

1.1 Introductory and Basic Aspects

Neurobiology of Mood Disorders

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1.1.1 Definition of mood disorders, impact on a global scale and unmet needs

The diagnosis of “mood disorders” or “depressive disorders” has evolved over the past 40 years with progressively more precise definitions in each edition of the Diagnostic and Statistical Manual of Mental Disorders (e.g., the “Text Revision” of the Fourth Edition: DSM-IV-TR; American Psychiatric Association, 2000) and International Classification of Diseases and Related Health Problems (ICD-10; WHO, 1993).

The group “depressive disorders” can be divided into three major illnesses:

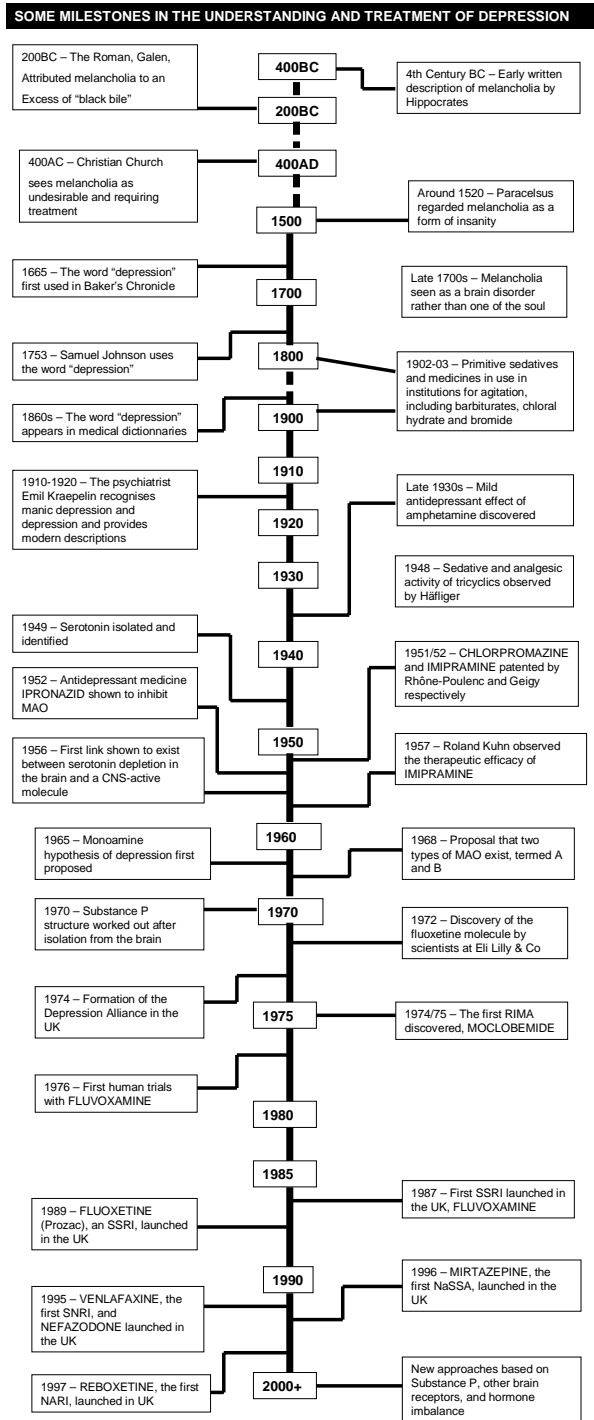
- Major (unipolar) depressive disorder
- Dysthymic disorder
- Bipolar disorder, also known as Manic-Depressive disorder

Depressive disorders arise from the complex interaction of multiple-susceptibility genes and environmental factors, and disease phenotypes include not only episodic and often profound mood disturbances, but also a range of cognitive, motor, autonomic, endocrine and sleep/wake abnormalities (Manji et al., 2001). Some milestones regarding recognition and treatment of depressive disorders are summarized in Fig. 1.

