1109

Canton H, Emeson RB, Barker EL, Backstrom JR, Lu JT, Chang MS, Sanders-Bush E (1996)
Identification, molecular cloning, and distribution of a short variant of the 5-hydroxytryptamine2C receptor produced by alternative splicing. Mol Pharmacol 50:799-807

Carboni E, Cadoni C, Tanda GL, Di Chiara G (1989)
Calcium-dependent, tetrodotoxin-sensitive stimulation of cortical serotonin release after a tryptophan load. J Neurochem 53:976-978

Carboni E, Di Chiara G (1989)
Serotonin release estimated by transcortical dialysis in freely-moving rats. Neuroscience 32:637-645

Carlsson A, Lindqvist M (1978)
Dependence of 5-HT and catecholamine synthesis on concentrations of precursor amino-acids in rat brain. Naunyn Schmiedebergs Arch Pharmacol 303:157-164

Carrasco JL, Sandner C (2005)
Clinical effects of pharmacological variations in selective serotonin reuptake inhibitors: an overview. Int J Clin Pract 59:1428-1434

Casanovas JM, Artigas F (1996)
Differential effects of ipsapirone on 5-hydroxytryptamine release in the dorsal and median raphe neuronal pathways. J Neurochem 67:1945-1952

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, 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

Castrén E (2005)
Is mood chemistry? Nat Rev Neurosci 6:241-246

Castrén E, Võikar V, Rantamäki T (2007)
Role of neurotrophic factors in depression. Curr Opin Pharmacol 7:18-21

Ceci A, Baschirotto A, Borsini F (1994)
The inhibitory effect of 8-OH-DPAT on the firing activity of dorsal raphe serotoninergic neurons in rats is attenuated by lesion of the frontal cortex. Neuropharmacology 33:709-713

Ceglia I, Carli M, Baviera M, Renoldi G, Calcagno E, Invernizzi RW (2004)
The 5-HT receptor antagonist M100,907 prevents extracellular glutamate rising in response to NMDA receptor blockade in the mPFC. J Neurochem 91:189-199

Celada P, Puig MV, Casanovas JM, Guillazo G, Artigas F (2001)
Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: Involvement of serotonin-1A, GABA(A), and glutamate receptors. J Neurosci 21:9917-9929

Celada P, Casanovas JM, Paez X, Artigas F (2002)
Control of serotonergic neurons in the dorsal raphe nucleus by the lateral hypothalamus. Brain Res 932:79-90

Cervo L, Samanin R (1991)
Effect of chronic treatment with 8-OH-DPAT in the forced swimming test requires the integrity of presynaptic serotonergic mechanisms. Psychopharmacologia 103:524-528

Cervo L, Canetta A, Calcagno E, Burbassi S, Sacchetti G, Caccia S, Fracasso C, Albani D, Forloni G, Invernizzi RW (2005)
Genotype-dependent activity of tryptophan hydroxylase-2 determines the response to citalopram in a mouse model of depression. J Neurosci 25:8165-8172

Chaurasia CS (1999)
In vivo microdialysis sampling: theory and applications. Biomed Chromatogr 13:317-332

Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT (2001)
Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 50:260-265

Chen KC, Höistad M, Kehr J, Fuxe K, Nicholson C (2002)
Theory relating in vitro and in vivo microdialysis with one or two probes. J Neurochem 81:108-121

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS (2006)
Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314:140-143

Chotai J, Serretti A, Lattuada E, Lorenzi C, Lilli R (2003)
Gene-environment interaction in psychiatric disorders as indicated by season of birth variations in tryptophan hydroxylase (TPH), serotonin transporter (5-HTTLPR) and dopamine receptor (DRD4) gene polymorphisms. Psychiatry Res 119:99-111

Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KC (2000)
Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. Neuropharmacology 39:123-132

Conti AC, Cryan JF, Dalvi A, Lucki I, Blendy JA (2002)
cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. J Neurosci 22:3262-3268

Coppell AL, Pei Q, Zetterström TSC (2003)
Bi-phasic change in BDNF gene expression following antidepressant drug treatment. Neuropharmacology 44:903-910

Coppen A, Wood K (1982)
5-Hydroxytryptamine in the pathogenesis of affective disorders. Adv Biochem Psychopharmacol 34:249-258

Corya SA, Andersen SW, Detke HC, Kelly LS, Van Campen LE, Sanger TM, Williamson DJ, Dubé S (2003)
Long-term antidepressant efficacy and safety of olanzapine/fluoxetine combination: a 76-week open-label study. J Clin Psychiatry 64:1349-1356

Cosford RJ, Vinson AP, Kukoyi S, Justice JB (1996)
Quantitative microdialysis of serotonin and norepinephrine: pharmacological influences on in vivo extraction fraction. J Neurosci Methods 68:39-47

Cremers TIFH, Giorgetti M, Bosker FJ, Hogg S, Arnt J, Mørk A, Honig G, Bøgesø KP, Westerink BHC, den Boer H, Wikstrom HV, Tecott LH (2004)
Inactivation of 5-HT(2C) receptors potentiates consequences of serotonin reuptake blockade. Neuropsychopharmacology 29:1782-1789

Cremers TIFH, Rea K, Bosker FJ, Wikström HV, Hogg S, Mørk A, Westerink BHC (2007)
Augmentation of SSRI effects on serotonin by 5-HT2C antagonists: mechanistic studies. Neuropsychopharmacology 32:1550-1557

Crespi F, Garratt JC, Sleight AJ, Marsden CA (1990)
In vivo evidence that 5-hydroxytryptamine (5-HT) neuronal firing and release are not necessarily correlated with 5-HT metabolism. Neuroscience 35:139-144

Crespi F, Ratti E, Trist DG (1994)
Melatonin, a hormone monitorable in vivo by voltammetry? Analyst 119:2193-2197

Crowley JJ, Blendy JA, Lucki I (2005)
Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. Psychopharmacologia 183:257-264

Cryan JF, Markou A, Lucki I (2002)
Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 23:238-245

Cryan JF, Mombereau C (2004)
In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. Mol Psychiatry 9:326-357

Cryan JF, O'Leary OF, Jin SH, Friedland JC, Ouyang M, Hirsch BR, Page ME, Dalvi A, Thomas SA, Lucki I (2004)
Norepinephrine-deficient mice lack responses to antidepressant drugs, including selective serotonin reuptake inhibitors. Proc Natl Acad Sci U S A 101:8186-8191

Cryan JF, Holmes A (2005)
The ascent of mouse: advances in modelling human depression and anxiety. Nat Rev Drug Discov 4:775-790

Cryan JF, Kaupmann K (2005)
Don't worry 'B' happy!: a role for GABA(B) receptors in anxiety and depression. Trends Pharmacol Sci 26:36-43

Cryan JF, Valentino RJ, Lucki I (2005**a**)
Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev 29:547-569

Cryan JF, Mombereau C, Vassout A (2005**b**)
The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev 29:571-625

Cryan JF, Slattery DA (2007)
Animal models of mood disorders: Recent developments. Curr Opin Psychiatry 20:1-7

D'Souza UM, Craig IW (2006)
Functional polymorphisms in dopamine and serotonin pathway genes. Hum Mutat 27:1-13

Dahlström A, Fuxe K (1964)
Localization of monoamines in the lower brain stem. Experientia 20:398-399

Dalvi A, Lucki I (1999)
Murine models of depression. Psychopharmacologia 147:14-16

Daniel PM, Moorhouse SR, Pratt OE (1976)
Amino acid precursors of monoamine neurotransmitters and some factors influencing their supply to the brain. Psychol Med 6:277-286

Daszuta A, Faudon M, Ternaux JP (1982)
Uptake of [3H]serotonin and [3H]noradrenaline in the raphe nuclei and the locus coeruleus of C57BL/6 Rholco and BALB/c Cenlco mice at three times of the day. Neurosci Lett 29:141-146

David DJ, Renard CE, Jolliet P, Hascoët M, Bourin M (2003)
Antidepressant-like effects in various mice strains in the forced swimming test. Psychopharmacologia 166:373-382

David DJ, Bourin M, Jego G, Przybylski C, Jolliet P, Gardier AM. (2003b)
Effects of acute treatment with paroxetine, citalopram and venlafaxine in vivo on noradrenaline and serotonin outflow: a microdialysis study in Swiss mice. Br J Pharmacol. 140(6):1128-36

De Foubert G, Carney SL, Robinson CS, Destexhe EJ, Tomlinson R, Hicks CA, Murray TK, Gaillard JP, Deville C, Xhenseval V, Thomas CE, O'Neill MJ, Zetterström TSC (2004)
Fluoxetine-induced change in rat brain expression of brain-derived neurotrophic factor varies depending on length of treatment. Neuroscience 128:597-604

de Groote L, Linthorst ACE (2007)
Exposure to novelty and forced swimming evoke stressor-dependent changes in extracellular GABA in the rat hippocampus. Neuroscience 148:794-805

Delgado PL, Charney DS, Price LH, Aghajanian GK, Landis H, Heninger GR (1990)
Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. Arch Gen Psychiatry 47:411-418

Delgado PL, Price LH, Miller HL, Salomon RM, Licinio J, Krystal JH, Heninger GR, Charney DS (1991)
Rapid serotonin depletion as a provocative challenge test for patients with major depression: relevance to antidepressant action and the neurobiology of depression. Psychopharmacol Bull 27:321-330

Delgado PL, Miller HL, Salomon RM, Licinio J, Heninger GR, Gelenberg AJ, Charney DS (1993)
Monoamines and the mechanism of antidepressant action: effects of catecholamine depletion on mood of patients treated with antidepressants. Psychopharmacol Bull 29:389-396

Delgado PL, Miller HL, Salomon RM, Licinio J, Krystal JH, Moreno FA, Heninger GR, Charney DS (1999)
Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. Biol Psychiatry 46:212-220

Di Cara B, Samuel D, Salin P, Kerkerian-Le Goff L, Daszuta A (2003)
Serotonergic regulation of the GABAergic transmission in the rat basal ganglia. Synapse 50:144-150

Di Chiara G (1990)
In-vivo brain dialysis of neurotransmitters. Trends Pharmacol Sci 11:116-121

Di Giovanni G, Di Matteo V, Pierucci M, Benigno A, Esposito E (2006)
Central serotonin2C receptor: from physiology to pathology. Curr Top Med Chem 6:1909-1925

Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E (2000)
Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin(2C) receptors. Brain Res 865:85-90

Dias BG, Banerjee SB, Duman RS, Vaidya VA (2003)
Differential regulation of brain derived neurotrophic factor transcripts by antidepressant treatments in the adult rat brain. Neuropharmacology 45:553-563

Donzanti BA, Yamamoto BK (1988)
An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. Life Sci 43:913-922

Drew KL, Ungerstedt U (1991)
Pergolide presynaptically inhibits calcium-stimulated release of gamma-aminobutyric acid. J Neurochem 57:1927-1930

Drijfhout WJ, Grol CJ, Westerink BH (1993)
Microdialysis of melatonin in the rat pineal gland: methodology and pharmacological applications. J Neurochem 61:936-942

Dubocovich ML, Mogilnicka E, Areso PM (1990)
Antidepressant-like activity of the melatonin receptor antagonist, luzindole (N-0774), in the mouse behavioral despair test. Eur J Pharmacol 182:313-325

Dulawa SC, Holick KA, Gundersen B, Hen R (2004)
Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 29:1321-1330

Duman RS, Heninger GR, Nestler EJ (1997)
A molecular and cellular theory of depression. Arch Gen Psychiatry 54:597-606

Duman RS (1998)
Novel therapeutic approaches beyond the serotonin receptor. Biol Psychiatry 44:324-335

Duman RS, Malberg J, Nakagawa S, D'Sa C (2000)
Neuronal plasticity and survival in mood disorders. Biol Psychiatry 48:732-739

Duman RS, Nakagawa S, Malberg J (2001)
Regulation of adult neurogenesis by antidepressant treatment. Neuropsychopharmacology 25:836-844

Duman RS (2002)
Pathophysiology of depression: the concept of synaptic plasticity. Eur Psychiatry 17 Suppl 3:306-310

Duman RS (2004**a**)
Depression: a case of neuronal life and death? Biol Psychiatry 56:140-145

Duman RS (2004**b**)
Role of neurotrophic factors in the etiology and treatment of mood disorders. Neuromolecular Med 5:11-25

Duman RS, Monteggia LM (2006)
A neurotrophic model for stress-related mood disorders. Biol Psychiatry 59:1116-1127

Duncan LE, Hutchison KE, Carey G, Craighead WE (2008)
Variation in brain-derived neurotrophic factor (BDNF) gene is associated with symptoms of depression. J Affect Disord

Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN (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

Ebmeier KP, Donaghey C, Steele JD (2006)
Recent developments and current controversies in depression. Lancet 367:153-167

Edwards JG, Anderson I (1999)
Systematic review and guide to selection of selective serotonin reuptake inhibitors. Drugs 57:507-533

Eisch AJ, Bolaños CA, de Wit J, Simonak RD, Pudiak CM, Barrot M, Verhaagen J, Nestler EJ (2003)
Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. Biol Psychiatry 54:994-1005

El Yacoubi M, Bouali S, Popa D, Naudon L, Leroux-Nicollet I, Hamon M, Costentin J, Adrien J, Vaugeois JM (2003)
Behavioral, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. Proc Natl Acad Sci U S A 100:6227-6232

El Yacoubi M, Vaugeois JM (2007)
Genetic rodent models of depression. Curr Opin Pharmacol 7:3-7

Elhwuegi AS (2004)
Central monoamines and their role in major depression. Prog Neuropsychopharmacol Biol Psychiatry 28:435-451

Englander MT, Dulawa SC, Bhansali P, Schmauss C (2005)
How stress and fluoxetine modulate serotonin 2C receptor pre-mRNA editing. J Neurosci 25:648-651

Esteban S, Nicolaus C, Garmundi A, Rial RV, Rodríguez AB, Ortega E, Ibars CB (2004)
Effect of orally administered L-tryptophan on serotonin, melatonin, and the innate immune response in the rat. Mol Cell Biochem 267:39-46

Fava M, Davidson KG (1996)
Definition and epidemiology of treatment-resistant depression. Psychiatr Clin North Am 19:179-200

Fava M, Kendler KS (2000)
Major depressive disorder. Neuron 28:335-341

Fava M (2003)
Diagnosis and definition of treatment-resistant depression. Biol Psychiatry 53:649-659

Ferraro G, Montalbano ME, Sardo P, La Grutta V (1996)
Lateral habenular influence on dorsal raphe neurons. Brain Res Bull 41:47-52

Filip M, Faron-Górecka A, Kusmider M, Golda A, Frankowska M, Dziedzicka-Wasylewska M (2006)
Alterations in BDNF and trkB mRNAs following acute or sensitizing cocaine treatments and withdrawal. Brain Res 1071:218-225

Fink KB, Göthert M (2007)
5-HT receptor regulation of neurotransmitter release. Pharmacol Rev 59:360-417

Forster EA, Cliffe IA, Bill DJ, Dover GM, Jones D, Reilly Y, Fletcher A (1995)
A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. Eur J Pharmacol 281:81-88

Franklin and Paxinos (1997)
The Mouse Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA, USA

Frankowska M, Filip M, Przegali_ski E (2007)
Effects of GABAB receptor ligands in animal tests of depression and anxiety. Pharmacol Rep 59:645-655

Freis ED (1954)
Mental depression in hypertensive patients treated for long periods with large doses of reserpine. N Engl J Med 251:1006-1008

Frodl T, Schüle C, Schmitt G, Born C, Baghai T, Zill P, Bottlender R, Rupprecht R, Bondy B, Reiser M, Möller HJ, Meisenzahl EM (2007)
Association of the brain-derived neurotrophic factor Val66Met polymorphism with reduced hippocampal volumes in major depression. Arch Gen Psychiatry 64:410-416

Fuller RW (1994)
Uptake inhibitors increase extracellular serotonin concentration measured by brain microdialysis. Life Sci 55:163-167

Gallager DW, Aghajanian GK (1976)
Effect of antipsychotic drugs on the firing of dorsal raphe cells. II. Reversal by picrotoxin. Eur J Pharmacol 39:357-364

Gardier AM, Malagié I, Trillat AC, Jacquot C, Artigas F (1996)
Role of 5-HT1A autoreceptors in the mechanism of action of serotoninergic antidepressant drugs: recent findings from in vivo microdialysis studies. Fundam Clin Pharmacol 10:16-27

Garratt JC, Kidd EJ, Wright IK, Marsden CA (1991)
Inhibition of 5-hydroxytryptamine neuronal activity by the 5-HT agonist, DOI. Eur J Pharmacol 199:349-355

Gartside SE, Umbers V, Hajós M, Sharp T (1995)
Interaction between a selective 5-HT1A receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. Br J Pharmacol 115:1064-1070

Gaspar P, Cases O, Maroteaux L (2003)
The developmental role of serotonin: news from mouse molecular genetics. Nat Rev Neurosci 4:1002-1012

Gavioli EC, Vaughan CW, Marzola G, Guerrini R, Mitchell VA, Zucchini S, De Lima TCM, Rae GA, Salvadori S, Regoli D, Calo' G (2004)
Antidepressant-like effects of the nociceptin/orphanin FQ receptor antagonist UFP-101: new evidence from rats and mice. Naunyn Schmiedebergs Arch Pharmacol 369:547-553

Gerald C, Adham N, Kao HT, Olsen MA, Laz TM, Schechter LE, Bard JA, Vaysse PJ, Hartig PR, Branchek TA (1995)
The 5-HT4 receptor: molecular cloning and pharmacological characterization of two splice variants. EMBO J 14:2806-2815

Gervasoni D, Peyron C, Rampon C, Barbagli B, Chouvet G, Urbain N, Fort P, Luppi PH (2000)
Role and origin of the GABAergic innervation of dorsal raphe serotonergic neurons. J Neurosci 20:4217-4225

Gervasoni N, Aubry JM, Bondolfi G, Osiek C, Schwald M, Bertschy G, Karege F (2005)
Partial normalization of serum brain-derived neurotrophic factor in remitted patients after a major depressive episode. Neuropsychobiology 51:234-238

Gilmer WS, Trivedi MH, Rush AJ, Wisniewski SR, Luther J, Howland RH, Yohanna D, Khan A, Alpert J (2005)
Factors associated with chronic depressive episodes: a preliminary report from the STAR-D project. Acta Psychiatr Scand 112:425-433

Gobbi M, Moia M, Pirona L, Ceglia I, Reyes-Parada M, Scorza C, Mennini T (2002)
p-Methylthioamphetamine and 1-(m-chlorophenyl)piperazine, two non-neurotoxic 5-HT releasers in vivo, differ from neurotoxic amphetamine derivatives in their mode of action at 5-HT nerve endings in vitro. J Neurochem 82:1435-1443

Gonul AS, Akdeniz F, Taneli F, Donat O, Eker C, Vahip S (2005)
Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. Eur Arch Psychiatry Clin Neurosci 255:381-386

Gould TD, Gottesman II (2006)
Psychiatric endophenotypes and the development of valid animal models. Genes Brain Behav 5:113-119

Graeff FG, Guimarães FS, De Andrade TG, Deakin JF (1996)
Role of 5-HT in stress, anxiety, and depression. Pharmacol Biochem Behav 54:129-141

Grahame-Smith DG (1964)
Tryptophan hydroxylation in brain. Biochem Biophys Res Commun 16:586-592

Gray E, Whittaker V.P. (1962)
The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. J Anat 96:79-88

Gray JA, Goodwin GM, Heal DJ, Green AR (1987)
Hypothermia induced by baclofen, a possible index of GABAB receptor function in mice, is enhanced by antidepressant drugs and ECS. Br J Pharmacol 92:863-870

Greenberg BD, Tolliver TJ, Huang SJ, Li Q, Bengel D, Murphy DL (1999)
Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. Am J Med Genet 88:83-87

Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y (2003)
Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci 23:742-747

Groves JO (2007)
Is it time to reassess the BDNF hypothesis of depression? Mol Psychiatry 12:1079-1088