1.1.1.1 Major (unipolar) depressive disorder

Major depression is a chronic, recurring and potentially life-threatening illness with varied origins, a broad range of symptoms (Box 1), complex genetics and obscure neurobiology that affects up to 20% of the population across the globe (lifetime prevalence), being 2–5 times as high in women as in men (Ayuso-Mateos et al., 2001; Manji et al., 2001; Nestler et al., 2002; Charney, 2004; Lesch, 2004; Gillespie and Nemeroff, 2005; Table 1). This means that in any one year, 19 million people in the United States alone, and 121 million people worldwide, will suffer from some kind of diagnosable depressive

Fig. 1. Milestones of depressive disorders



Box. 1. Diagnostic criteria for depression

Diagnostic Statistical Manual of Mental Disorders defines a "major depressive episode" as being characterized by at least five of the following nine symptoms:

- Depressed or irritable mood
- Decreased interest in pleasurable activities and ability to experience pleasure
- Significant weight gain or loss (>5% change in a month)
- Insomnia or hypersomnia; early-morning awakening
- Psychomotor agitation or retardation
- Fatigue or loss of energy
- Feelings of worthlessness or excessive guilt
- Diminished ability to think or concentrate
- Recurrent thoughts of death or suicide

Each symptom must be evident daily or almost every day for at least 2 weeks. The severity of the disorder can range from the very mild to the very severe, and it occurs most commonly in episodes.

"Melancholic" subtype describes particularly severe cases, with prominent circadian variations in symptoms. Manifestations of psychosis (for example, hallucinations or delusions) and anxiety are also seen in many individuals with depression. Individuals with relatively mild but prolonged symptoms, those which persist for at least 2 years, are considered to have "dysthymia".

"Adjustment disorder with depressed mood" describes depressive symptoms that occur after a significant trauma (loss of a job, serious illness, death of a loved one, breakup of a marriage, etc.) and that last for an abnormally long time. If it does not resolve, adjustment disorder can progress to major depression.

"Subsyndromal depression" (or minor depression), is less severe than major depression but carries an increased risk for major depression.

"Seasonal affective disorder" (SAD) strikes during the winter months. SAD is characterized by lethargy, joylessness, hopelessness, anxiety and social withdrawal as well as increased need for sleep and weight gain.

"Premenstrual dysphoric disorder" (PMDD) is a severe distressing and debilitating condition that affects 3-5% of all women. Symptoms – typically depression, anxiety, cognitive and physical manifestations – may occur only during the premenstrual period or may become exacerbated during that time.

Some depressed patients have a variation of the disorder called "bipolar disorder", also known as manic-depressive illness. Bipolar affective disorder refers to patients with depressive "low" episodes alternating with periods of mania, or inappropriate "highs", characterized by an elated mood, increased activity, over-confidence, also known as mania, and impaired concentration.

Other subtypes of depression include "postpartum depression", "psychotic depression" and "atypical depression"

The range of symptoms that comprise depression and the range of diagnostic categories highlight the probable heterogeneity of the illness and the difficulty in establishing any given diagnosis with certainty (Criteria adapted from Diagnostic Statistical Manual of Mental Disorders, 2000; Gruenberg et al., 2005; Berton and Nestler, 2006).

disorders. It is now the leading cause of disability globally and ranks fourth in the top ten leading causes of the global

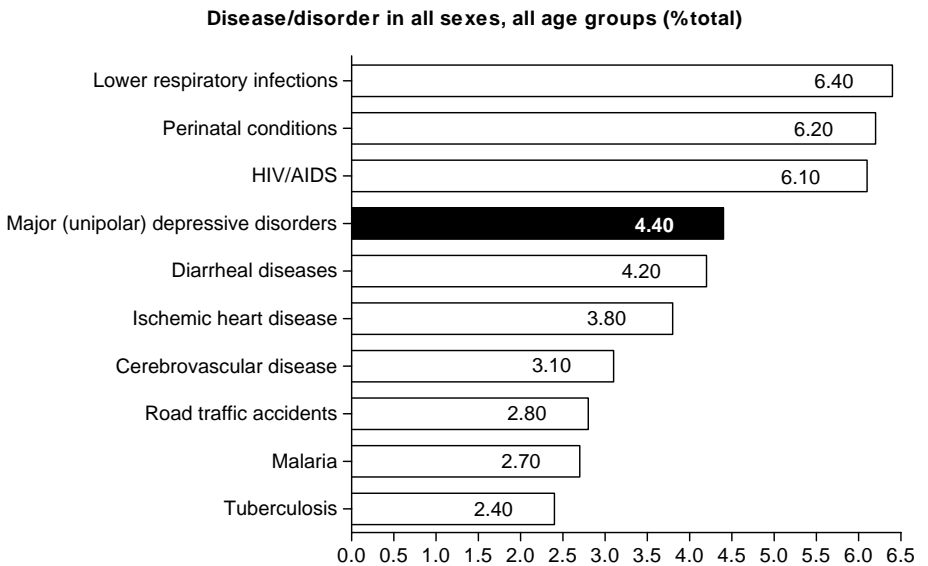
burden of disease based on a survey by the World Health Organization (Figs. 2, 3).