Guiard BP, Przybylski C, Guilloux JP, Seif I, Froger N, De Felipe C, Hunt SP, Lanfumey L, Gardier AM (2004)
Blockade of substance P (neurokinin 1) receptors enhances extracellular serotonin when combined with a selective serotonin reuptake inhibitor: an in vivo microdialysis study in mice. J Neurochem 89:54-63

Guilloux JP, David DJP, Guiard BP, Chenu F, Repérant C, Toth M, Bourin M, Gardier AM (2006)
Blockade of 5-HT1A receptors by (+/-)-pindolol potentiates cortical 5-HT outflow, but not antidepressant-like activity of paroxetine: microdialysis and behavioral approaches in 5-HT1A receptor knockout mice. Neuropsychopharmacology 31:2162-2172

Gundlah C, Martin KF, Heal DJ, Auerbach SB (1997)
In vivo criteria to differentiate monoamine reuptake inhibitors from releasing agents: sibutramine is a reuptake inhibitor. J Pharmacol Exp Ther 283:581-591

Gurevich I, Englander MT, Adlersberg M, Siegal NB, Schmauss C (2002a)
Modulation of serotonin 2C receptor editing by sustained changes in serotonergic neurotransmission. J Neurosci 22:10529-10532

Gurevich I, Tamir H, Arango V, Dwork AJ, Mann JJ, Schmauss C (2002b)
Altered editing of serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. Neuron 34:349-356

Guscott M, Bristow LJ, Hadingham K, Rosahl TW, Beer MS, Stanton JA, Bromidge F, Owens AP, Huscroft I, Myers J, Rupniak NM, Patel S, Whiting PJ, Hutson PH, Fone KC, Biello SM, Kulagowski JJ, McAllister G (2005)
Genetic knockout and pharmacological blockade studies of the 5-HT7 receptor suggest therapeutic potential in depression. Neuropharmacology 48:492-502

Gutknecht L, Waider J, Kraft S, Kriegebaum C, Holtmann B, Reif A, Schmitt A, Lesch KP (2008)
Deficiency of brain 5-HT synthesis but serotonergic neuron formation in Tph2 knockout mice. J Neural Transm 115:1127-1132

Guzzetti S, Calcagno E, Canetta A, Sacchetti G, Fracasso C, Caccia S, Cervo L, Invernizzi RW (2008)
Strain differences in paroxetine-induced reduction of immobility time in the forced swimming test in mice: role of serotonin. Eur J Pharmacol 594:117-124

Gören MZ, Küçükibrahimoglu E, Berkman K, Terzioglu B (2007)
Fluoxetine partly exerts its actions through GABA: a neurochemical evidence. Neurochem Res 32:1559-1565

Hackler EA, Airey DC, Shannon CC, Sodhi MS, Sanders-Bush E (2006)
5-HT(2C) receptor RNA editing in the amygdala of C57BL/6J, DBA/2J, and BALB/cJ mice. Neurosci Res 55:96-104

Haghighi F, Bach-Mizrachi H, Huang YY, Arango V, Shi S, Dwork AJ, Rosoklija G, Sheng HT, Morozova I, Ju J, Russo JJ, Mann JJ (2008)
Genetic architecture of the human tryptophan hydroxylase 2 Gene: existence of neural isoforms and relevance for major depression. Mol Psychiatry 13:813-820

Hajak G, Huether G, Blanke J, Blömer M, Freyer C, Poeggeler B, Reimer A, Rodenbeck A, Schulz-Varszegi M, Rüther E (1991)
The influence of intravenous L-tryptophan on plasma melatonin and sleep in men. Pharmacopsychiatry 24:17-20

Hajós M, Richards CD, Székely AD, Sharp T (1998)
An electrophysiological and neuroanatomical study of the medial prefrontal cortical projection to the midbrain raphe nuclei in the rat. Neuroscience 87:95-108

Hajós M, Hajós-Korcsok E, Sharp T (1999)
Role of the medial prefrontal cortex in 5-HT1A receptor-induced inhibition of 5-HT neuronal activity in the rat. Br J Pharmacol 126:1741-1750

Hamilton M (1960)
A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56-62

Hanna GL, Himle JA, Curtis GC, Koram DQ, Veenstra-VanderWeele J, Leventhal BL, Cook EH (1998)
Serotonin transporter and seasonal variation in blood serotonin in families with obsessive-compulsive disorder. Neuropsychopharmacology 18:102-111

Harandi M, Aguera M, Gamrani H, Didier M, Maitre M, Calas A, Belin MF (1987)
gamma-Aminobutyric acid and 5-hydroxytryptamine interrelationship in the rat nucleus raphe dorsalis: combination of radioautographic and immunocytochemical techniques at light and electron microscopy levels. Neuroscience 21:237-251

He M, Sibille E, Benjamin D, Toth M, Shippenberg T (2001)
Differential effects of 5-HT1A receptor deletion upon basal and fluoxetine-evoked 5-HT concentrations as revealed by in vivo microdialysis. Brain Res 902:11-17

Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, Lesch KP (1996)
Allelic variation of human serotonin transporter gene expression. J Neurochem 66:2621-2624

Henningsson S, Annerbrink K, Olsson M, Allgulander C, Andersch S, Sjödin I, Gustafson D, Skoog I, Eriksson E, Westberg L (2007)
Absence of the Arg441His polymorphism in the tryptophan hydroxylase 2 gene in adults with anxiety disorders and depression. Am J Med Genet B Neuropsychiatr Genet 144B:816-817

Hensler JG, Kovachich GB, Frazer A (1991)
A quantitative autoradiographic study of serotonin1A receptor regulation. Effect of 5,7-dihydroxytryptamine and antidepressant treatments. Neuropsychopharmacology 4:131-144

Herrera-Marschitz M, You ZB, Goiny M, Meana JJ, Silveira R, Godukhin OV, Chen Y, Espinoza S, Pettersson E, Loidl CF, Lubec G, Andersson K, Nylander I, Terenius L, Ungerstedt U (1996)
On the origin of extracellular glutamate levels monitored in the basal ganglia of the rat by in vivo microdialysis. J Neurochem 66:1726-1735

Hervás I, Queiroz CM, Adell A, Artigas F (2000)
Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat. Br J Pharmacol 130:160-166

Hervás I, Vilaró MT, Romero L, Scorza MC, Mengod G, Artigas F (2001)
Desensitization of 5-HT(1A) autoreceptors by a low chronic fluoxetine dose effect of the concurrent administration of WAY-100635. Neuropsychopharmacology 24:11-20

Hery F, Faudon M, Ternaux JP (1982)
In vivo release of serotonin in two raphe nuclei (raphe dorsalis and magnus) of the cat. Brain Res Bull 8:123-129

Hjorth S (1993)
Serotonin 5-HT1A autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study. J Neurochem 60:776-779

Hjorth S, Auerbach SB (1994)
Further evidence for the importance of 5-HT1A autoreceptors in the action of selective serotonin reuptake inhibitors. Eur J Pharmacol 260:251-255

Holtzheimer PE, Nemeroff CB (2006)
Advances in the treatment of depression. NeuroRx 3:42-56

Hood SD, Bell CJ, Nutt DJ (2005)
Acute tryptophan depletion. Part I: rationale and methodology. Aust N Z J Psychiatry 39:558-564

Hoshaw BA, Malberg JE, Lucki I (2005)
Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. Brain Res 1037:204-208

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP (1994)
International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev 46:157-203

Hrdina PD, Foy B, Hepner A, Summers RJ (1990)
Antidepressant binding sites in brain: autoradiographic comparison of [3H]paroxetine and [3H]imipramine localization and relationship to serotonin transporter. J Pharmacol Exp Ther 252:410-418

Huang TL, Lee CT, Liu YL (2008)
Serum brain-derived neurotrophic factor levels in patients with major depression: effects of antidepressants. J Psychiatr Res 42:521-525

Hwang BH, Kunkler PE, Tarricone BJ, Hingtgen JN, Nurnberger JI (1999)
Stress-induced changes of norepinephrine uptake sites in the locus coeruleus of C57BL/6J and DBA/2J mice: a quantitative autoradiographic study using [3H]-tomoxetine. Neurosci Lett 265:151-154

Hyttel J (1977)
Neurochemical characterization of a new potent and selective serotonin uptake inhibitor: Lu 10-171. Psychopharmacologia 51:225-233

Invernizzi R, Carli M, Di Clemente A, Samanin R (1991)
Administration of 8-hydroxy-2-(Di-n-propylamino)tetralin in raphe nuclei dorsalis and medianus reduces serotonin synthesis in the rat brain: differences in potency and regional sensitivity. J Neurochem 56:243-247

Invernizzi R, Belli S, Samanin R (1992)
Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex. Brain Res 584:322-324

Invernizzi R, Bramante M, Samanin R (1994)
Chronic treatment with citalopram facilitates the effect of a challenge dose on cortical serotonin output: role of presynaptic 5-HT1A receptors. Eur J Pharmacol 260:243-246

Invernizzi R, Bramante M, Samanin R (1996)
Role of 5-HT1A receptors in the effects of acute chronic fluoxetine on extracellular serotonin in the frontal cortex. Pharmacol Biochem Behav 54:143-147

Invernizzi R, Velasco C, Bramante M, Longo A, Samanin R (1997)
Effect of 5-HT1A receptor antagonists on citalopram-induced increase in extracellular serotonin in the frontal cortex, striatum and dorsal hippocampus. Neuropharmacology 36:467-473

Isles AR, Hathway GJ, Humby T, de la Riva C, Kendrick KM, Wilkinson LS (2005)
An mTph2 SNP gives rise to alterations in extracellular 5-HT levels, but not in performance on a delayed-reinforcement task. Eur J Neurosci 22:997-1000

Jacobs BL, Azmitia EC (1992)
Structure and function of the brain serotonin system. Physiol Rev 72:165-229

Jacobsen JPR, Nielsen EØ, Hummel R, Redrobe JP, Mirza N, Weikop P (2008)
Insensitivity of NMRI mice to selective serotonin reuptake inhibitors in the tail suspension test can be reversed by co-treatment with 5-hydroxytryptophan. Psychopharmacology 199:137-150

Jacobsen JP, Mørk A. (2004)
The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. Brain Res. 1024(1-2):183-92.