Table 1. Prevalence of major depression.

	Single point	6–12 months	Lifetime
Unipolar depression	3.9% (1.5–6.0)	4.7% (2.6–9.0)	12.6% (4.4–19.5)
Bipolar depression	0.7% (0–2.3)	1.5% (0.1–4.0)	1.3% (0.6–3.3)

Data from 16 epidemiological studies (6 Europe, 2 Canada, 7 USA; Angst, 1995).

Fig. 2. Depression, a major cause of disability worldwide: leading causes of Disability-Adjusted Life Years (DALYs-2000; adapted from WHO Mental Health Report, 2001).



It has been estimated that, by 2020, unipolar depression will have the dubious distinction of becoming the second cause of the global disease burden (Table 2), a position that the disease already holds for American women.

In 2000, the economic burden (direct and indirect) of depressive disorders in the United States was estimated to be \$83.1 billion (Greenberg et al., 2003) and it is estimated that only around 50% of patients suffering from major depressive disorder are assigned the correct diagnosis and go on to receive appropriate treatment. Of those that do receive suitable treatment, approximately 50% reach full remission (Table 3). The recurrence rate for those who recover from the first episode is typically

around 35% within 2 years and increases to 60% in 12 years. After three episodes, the likelihood of recurrence is 90%.

Fig. 3. Leading causes of Years of Life Lived with Disability (YLDs-2000; adapted from WHO Mental Health Report 2001).

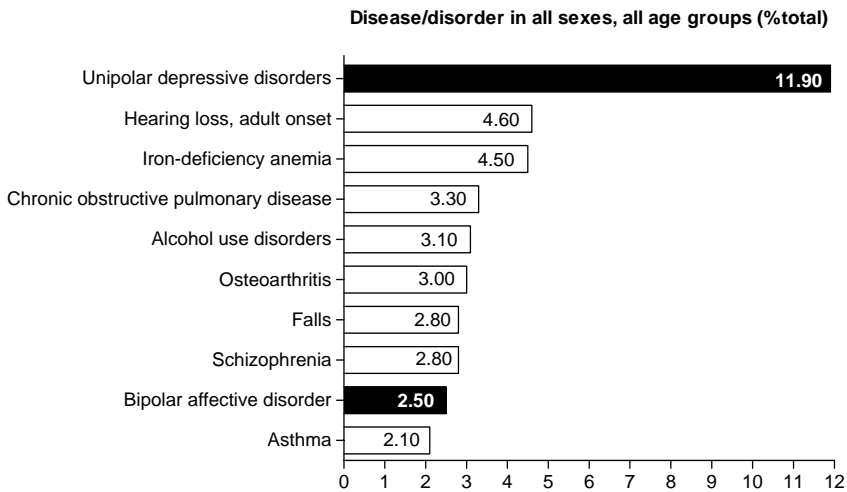


Table 2. Leading causes of Disability-Adjusted Life Years (DALYs-Estimated 2020).

RANK	2020 (Murray and Lopez, 1996)
1	Ischemic heart disease
2	Unipolar major depression
3	Road traffic accidents
4	Cerebrovascular disease
5	Chronic obstructive pulmonary disease

Table 3. Effectiveness of interventions for depression (Mynors-Wallis, 1996; Schulberg et al., 1996).

Intervention	% remission after 3–8 months
Placebo	27
Tricyclics	48–52
Psychotherapy (cognitive or interpersonal)	48–60

During 2002, the market of antidepressants accounted for around \$17 billion of the \$49 billion market for the Central Nervous System (CNS).

One of the particularly tragic outcomes of a depressive disorder is suicide. Approximately 15–20% of depressive patients end their own lives (Goodwin and Jamison, 1990; Angst et al., 1999; Ebmeier et al., 2006). About 60% of all

suicides occur in relation to mood disorders (Mann, 2003). Suicide remains one of the most common and unavoidable outcomes of depression and is now the eighth leading cause of death in the United States.

Several lines of evidence indicate an important contribution of depression to medical morbidity. Depressive disorders often co-occur with anxiety disorders, bulimia, anorexia and substance abuse. Depression is also associated with an increased risk of coronary heart disease (Ferketich et al., 2000) and depressed patients have an increased risk of premature death compared with control subjects (Harris and Barraclough, 1998).

To date, no single antidepressant drug is effective in all patients treated, probably due to the heterogeneity of the disease and to individual differences in the response to the agents used. Moreover, all antidepressant pharmacological treatments need to be administered for several weeks before amelioration signs begin to emerge.

To summarize, depression is a common mental disorder, causing a very high level of disease burden, and is expected to be a rising trend during the coming 15 years. Nowadays, antidepressant drugs of choice are, in the first instance, serotonin selective reuptake inhibitors, such as fluoxetine, paroxetine, fluvoxamine, citalopram and sertraline (NICE, 2004). The main risks associated with serotonin selective reuptake inhibitors are treatment-emergent suicidal behavior and withdrawal symptoms, especially in children and adolescents (Weller et al., 2004). Moreover, existing antidepressant treatments exhibit limited efficacy and a slow onset of action.

The search for an adequate treatment of major depression is one of the main challenges of Neuropharmacology.

1.1.1.2 Dysthymic disorder

Dysthymic disorder (or dysthymia) is a less severe but more prolonged form of depression that lasts for years. A person with dysthymia is unable to obtain any enjoyment from life. Dysthymic adults may be pessimistic, guilt-ridden, irritable, easily hurt by others or withdrawn. According to the American Medical Association, the condition is diagnosed when an individual has a generally depressed mood for most of the day, the majority of the time and for a period of at least two years (one year in a child). Children with the condition may be irritable, cranky, difficult, generally sad or have low self-esteem.

Many patients with dysthymia will at some point experience a superimposed major depressive episode. This condition has been termed *double depression*. Double depression is experienced by as many as 75% of all dysthymic individuals at some point in their lives (Kocsis, 2000).

1.1.1.3 Bipolar disorder (manic depressive disorder)

Bipolar disorder is characterized by cycling mood changes: severe “lows” (depression; Box 1) and “highs” (mania; Box 2).

Box 2. Diagnostic criteria for manic phase of bipolar disorders

Symptoms of mania include abnormally and persistently elevated mood accompanied by at least three of the following:

- Abnormal or excessive elation
- Unusual irritability
- Decreased need for sleep
- Grandiose notions
- Increased talkativeness
- Racing thoughts
- Excessive involvement in risky behaviors or activities, including increased sexual behavior, and use of alcohol and illicit drugs
- Increased energy, activity or restlessness
- Poor judgment
- Overly-inflated self-esteem
- Distractibility or irritability
- Inappropriate social behavior (provocative, intrusive or aggressive behavior)
- Denial that anything is wrong

Criteria adapted from Diagnostic Statistical Manual of Mental Disorders, 2000

According to the National Institutes of Mental Health, bipolar disorder affects approximately 2.3 million adult Americans. The lifetime prevalence of bipolar disorder is in the range 1.3–1.6% and does not differ significantly by age, sex, race or ethnicity. There is an exception in rapid cycling, a severe and difficult to treat variant of the disorder, which arises mostly in women (Muller-Oerlinhausen et al., 2002). Bipolar disorder is believed to be widely under-recognized in the primary care setting, which implies that its prevalence could be much higher (Das et al., 2005; Kupfer, 2005).

1.1.2 Causes and associations in mood disorders: genetics and pharmacogenetics

Depression and other mood disorders tend to proliferate as a result of multiple, complex, biological, psychological and social factors, including war and other violent conflicts, natural disasters, poverty and limited access to resources. A combination of genetic and environmental factors join to unchain depression (Sullivan et al., 2000; Wong and Licinio, 2001; Lesch, 2004; Hamet and Tremblay, 2005; Ebmeier et al., 2006). Multiple susceptibility genes of major or small effect, in interaction with each other and in conjunction with environmental events, produce vulnerability to the disorder (Fig. 4).

A significant determinant of depression is the individual's "personal threshold," or vulnerability to depression. Some people are more likely than others to become depressed, but no one is immune. Depression can affect individuals at any stage of the life span, although the incidence is highest during middle age. There is, however, an increasing recognition of depression during adolescence and young adulthood (Lewinsohn et al., 1993; Taylor et al., 2002).