Jacobson LH, Cryan JF (2007)
Feeling strained? Influence of genetic background on depression-related behavior in mice: a review. Behav Genet 37:171-213

Jankowski MP, Sesack SR (2004)
Prefrontal cortical projections to the rat dorsal raphe nucleus: ultrastructural features and associations with serotonin and gamma-aminobutyric acid neurons. J Comp Neurol 468:518-529

Jans LAW, Riedel WJ, Markus CR, Blokland A (2007)
Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. Mol Psychiatry 12:522-543

Jaworek J, Nawrot K, Konturek SJ, Leja-Szpak A, Thor P, Pawlik WW (2004)
Melatonin and its precursor, L-tryptophan: influence on pancreatic amylase secretion in vivo and in vitro. J Pineal Res 36:155-164

Jazrawi SP, Yamaguchi Y, Horton RW (1987)
Synaptosomal high-affinity noradrenaline uptake does not differ between mice susceptible (DBA/2J) and resistant (C57 BL/6) to audiogenic seizures. Epilepsy Res 1:139-141

Jimenez-Jorge S, Guerrero JM, Jimenez-Caliani AJ, Naranjo MC, Lardone PJ, Carrillo-Vico A, Osuna C, Molinero P (2007)
Evidence for melatonin synthesis in the rat brain during development. J Pineal Res 42:240-246

Joiner TE, Johnson F, Soderstrom K, Brown JS (2003)
Is there an association between serotonin transporter gene polymorphism and family history of depression? J Affect Disord 77:273-275

Jolas T, Aghajanian GK (1997)
Opioids suppress spontaneous and NMDA-induced inhibitory postsynaptic currents in the dorsal raphe nucleus of the rat in vitro. Brain Res 755:229-245

Kalia M (2005)
Neurobiological basis of depression: an update. Metabolism 54:24-27

Kalueff AV, Nutt DJ (2007)
Role of GABA in anxiety and depression. Depress Anxiety 24:495-517

Kalén P, Strecker RE, Rosengren E, Björklund A (1989)
Regulation of striatal serotonin release by the lateral habenula-dorsal raphe pathway in the rat as demonstrated by in vivo microdialysis: role of excitatory amino acids and GABA. Brain Res 492:187-202

Kalén P, Strecker RE, Rosengren E, Björklund A (1988)
Endogenous release of neuronal serotonin and 5-hydroxyindoleacetic acid in the caudate-putamen of the rat as revealed by intracerebral dialysis coupled to high-performance liquid chromatography with fluorimetric detection. J Neurochem 51:1422-1435

Kaplan, Sadock (2000)
Comprehensive textbook of psychiatry. Sadock IIV ed. (ed.)

Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM (2002**a**)
Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 109:143-148

Karege F, Schwald M, Cisse M (2002**b**)
Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. Neurosci Lett 328:261-264

Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R (2005)
Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res 136:29-37

Ke L, Qi ZY, Ping Y, Ren CY (2006)
Effect of SNP at position 40237 in exon 7 of the TPH2 gene on susceptibility to suicide. Brain Res 1122:24-26

Keck ME (2006)
Corticotropin-releasing factor, vasopressin and receptor systems in depression and anxiety. Amino Acids 31:241-250

Kempf E, Greilsamer J, Mack G, Mandel P (1974)
Correlation of behavioural differences in three strains of mice with differences in brain amines. Nature 247:483-485

Kendler KS, Gardner CO, Prescott CA (1999)
Clinical characteristics of major depression that predict risk of depression in relatives. Arch Gen Psychiatry 56:322-327

Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B (2005)
The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. Arch Gen Psychiatry 62:529-535

Kennett GA, Lightowler S, de Biasi V, Stevens NC, Wood MD, Tulloch IF, Blackburn TP (1994)
Effect of chronic administration of selective 5-hydroxytryptamine and noradrenaline uptake inhibitors on a putative index of 5-HT2C/2B receptor function. Neuropharmacology 33:1581-1588

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes IT, Brown AM, Middlemiss DN, Blackburn TP (1997)
SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. Neuropharmacology 36:609-620

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

Kim DK, Lim SW, Lee S, Sohn SE, Kim S, Hahn CG, Carroll BJ (2000)
Serotonin transporter gene polymorphism and antidepressant response. Neuroreport 11:215-219

Kim DK, Tolliver TJ, Huang SJ, Martin BJ, Andrews AM, Wichems C, Holmes A, Lesch KP, Murphy DL (2005)
Altered serotonin synthesis, turnover and dynamic regulation in multiple brain regions of mice lacking the serotonin transporter. Neuropharmacology 49:798-810

Kim H, Lim SW, Kim S, Kim JW, Chang YH, Carroll BJ, Kim DK (2006)
Monoamine transporter gene polymorphisms and antidepressant response in koreans with late-life depression. JAMA 296:1609-1618

King M, Nazareth I, Levy G, Walker C, Morris R, Weich S, Bellón-Saameño JA, Moreno B, Svab I, Rotar D, Rifel J, Maaroos HI, Aluoja A, Kalda R, Neeleman J, Geerlings MI, Xavier M, de Almeida MC, Correa B, Torres-Gonzalez F (2008)
Prevalence of common mental disorders in general practice attendees across Europe. Br J Psychiatry 192:362-367

Kirsch I, Deacon BJ, Huedo-Medina TB, Scoboria A, Moore TJ, Johnson BT (2008)
Initial severity and antidepressant benefits: a meta-analysis of data submitted to the Food and Drug Administration. PLoS Med 5:e45

Koe BK, Weissman A (1966)
p-Chlorophenylalanine: a specific depletor of brain serotonin. J Pharmacol Exp Ther 154:499-516

Kosofsky BE, Molliver ME (1987)
The serotoninergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. Synapse 1:153-168

Kovacs M, Devlin B, Pollock M, Richards C, Mukerji P (1997)
A controlled family history study of childhood-onset depressive disorder. Arch Gen Psychiatry 54:613-623

Kozisek ME, Middlemas D, Bylund DB (2008)
Brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B in the mechanism of action of antidepressant therapies. Pharmacol Ther 117:30-51

Kraft JB, Peters EJ, Slager SL, Jenkins GD, Reinalda MS, McGrath PJ, Hamilton SP (2007)
Analysis of association between the serotonin transporter and antidepressant response in a large clinical sample. Biol Psychiatry 61:734-742

Krishnan V V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ. (2007)
Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131:391-404

Krishnan V, Nestler EJ (2008)
The molecular neurobiology of depression. Nature 455:894-902

Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, Epperson CN, Goddard A, Mason GF (2002)
Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. Mol Psychiatry 7 Suppl 1:S71-S80

Kulikov AV, Osipova DV, Naumenko VS, Popova NK (2005)
Association between Tph2 gene polymorphism, brain tryptophan hydroxylase activity and aggressiveness in mouse strains. Genes Brain Behav 4:482-485

Lahmame A, Armario A (1996)
Differential responsiveness of inbred strains of rats to antidepressants in the forced swimming test: are Wistar Kyoto rats an animal model of subsensitivity to antidepressants? Psychopharmacologia 123:191-198

Lahmame A, del Arco C, Pazos A, Yritia M, Armario A (1997)
Are Wistar-Kyoto rats a genetic animal model of depression resistant to antidepressants? Eur J Pharmacol 337:115-123

Larsen MH, Hay-Schmidt A, Rønn LCB, Mikkelsen JD (2008)
Temporal expression of brain-derived neurotrophic factor (BDNF) mRNA in the rat hippocampus after treatment with selective and mixed monoaminergic antidepressants. Eur J Pharmacol 578:114-122

Lasley SM, Michaelson IA, Greenland RD, McGinnis PM (1984)
Simultaneous measurement of tyrosine, tryptophan and related monoamines for determination of neurotransmitter turnover in discrete rat brain regions by liquid chromatography with electrochemical detection. J Chromatogr 305:27-42

Lechin F, van der Dijs B, Hernández-Adrián G (2006)
Dorsal raphe vs. median raphe serotonergic antagonism. Anatomical, physiological, behavioral, neuroendocrinological, neuropharmacological and clinical evidences: relevance for neuropharmacological therapy. Prog Neuropsychopharmacol Biol Psychiatry 30:565-585

Lee SH, Lee KJ, Lee HJ, Ham BJ, Ryu SH, Lee MS (2005)
Association between the 5-HT6 receptor C267T polymorphism and response to antidepressant treatment in major depressive disorder. Psychiatry Clin Neurosci 59:140-145

Lehner M, Taracha E, Turzynska D, Sobolewska A, Hamed A, Kolomanska P, Skórzewska A, Maciejak P, Szyndler J, Bidzinski A, Plaznik A (2008)
The role of the dorsomedial part of the prefrontal cortex serotonergic innervation in rat responses to the aversively conditioned context: behavioral, biochemical and immunocytochemical studies. Behav Brain Res 192:203-215

Leja-Szpak A, Jaworek J, Tomaszewska R, Nawrot K, Bonior J, Kot M, Palonek M, Stachura J, Czupryna A, Konturek SJ, Pawlik WW (2004)
Melatonin precursor; L-tryptophan protects the pancreas from development of acute pancreatitis through the central site of action. J Physiol Pharmacol 55:239-254

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL (1996)
Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274:1527-1531

Lesch KP (2004)
Gene-environment interaction and the genetics of depression. J Psychiatry Neurosci 29:174-184

Lesch KP, Gutknecht L (2005)
Pharmacogenetics of the serotonin transporter. Prog Neuropsychopharmacol Biol Psychiatry 29:1062-1073

Levine ES, Jacobs BL (1992)
Neurochemical afferents controlling the activity of serotonergic neurons in the dorsal raphe nucleus: microiontophoretic studies in the awake cat. J Neurosci 12:4037-4044

Levinson DF (2006)
The genetics of depression: a review. Biol Psychiatry 60:84-92

Li D, He L (2006)
Further clarification of the contribution of the tryptophan hydroxylase (TPH) gene to suicidal behavior using systematic allelic and genotypic meta-analyses. Hum Genet 119:233-240

Licinio J, Wong ML (2005)
Depression, antidepressants and suicidality: a critical appraisal. Nat Rev Drug Discov 4:165-171

Lidov HG, Molliver ME (1982)
Immunohistochemical study of the development of serotonergic neurons in the rat CNS. Brain Res Bull 9:559-604

Lin E, Chen PS (2008)
*Pharmacogenomics with antidepressants in the STAR*D study.* Pharmacogenomics 9:935-946

Lindsay RM (1994)
Neurotrophic growth factors and neurodegenerative diseases: therapeutic potential of the neurotrophins and ciliary neurotrophic factor. Neurobiol Aging 15:249-251

Lindvall O, Kokaia Z, Bengzon J, Elmér E, Kokaia M (1994)
Neurotrophins and brain insults. Trends Neurosci 17:490-496

Little KY, McLaughlin DP, Zhang L, Livermore CS, Dalack GW, McFinton PR, DelProposto ZS, Hill E, Cassin BJ, Watson SJ, Cook EH (1998)
Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. Am J Psychiatry 155:207-213

Liu RJ, Lambe EK, Aghajanian GK (2005)
Somatodendritic autoreceptor regulation of serotonergic neurons: dependence on L-tryptophan and tryptophan hydroxylase-activating kinases. Eur J Neurosci 21:945-958

Liu R, Jolas T, Aghajanian G (2000)
Serotonin 5-HT(2) receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. Brain Res 873:34-45

Lloyd KG, Thuret F, Pilc A (1985)
Upregulation of gamma-aminobutyric acid (GABA) B binding sites in rat frontal cortex: a common action of repeated administration of different classes of antidepressants and electroshock. J Pharmacol Exp Ther 235:191-199

Lorens SA, Guldberg HC (1974)
Regional 5-hydroxytryptamine following selective midbrain raphe lesions in the rat. Brain Res 78:45-56

Lucki I, Dalvi A, Mayorga AJ (2001)
Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. Psychopharmacologia 155:315-322

Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW, Bora SH, Wihler C, Koliatsos VE, Tessarollo L (1999)
Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc Natl Acad Sci U S A 96:15239-15244

López-Rubalcava C, Lucki I (2000)
Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. Neuropsychopharmacology 22:191-199

Lönnroth P, Jansson PA, Smith U (1987)
A microdialysis method allowing characterization of intercellular water space in humans. Am J Physiol 253:E228-E231

Maier SF, Watkins LR (2005)
Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci Biobehav Rev 29:829-841

Malberg JE, Blendy JA (2005)
Antidepressant action: to the nucleus and beyond. Trends Pharmacol Sci 26:631-638

Malison RT, Price LH, Berman R, van Dyck CH, Pelton GH, Carpenter L, Sanacora G, Owens MJ, Nemeroff CB, Rajeevan N, Baldwin RM, Seibyl JP, Innis RB, Charney DS (1998)
Reduced brain serotonin transporter availability in major depression as measured by [123I]-2 beta-carbomethoxy-3 beta-(4-iodophenyl)tropane and single photon emission computed tomography.
Biol Psychiatry 44:1090-1098

Malkesman O, Maayan R, Weizman A, Weller A (2006)
Aggressive behavior and HPA axis hormones after social isolation in adult rats of two different genetic animal models for depression. Behav Brain Res 175:408-414

Mamounas LA, Blue ME, Siuciak JA, Altar CA (1995)
Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci 15:7929-7939

Mamounas LA, Altar CA, Blue ME, Kaplan DR, Tessarollo L, Lyons WE (2000)
BDNF promotes the regenerative sprouting, but not survival, of injured serotonergic axons in the adult rat brain. J Neurosci 20:771-782

Mann JJ, Huang YY, Underwood MD, Kassir SA, Oppenheim S, Kelly TM, Dwork AJ, Arango V (2000)
A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. Arch Gen Psychiatry 57:729-738

Marano CM, Phatak P, Vemulapalli UR, Sasan A, Nalbandyan MR, Ramanujam S, Soekadar S, Demosthenous M, Regenold WT (2007)
Increased plasma concentration of brain-derived neurotrophic factor with electroconvulsive therapy: a pilot study in patients with major depression. J Clin Psychiatry 68:512-517

Martin JR, Bös M, Jenck F, Moreau J, Mutel V, Sleight AJ, Wichmann J, Andrews JS, Berendsen HH, Broekkamp CL, Ruigt GS, Köhler C, Delft AM (1998)
5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. J Pharmacol Exp Ther 286:913-924

Martinowich K, Manji H, Lu B (2007)
New insights into BDNF function in depression and anxiety. Nat Neurosci 10:1089-1093

Martín-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G, Artigas F (2001)
Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. J Neurosci 21:9856-9866

Martínez-Turrillas R, Del Río J, Frechilla D (2005)
Sequential changes in BDNF mRNA expression and synaptic levels of AMPA receptor subunits in rat hippocampus after chronic antidepressant treatment. Neuropharmacology 49:1178-1188

Matos FF, Urban C, Yocca FD (1996)
Serotonin (5-HT) release in the dorsal raphé and ventral hippocampus: raphé control of somatodendritic and terminal 5-HT release. J Neural Transm 103:173-190

Mattson MP, Maudsley S, Martin B (2004)
BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci 27:589-594

Mayberg HS (2003)
Positron emission tomography imaging in depression: a neural systems perspective. Neuroimaging Clin N Am 13:805-815

McAllister-Williams RH (2006)
Relapse prevention in bipolar disorder: a critical review of current guidelines. J Psychopharmacol 20:12-16

McArthur R, Borsini F (2006)
Animal models of depression in drug discovery: a historical perspective. Pharmacol Biochem Behav 84:436-452

McKinney WT, Bunney WE (1969)
Animal model of depression. I. Review of evidence: implications for research. Arch Gen Psychiatry 21:240-248

Meltzer HY, Li Z, Kaneda Y, Ichikawa J (2003)
Serotonin receptors: their key role in drugs to treat schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 27:1159-1172

Micale V, Arezzi A, Rampello L, Drago F (2006)
Melatonin affects the immobility time of rats in the forced swim test: the role of serotonin neurotransmission. Eur Neuropsychopharmacol 16:538-545

Michelsen KA, Schmitz C, Steinbusch HWM (2007)
The dorsal raphe nucleus--from silver stainings to a role in depression. Brain Res Rev 55:329-342

Middlemiss DN, Price GW, Watson JM (2002)
Serotonergic targets in depression. Curr Opin Pharmacol 2:18-22

Millan MJ (2006)
Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. Pharmacol Ther 110:135-370

Minov C, Baghai TC, Schüle C, Zwanzger P, Schwarz MJ, Zill P, Rupprecht R, Bondy B (2001)
Serotonin-2A-receptor and -transporter polymorphisms: lack of association in patients with major depression. Neurosci Lett 303:119-122

Miquel MC, Doucet E, Riad M, Adrien J, Vergé D, Hamon M (1992)
Effect of the selective lesion of serotoninergic neurons on the regional distribution of 5-HT1A receptor mRNA in the rat brain. Brain Res Mol Brain Res 14:357-362

Miró X, Pérez-Torres S, Artigas F, Puigdomènech P, Palacios JM, Mengod G (2002)
Regulation of cAMP phosphodiesterase mRNAs expression in rat brain by acute and chronic fluoxetine treatment. An in situ hybridization study. Neuropharmacology 43:1148-1157

Molliver ME (1987)
Serotonergic neuronal systems: what their anatomic organization tells us about function. J Clin Psychopharmacol 7:3S-23S

Molteni R, Calabrese F, Bedogni F, Tongiorgi E, Fumagalli F, Racagni G, Riva MA (2006)
Chronic treatment with fluoxetine up-regulates cellular BDNF mRNA expression in rat dopaminergic regions. Int J Neuropsychopharmacol 9:307-317

Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, Cryan JF (2004)
Genetic and pharmacological evidence of a role for GABA(B) receptors in the modulation of anxiety- and antidepressant-like behavior. Neuropsychopharmacology 29:1050-1062

Montgomery SA, Asberg M (1979)
A *new depression scale designed to be sensitive to change.* Br J Psychiatry 134:382-389

Moreno FA, Rowe DC, Kaiser B, Chase D, Michaels T, Gelernter J, Delgado PL (2002)
Association between a serotonin transporter promoter region polymorphism and mood response during tryptophan depletion. Mol Psychiatry 7:213-216

Moser PC, Sanger DJ (1999)
5-HT1A receptor antagonists neither potentiate nor inhibit the effects of fluoxetine and befloxatone in the forced swim test in rats. Eur J Pharmacol 372:127-134

Mrazek DA, Rush AJ, Biernacka JM, O'Kane DJ, Cunningham JM, Wieben ED, Schaid DJ, Drews MS, Courson VL, Snyder KA, Black JL, Weinshilboum RM (2008)
SLC6A4 variation and citalopram response. Am J Med Genet B Neuropsychiatr Genet

Mueller TI, Leon AC (1996)
Recovery, chronicity, and levels of psychopathology in major depression. Psychiatr Clin North Am 19:85-102

Mullins UL, Gianutsos G, Eison AS (1999)
Effects of antidepressants on 5-HT7 receptor regulation in the rat hypothalamus. Neuropsychopharmacology 21:352-367

Murphy DL, Lesch KP (2008)
Targeting the murine serotonin transporter: insights into human neurobiology. Nat Rev Neurosci 9:85-96

Murray CJ, Lopez AD (1997)
Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 349:1498-1504

Nakagawa Y, Sasaki A, Takashima T (1999)
The GABA(B) receptor antagonist CGP36742 improves learned helplessness in rats. Eur J Pharmacol 381:1-7

Nelson JC (2000)
Augmentation strategies in depression 2000. J Clin Psychiatry 61 Suppl 2:13-19

Nemeroff CB (2007)
The burden of severe depression: a review of diagnostic challenges and treatment alternatives. J Psychiatr Res 41:189-206

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002)
Neurobiology of depression. Neuron 34:13-25

Neumeister A (2003)
Tryptophan depletion, serotonin, and depression: where do we stand? Psychopharmacol Bull 37:99-115

Neumeister A, Konstantinidis A, Stastny J, Schwarz MJ, Vitouch O, Willeit M, Praschak-Rieder N, Zach J, de Zwaan M, Bondy B, Ackenheil M, Kasper S (2002)
Association between serotonin transporter gene promoter polymorphism (5HTTLPR) and behavioral responses to tryptophan depletion in healthy women with and without family history of depression. Arch Gen Psychiatry 59:613-620

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

Nibuya M, Nestler EJ, Duman RS (1996)
Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 16:2365-2372

Nishikawa T, Scatton B (1983)
Evidence for a GABAergic inhibitory influence on serotonergic neurons originating from the dorsal raphe. Brain Res 279:325-329

Nishikawa T, Scatton B (1985)
Inhibitory influence of GABA on central serotonergic transmission. Raphé nuclei as the neuroanatomical site of the GABAergic inhibition of cerebral serotonergic neurons. Brain Res 331:91-103

Nitta A, Ohmiya M, Sometani A, Itoh M, Nomoto H, Furukawa Y, Furukawa S (1999)
Brain-derived neurotrophic factor prevents neuronal cell death induced by corticosterone. J Neurosci Res 57:227-235

O'Leary OF, Bechtholt AJ, Crowley JJ, Hill TE, Page ME, Lucki I (2007)
Depletion of serotonin and catecholamines block the acute behavioral response to different classes of antidepressant drugs in the mouse tail suspension test. Psychopharmacologia 192:357-371

O'Neill MF, Conway MW (2001)
Role of 5-HT(1A) and 5-HT(1B) receptors in the mediation of behavior in the forced swim test in mice. Neuropsychopharmacology 24:391-398

Oluyomi AO, Gibson EL, Barnfield AM, Curzon G (1994)
d-Fenfluramine and d-norfenfluramine hypophagias do not require increased hypothalamic 5-hydroxytryptamine release. Eur J Pharmacol 264:111-115

Osborne PG, O'Connor WT, Drew KL, Ungerstedt U (1990)
An in vivo microdialysis characterization of extracellular dopamine and GABA in dorsolateral striatum of awake freely moving and halothane anaesthetised rats. J Neurosci Methods 34:99-105

Overstreet DH, Pucilowski O, Rezvani AH, Janowsky DS (1995)
Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of Flinders Sensitive Line rats as an animal model of depression. Psychopharmacologia 121:27-37

Overstreet DH, Friedman E, Mathé AA, Yadid G (2005)
The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. Neurosci Biobehav Rev 29:739-759

Owens MJ, Nemeroff CB (1994)
Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem 40:288-295

Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I (1999)
Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacologia 147:162-167

Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ (1998)
Transport of brain-derived neurotrophic factor across the blood-brain barrier. Neuropharmacology 37:1553-1561

Pariante CM, Lightman SL (2008)
The HPA axis in major depression: classical theories and new developments. Trends Neurosci. Sep;31(9):464-8

Paykel ES, Brugha T, Fryers T (2005)
Size and burden of depressive disorders in Europe. Eur Neuropsychopharmacol 15:411-423

Pazos A, Cortés R, Palacios JM (1985)
Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res 346:231-249

Penington NJ, Fox AP (1994)
Effects of LSD on Ca++ currents in central 5-HT-containing neurons: 5-HT1A receptors may play a role in hallucinogenesis. J Pharmacol Exp Ther 269:1160-1165

Perona MTG, Waters S, Hall FS, Sora I, Lesch KP, Murphy DL, Caron M, Uhl GR (2008)
Animal models of depression in dopamine, serotonin, and norepinephrine transporter knockout mice: prominent effects of dopamine transporter deletions. Behav Pharmacol 19:566-574

Petit-Demouliere B, Chenu F, Bourin M (2005)
Forced swimming test in mice: a review of antidepressant activity. Psychopharmacologia 177:245-255

Peña F, Tapia R (2000)
Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus in vivo: role of glutamate- and GABA-mediated neurotransmission and of ion channels. Neuroscience 101:547-561

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

Piñeyro G, Blier P (1999)
Autoregulation of serotonin neurons: role in antidepressant drug action. Pharmacol Rev 51:533-591

Pollock BG (2001)
Citalopram: a comprehensive review. Expert Opin Pharmacother 2:681-698

Porsolt RD, Le Pichon M, Jalfre M (1977**a**)
Depression: a new animal model sensitive to antidepressant treatments. Nature 266:730-732

Porsolt RD, Bertin A, Jalfre M (1977**b**)
Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229:327-336

Porsolt RD, Anton G, Blavet N, Jalfre M (1978)
Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 47:379-391

Pozzi L, Invernizzi R, Garavaglia C, Samanin R (1999)
Fluoxetine increases extracellular dopamine in the prefrontal cortex by a mechanism not dependent on serotonin: a comparison with citalopram. J Neurochem 73:1051-1057

Pozzi L, Acconcia S, Ceglia I, Invernizzi RW, Samanin R (2002)
Stimulation of 5-hydroxytryptamine (5-HT(2C)) receptors in the ventrotegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. J Neurochem 82:93-100

Prakhie IV, Oxenkrug GF (1998)
The effect of nifedipine, Ca(2+) antagonist, on activity of MAO inhibitors, N-acetylserotonin and melatonin in the mouse tail suspension test. Int J Neuropsychopharmacol 1:35-40

Pratt GD, Bowery NG (1993)
Repeated administration of desipramine and a GABAB receptor antagonist, CGP 36742, discretely up-regulates GABAB receptor binding sites in rat frontal cortex. Br J Pharmacol 110:724-735

Pérez V, Gilaberte I, Faries D, Alvarez E, Artigas F (1997)
Randomised, double-blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment. Lancet 349:1594-1597

Queree P, Sharp T (2006)
Electrophysiological evidence for the 5-HT2C receptor-mediated control of 5-HT cell firing in the anaesthetised rat. Proceeding of the British Pharmacological Society, http://www.pA2online.org/abstracts/Vol4Issue2abst050P.pdf.

Quitkin FM (1985)
The importance of dosage in prescribing antidepressants. Br J Psychiatry 147:593-597

Quitkin FM, Rabkin JG, Stewart JW, McGrath PJ, Harrison W (1986)
Study duration in antidepressant research: advantages of a 12-week trial. J Psychiatr Res 20:211-216

Raghavendra V, Kaur G, Kulkarni SK (2000)
Anti-depressant action of melatonin in chronic forced swimming-induced behavioral despair in mice, role of peripheral benzodiazepine receptor modulation. Eur Neuropsychopharmacol 10:473-481

Rakovska A, Giovannini MG, Della Corte L, Kalfin R, Bianchi L, Pepeu G (1998)
Neurotensin modulation of acetylcholine and GABA release from the rat hippocampus: an in vivo microdialysis study. Neurochem Int 33:335-340

Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V, Blakely RD (1993)
Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. Proc Natl Acad Sci U S A 90:2542-2546

Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R (1998)
Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc Natl Acad Sci U S A 95:14476-14481

Rattray M, Michael GJ, Lee J, Wotherspoon G, Bendotti C, Priestley JV (1999)
Intraregional variation in expression of serotonin transporter messenger RNA by 5-hydroxytryptamine neurons. Neuroscience 88:169-183

Rea K, Cremers TIFH, Westerink BHC (2005)
HPLC conditions are critical for the detection of GABA by microdialysis. J Neurochem 94:672-679

Redrobe JP, Bourin M, Colombel MC, Baker GB (1998)
Dose-dependent noradrenergic and serotonergic properties of venlafaxine in animal models indicative of antidepressant activity. Psychopharmacologia 138:1-8

Ressler KJ, Mayberg HS (2007)
Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nat Neurosci 10:1116-1124

Rickels K, Amsterdam J, Clary C, Fox I, Schweizer E, Weise C (1992)
The efficacy and safety of paroxetine compared with placebo in outpatients with major depression. J Clin Psychiatry 53 Suppl:30-32

Robinson TE, Whishaw IQ (1988)
Normalization of extracellular dopamine in striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats. Brain Res 450:209-224

Rodrigues ALS, da Silva GL, Mateussi AS, Fernandes ES, Miguel OG, Yunes RA, Calixto JB, Santos ARS (2002)
Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of Siphocampylus verticillatus. Life Sci 70:1347-1358

Romero L, Artigas F (1997)
Preferential potentiation of the effects of serotonin uptake inhibitors by 5-HT1A receptor antagonists in the dorsal raphe pathway: role of somatodendritic autoreceptors. J Neurochem 68:2593-2603

Ruhé HG, Mason NS, Schene AH (2007)
Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol Psychiatry 12:331-359

Rush AJ, Trivedi M, Fava M (2003)
*Depression, IV: STAR*D treatment trial for depression.* Am J Psychiatry 160:237

Russo-Neustadt A, Ha T, Ramirez R, Kesslak JP (2001)
Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav Brain Res 120:87-95

Rutter JJ, Auerbach SB (1993)
Acute uptake inhibition increases extracellular serotonin in the rat forebrain. J Pharmacol Exp Ther 265:1319-1324

Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, Agerman K, Haapasalo A, Nawa H, Aloyz R, Ernfors P, Castrén E (2003)
Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. J Neurosci 23:349-357

Sakowski SA, Geddes TJ, Kuhn DM (2006)
Mouse tryptophan hydroxylase isoform 2 and the role of proline 447 in enzyme function. J Neurochem 96:758-765

Sanacora G, Mason GF, Rothman DL, Krystal JH (2002)
Increased occipital cortex GABA concentrations in depressed patients after therapy with selective serotonin reuptake inhibitors. Am J Psychiatry 159:663-665

Sanacora G, Mason GF, Rothman DL, Hyder F, Ciarcia JJ, Ostroff RB, Berman RM, Krystal JH (2003)
Increased cortical GABA concentrations in depressed patients receiving ECT. Am J Psychiatry 160:577-579

Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, Krystal JH, Mason GF (2004)
Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. Arch Gen Psychiatry 61:705-713

Sands SA, Reisman SA, Enna SJ (2004)
Effect of antidepressants on GABA(B) receptor function and subunit expression in rat hippocampus. Biochem Pharmacol 68:1489-1495

Sapolsky (1996)
Stress, Glucocorticoids, and Damage to the Nervous System: The Current State of Confusion. Stress 1:1-19

Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J, Gunn RN, Grasby PM, Cowen PJ (2000)
Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. Arch Gen Psychiatry 57:174-180

Sarna GS, Hutson PH, O'Connell MT, Curzon G (1991)
Effect of tryptophan on extracellular concentrations of tryptophan and 5-hydroxyindoleacetic acid in the striatum and cerebellum. J Neurochem 56:1564-1568

Savelieva KV, Zhao S, Pogorelov VM, Rajan I, Yang Q, Cullinan E, Lanthorn TH (2008)
Genetic disruption of both tryptophan hydroxylase genes dramatically reduces serotonin and affects behavior in models sensitive to antidepressants. PLoS ONE 3:e3301

Schechter LE, Ring RH, Beyer CE, Hughes ZA, Khawaja X, Malberg JE, Rosenzweig-Lipson S (2005)
Innovative approaches for the development of antidepressant drugs: current and future strategies. NeuroRx 2:590-611

Schildkraut JJ (1965)
The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry 122:509-522

Schousboe A, Westergaard N, Hertz L (1993)
Neuronal-astrocytic interactions in glutamate metabolism. Biochem Soc Trans 21:49-53

Schwarcz R (2004)
The kynurenine pathway of tryptophan degradation as a drug target. Curr Opin Pharmacol 4:12-17

Selvaraj S, Wylezinska M, Evans J, Jezzard P, Matthews PM, Cowen PJ (2006)
Tryptophan depletion does not lower brain GABA levels in healthy volunteers. Psychopharmacologia 187:131-132

Serrats J, Mengod G, Cortés R (2005)
Expression of serotonin 5-HT2C receptors in GABAergic cells of the anterior raphe nuclei. J Chem Neuroanat 29:83-91

Serretti A, Artioli P (2004)
From molecular biology to pharmacogenetics: a review of the literature on antidepressant treatment and suggestions of possible candidate genes. Psychopharmacologia 174:490-503

Serretti A, Benedetti F, Zanardi R, Smeraldi E (2005**a**)
The influence of Serotonin Transporter Promoter Polymorphism (SERTPR) *and other polymorphisms of the serotonin pathway on the efficacy of antidepressant treatments.* Prog Neuropsychopharmacol Biol Psychiatry 29:1074-1084

Serretti A, Artioli P, Quartesan R (2005**b**)
Pharmacogenetics in the treatment of depression: pharmacodynamic studies. Pharmacogenet Genomics 15:61-67

Serretti A, Kato M, Kennedy JL (2008)
Pharmacogenetic studies in depression: a proposal for methodologic guidelines. Pharmacogenomics J 8:90-100

Sharma H, Olsson Y., Dey P.K. (1990)
Changes in blood-brain barrier and cerebral blood flow following elevation of circulating serotonin level in anesthetized rats. Brain Res 517:215-223

Sharp T, Boothman L, Raley J, Quérée P (2007)
Important messages in the 'post': recent discoveries in 5-HT neurone feedback control. Trends Pharmacol Sci 28:629-636

Shaw K, Turner J, Del Mar C (2002)
Tryptophan and 5-hydroxytryptophan for depression. Cochrane Database Syst Rev :CD003198

Shelton RC, Tollefson GD, Tohen M, Stahl S, Gannon KS, Jacobs TG, Buras WR, Bymaster FP, Zhang W, Spencer KA, Feldman PD, Meltzer HY (2001)
A novel augmentation strategy for treating resistant major depression. Am J Psychiatry 158:131-134

Shelton R (2007)
The molecular neurobiology of depression. Psychiatr Clin North Am 30:1-11

Shen RY, Andrade R (1998)
5-Hydroxytryptamine2 receptor facilitates GABAergic neurotransmission in rat hippocampus. J Pharmacol Exp Ther 285:805-812

Shiah IS, Yatham LN (1998)
GABA function in mood disorders: an update and critical review. Life Sci 63:1289-1303

Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, Nakazato M, Watanabe H, Shinoda N, Okada SI, Iyo M (2003)
Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry 54:70-75

Shirayama Y, Chen ACH, Nakagawa S, Russell DS, Duman RS (2002)
Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22:3251-3261

Shirayama Y, Chaki S (2006)
Neurochemistry of the nucleus accumbens and its relevance to depression and antidepressant action in rodents. Current neuropharmacology 4:277-291

Siegal GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD (1999)
chapter 13: serotonin. In: Basic Neurochemistry, Molecular, Cellular, and Medical Aspects (Philadelphia, ed), Lippincott, Williams & Wilkins.

Singewald N, Sharp T (2000)
Neuroanatomical targets of anxiogenic drugs in the hindbrain as revealed by Fos immunocytochemistry. Neuroscience 98:759-770

Sinton CM, Fallon SL (1988)
Electrophysiological evidence for a functional differentiation between subtypes of the 5-HT1 receptor. Eur J Pharmacol 157:173-181

Siuciak JA, Boylan C, Fritsche M, Altar CA, Lindsay RM (1996)
BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. Brain Res 710:11-20

Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM (1997)
Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol Biochem Behav 56:131-137

Siuciak JA, Clark MS, Rind HB, Whittemore SR, Russo AF (1998)
BDNF induction of tryptophan hydroxylase mRNA levels in the rat brain. J Neurosci Res 52:149-158

Slattery DA, Desrayaud S, Cryan JF (2005)
GABAB receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. J Pharmacol Exp Ther 312:290-296

Smith M, Makino S, Kvetnansky R, Post R (1995)
Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15:1768-1777

Sobocki P, Jönsson B, Angst J, Rehnberg C (2006)
Cost of depression in Europe. J Ment Health Policy Econ 9:87-98

Sprouse JS, Aghajanian GK (1986)
(-)-Propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT1A selective agonists. Eur J Pharmacol 128:295-298

Stahl SM (1998)
Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. J Affect Disord 51:215-235

Stam NJ, Roesink C, Dijcks F, Garritsen A, van Herpen A, Olijve W (1997)
Human serotonin 5-HT7 receptor: cloning and pharmacological characterisation of two receptor variants. FEBS Lett 413:489-494

Starkey SJ, Skingle M (1994)
5-HT1D as well as 5-HT1A autoreceptors modulate 5-HT release in the guinea-pig dorsal raphé nucleus. Neuropharmacology 33:393-402

Steinbusch HW (1981)
Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience 6:557-618

Steru L, Chermat R, Thierry B, Simon P (1985)
The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacologia 85:367-370

Stimpson N, Agrawal N, Lewis G (2002)
Randomised controlled trials investigating pharmacological and psychological interventions for treatment-refractory depression. Systematic review. Br J Psychiatry 181:284-294

Stockmeier CA, Shapiro LA, Dilley GE, Kolli TN, Friedman L, Rajkowska G (1998)
Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. J Neurosci 18:7394-7401

Stone TW, Darlington LG (2002)
Endogenous kynurenines as targets for drug discovery and development. Nat Rev Drug Discov 1:609-620

Sullivan PF, Neale MC, Kendler KS (2000)
Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 157:1552-1562

Sunal R, Gümü_el B, Kayaalp SO (1994)
Effect of changes in swimming area on results of "behavioral despair test". Pharmacol Biochem Behav 49:891-896

Svenningsson P, Chergui K, Rachleff I, Flajolet M, Zhang X, El Yacoubi M, Vaugeois JM, Nomikos GG, Greengard P (2006)
Alterations in 5-HT1B receptor function by p11 in depression-like states. Science 311:77-80

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

Szapacs ME, Mathews TA, Tessarollo L, Ernest Lyons W, Mamounas LA, Andrews AM (2004)
Exploring the relationship between serotonin and brain-derived neurotrophic factor: analysis of BDNF protein and extraneuronal 5-HT in mice with reduced serotonin transporter or BDNF expression. J Neurosci Methods 140:81-92

Tao R, Hjorth S (1992)
Differences in the in vitro and in vivo 5-hydroxytryptamine extraction performance among three common microdialysis membranes. J Neurochem 59:1778-1785

Tao R, Ma Z, Auerbach SB (1996)
Differential regulation of 5-hydroxytryptamine release by GABAA and GABAB receptors in midbrain raphe nuclei and forebrain of rats. Br J Pharmacol 119:1375-1384

Tao R, Ma Z, Auerbach SB (2000)
Differential effect of local infusion of serotonin reuptake inhibitors in the raphe versus forebrain and the role of depolarization-induced release in increased extracellular serotonin. J Pharmacol Exp Ther 294:571-579

Tao R, Auerbach SB (2002)
GABAergic and glutamatergic afferents in the dorsal raphe nucleus mediate morphine-induced increases in serotonin efflux in the rat central nervous system. J Pharmacol Exp Ther 303:704-710

Tao R, Auerbach SB (2003)
Influence of inhibitory and excitatory inputs on serotonin efflux differs in the dorsal and median raphe nuclei. Brain Res 961:109-120

Tatarczy_ska E, K_odzi_ska A, Stachowicz K, Chojnacka-Wójcik E (2004)
Effect of combined administration of 5-HT1A or 5-HT1B/1D receptor antagonists and antidepressants in the forced swimming test. Eur J Pharmacol 487:133-142

Taylor M, Bhagwagar Z, Cowen PJ, Sharp T (2003)
GABA and mood disorders. Psychol Med 33:387-393

Tejani-Butt S, Kluczynski J, Paré WP (2003)
Strain-dependent modification of behavior following antidepressant treatment. Prog Neuropsychopharmacol Biol Psychiatry 27:7-14

Tenner K, Qadri F, Bert B, Voigt JP, Bader M (2008)
The mTPH2 C1473G single nucleotide polymorphism is not responsible for behavioural differences between mouse strains. Neurosci Lett 431:21-25

Thase ME, Rush AJ (1997)
When at first you don't succeed: sequential strategies for antidepressant nonresponders. J Clin Psychiatry 58 Suppl 13:23-29

Thoenen H (1991)
The changing scene of neurotrophic factors. Trends Neurosci. May;14(5):165-70.

Thomas DR, Nelson DR, Johnson AM (1987)
Biochemical effects of the antidepressant paroxetine, a specific 5-hydroxytryptamine uptake inhibitor. Psychopharmacologia 93:193-200

Timmerman W, Westerink BH (1997)
Brain microdialysis of GABA and glutamate: what does it signify? Synapse 27:242-261

Trillat AC, Malagié I, Mathé-Allainmat M, Anmela MC, Jacquot C, Langlois M, Gardier AM (1998)
Effects of WAY 100635 and (-)-5-Me-8-OH-DPAT, a novel 5-HT1A receptor antagonist, on 8-OH-DPAT responses. Eur J Pharmacol 347:41-49

Tsai SJ, Cheng CY, Yu YWY, Chen TJ, Hong CJ (2003)
Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. Am J Med Genet B Neuropsychiatr Genet 123B:19-22

Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006)
Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 9:519-525

Turner EH, Matthews AM, Linardatos E, Tell RA, Rosenthal R (2008)
Selective publication of antidepressant trials and its influence on apparent efficacy. N Engl J Med 358:252-260

Tzvetkov MV, Brockmöller J, Roots I, Kirchheiner J (2008)
Common genetic variations in human brain-specific tryptophan hydroxylase-2 and response to antidepressant treatment. Pharmacogenet Genomics 18:495-506

Törk I (1990)
Anatomy of the serotonergic system. Ann N Y Acad Sci 600:9-34; discussion 34

Van Den Bogaert A, Sleegers K, De Zutter S, Heyrman L, Norrback KF, Adolfsson R, Van Broeckhoven C, Del-Favero J (2006)
Association of brain-specific tryptophan hydroxylase, TPH2, with unipolar and bipolar disorder in a Northern Swedish, isolated population. Arch Gen Psychiatry 63:1103-1110

Van der Does AJ (2001)
The effects of tryptophan depletion on mood and psychiatric symptoms. J Affect Disord 64:107-119

van der Zeyden M, Oldenziel WH, Rea K, Cremers TI, Westerink BH (2008)
Microdialysis of GABA and glutamate: analysis, interpretation and comparison with microsensors. Pharmacol Biochem Behav 90:135-147

Varga V, Székely AD, Csillag A, Sharp T, Hajós M (2001)
Evidence for a role of GABA interneurones in the cortical modulation of midbrain 5-hydroxytryptamine neurones. Neuroscience 106:783-792

Varga V, Kocsis B, Sharp T (2003)
Electrophysiological evidence for convergence of inputs from the medial prefrontal cortex and lateral habenula on single neurons in the dorsal raphe nucleus. Eur J Neurosci 17:280-286

Ventimiglia R, Mather PE, Jones BE, Lindsay RM (1995)
The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. Eur J Neurosci 7:213-222

Vollmayr B, Henn FA (2001)
Learned helplessness in the rat: improvements in validity and reliability. Brain Res Brain Res Protoc 8:1-7

Volosin M, Song W, Almeida RD, Kaplan DR, Hempstead BL, Friedman WJ (2006)
Interaction of survival and death signaling in basal forebrain neurons: roles of neurotrophins and proneurotrophins. J Neurosci 26:7756-7766

Wallace JA, Lauder JM (1983)
Development of the serotonergic system in the rat embryo: an immunocytochemical study. Brain Res Bull 10:459-479

Walther DJ, Peter JU, Bashammakh S, Hörtnagl H, Voits M, Fink H, Bader M (2003)
Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science 299:76

Wang RY, Aghajanian GK (1977**a**)
Antidromically identified serotonergic neurons in the rat midbrain raphe: evidence for collateral inhibition. Brain Res 132:186-193

Wang RY, Aghajanian GK (1977**b**)
Physiological evidence for habenula as major link between forebrain and midbrain raphe. Science 197:89-91

Wang QP, Ochiai H, Nakai Y (1992)
GABAergic innervation of serotonergic neurons in the dorsal raphe nucleus *of the rat studied by electron microscopy double immunostaining.* Brain Res Bull 29:943-948

Warden D, Rush AJ, Trivedi MH, Fava M, Wisniewski SR (2007)
*The STAR*D Project results: a comprehensive review of findings.* Curr Psychiatry Rep 9:449-459

Waselus M, Galvez JP, Valentino RJ, Van Bockstaele EJ (2006)
Differential projections of dorsal raphe nucleus neurons to the lateral septum and striatum. J Chem Neuroanat 31:233-242

Weber LJ, Horita A (1965)
A study of 5-hydroxytryptamine formation from L-tryptophan in the brain and other tissues. Biochem Pharmacol 14:1141-1149

Weissbach H, Redfield BG, Axelrod J (1960)
Biosynthesis of melatonin: enzymic conversion of serotonin to N-acetylserotonin. Biochim Biophys Acta 43:352-353

Weissmann-Nanopoulos D, Mach E, Magre J, Demassey Y, Pujol J (1985)
Evidence for the localization of 5HT1A binding sites on serotonin containing neurons in the raphe dorsalis and raphe centralis nuclei of the rat brain. Neurochemistry Int. 7:1061-1072

Werner P, Kawashima E, Reid J, Hussy N, Lundström K, Buell G, Humbert Y, Jones KA (1994)
Organization of the mouse 5-HT3 receptor gene and functional expression of two splice variants. Brain Res Mol Brain Res 26:233-241

Westerink BH, De Vries JB (1991)
Effect of precursor loading on the synthesis rate and release of dopamine and serotonin in the striatum: a microdialysis study in conscious rats. J Neurochem 56:228-233

Westerink BH (1995)
Brain microdialysis and its application for the study of animal behaviour. Behav Brain Res 70:103-124

White KJ, Walline CC, Barker EL (2005)
Serotonin transporters: implications for antidepressant drug development. AAPS J 7:E421-E433

Willner P (1995)
Animal models of depression: validity and applications. Adv Biochem Psychopharmacol 49:19-41

Willner P (1997)
Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacologia 134:319-329

Willner P, Mitchell PJ (2002)
The validity of animal models of predisposition to depression. Behav Pharmacol 13:169-188

Wong ML, Licinio J (2001)
Research and treatment approaches to depression. Nat Rev Neurosci 2:343-351

Wong ML, Licinio J (2004)
From monoamines to genomic targets: a paradigm shift for drug discovery in depression. Nat Rev Drug Discov 3:136-151

Wong PT, Ong YP (2001)
Acute antidepressant-like and antianxiety-like effects of tryptophan in mice. Pharmacology 62:151-156

World Health Organization (1992)
ICD-10: The ICD-10 Classification of Mental and Behavioural Disorders: Clinical Descriptions and Diagnostic Guidelines Gaskell, Royal College of Psychiatrists, Geneva.

Wright IK, Garratt JC, Marsden CA (1990)
Effects of a selective 5-HT2 agonist, DOI, on 5-HT neuronal firing in the dorsal raphe nucleus and 5-HT release and metabolism in the frontal cortex. Br J Pharmacol 99:221-222

Wright DE, Seroogy KB, Lundgren KH, Davis BM, Jennes L (1995)
Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. J Comp Neurol 351:357-373

Wu WH, Huo SJ, Cheng CY, Hong CJ, Tsai SJ (2001)
Association study of the 5-HT(6) receptor polymorphism (C267T) and symptomatology and antidepressant response in major depressive disorders. Neuropsychobiology 44:172-175

Wurtman RJ (2005)
Genes, stress, and depression. Metabolism 54:16-19

Xi ZX, Baker DA, Shen H, Carson DS, Kalivas PW (2002)
Group II metabotropic glutamate receptors modulate extracellular glutamate in the nucleus accumbens. J Pharmacol Exp Ther 300:162-171

Xi ZX, Shen H, Baker DA, Kalivas PW (2003)
Inhibition of non-vesicular glutamate release by group III metabotropic glutamate receptors in the nucleus accumbens. J Neurochem 87:1204-1212

Yalcin I, Belzung C, Surget A (2008)
Mouse strain differences in the unpredictable chronic mild stress: a four-antidepressant survey. Behav Brain Res 193:140-143

Yang J, Siao CJ, Nagappan G, Marinic T, Jing D, McGrath K, Chen ZY, Mark W, Tessarollo L, Lee FS, Lu B, Hempstead BL (2009)
Neuronal release of proBDNF. Nat Neurosci 12:113-115

Yoshida K, Takahashi H, Higuchi H, Kamata M, Ito KI, Sato K, Naito S, Shimizu T, Itoh K, Inoue K, Suzuki T, Nemeroff CB (2004)
Prediction of antidepressant response to milnacipran by norepinephrine transporter gene polymorphisms. Am J Psychiatry 161:1575-1580

Zellweger MJ, Osterwalder RH, Langewitz W, Pfisterer ME (2004)
Coronary artery disease and depression. Eur Heart J 25:3-9

Zetterström T, Sharp T, Marsden CA, Ungerstedt U (1983)
In vivo measurement of dopamine and its metabolites by intracerebral dialysis: changes after d-amphetamine. J Neurochem 41:1769-1773

Zetterström TS, Pei Q, Madhav TR, Coppell AL, Lewis L, Grahame-Smith DG (1999)
Manipulations of brain 5-HT levels affect gene expression for BDNF in rat brain. Neuropharmacology 38:1063-1073

Zhang X, Beaulieu JM, Sotnikova TD, Gainetdinov RR, Caron MG (2004)
Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science 305:217

Zhang X, Gainetdinov RR, Beaulieu JM, Sotnikova TD, Burch LH, Williams RB, Schwartz DA, Krishnan KRR, Caron MG (2005)
Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. Neuron 45:11-16

Zhou J, Li L, Tang S, Cao X, Li Z, Li W, Li C, Zhang X (2008)
Effects of serotonin depletion on the hippocampal GR/MR and BDNF expression during the stress adaptation. Behav Brain Res 195:129-138

Zill P, Baghai TC, Zwanzger P, Schüle C, Eser D, Rupprecht R, Möller HJ, Bondy B, Ackenheil M (2004**a**)
SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. Mol Psychiatry 9:1030-1036

Zill P, Büttner A, Eisenmenger W, Möller HJ, Bondy B, Ackenheil M (2004**b**)
Single nucleotide polymorphism and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene in suicide victims. Biol Psychiatry 56:581-586

Zill P, Büttner A, Eisenmenger W, Möller HJ, Ackenheil M, Bondy B (2007)
Analysis of tryptophan hydroxylase I and II mRNA expression in the human brain: a post-mortem study. J Psychiatr Res 41:168-173

Zilles K, Wu J, Crusio WE, Schwegler H (2000)
Water maze and radial maze learning and the density of binding sites of glutamate, GABA, and serotonin receptors in the hippocampus of inbred mouse strains. Hippocampus 10:213-225