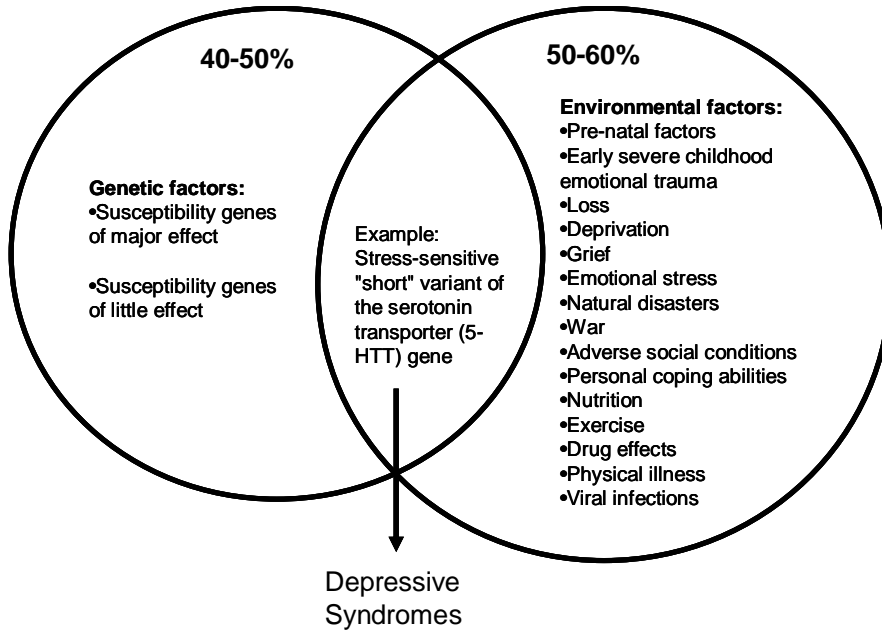
Based on family aggregation and contrasting results from studies in monozygotic and dizygotic twins, major depression has an estimated heritability of 40–50% (major depression) and 40–80% (bipolar disorders), although the specific genes that underlie this risk have not yet been identified. It does not show classic Mendelian inheritance that could be attributable to a single gene. Depression has therefore been classified as a genetically complex disorder, polygenic and epistatic, much like heart disease, hypertension, diabetes and cancer (Lander and Schork, 1994; Sullivan et al., 2000).

Depression with recurrent episodes and possibly early onset may have higher genetic influence and may be thus associated with greater familial aggregation (Sullivan et al., 2000).

Traditional genetic linkage studies and candidate gene methods have been used with fairly limited success in major depression. Genetic models of etiology generally assume a large number of genes with relatively small contributions to liability. Advances in high throughput genotyping and microarray techniques have made it more feasible to identify genes with small effect sizes (Hong and Tsai, 2003). Of special clinical interest are those studies that are based on pathophysiological notions (candidate

genes) and in particular those that examine the probability of patients to respond to particular treatments (Ebmeier et al., 2006).

Fig. 4. An integrative approach to depression: genetic and environmental factors (adapted from Wong and Licinio, 2001).



Genetic vulnerability may involve serotonin (5-HT) systems because tryptophan depletion in healthy subjects is reported to induce depressive symptoms only if they have affected relatives (Benkelfat et al., 1994). Allelic variations in the serotonin transporter (5-HTT) gene are of great interest in depression. A polymorphism in the 5' promoter region consisting of a short (s) and a long (l) allele with the s-allele being associated with decreased 5-HT transmembrane transport and reduced transporter mRNA has been described. The gene frequencies in Caucasians have been reported to be 41% s-allele and 59% l-allele (Mellerup et al., 2001). The s-allele has been associated with neuroticism in a number of studies (Bellivier et al., 2002), with depressive symptoms, diagnosable depression and suicidality in response to stressful life events in some, but not all, the cases and with the response of patients to selective serotonin reuptake inhibitors (SSRIs; Mann et al., 2000; Caspi et al., 2003). In a study, dietary depletion of circulating

tryptophan caused depressive symptoms only in subjects homozygous for the s-allele (Neumeister et al., 2002).

Some patients with depression carry a polymorphism, or genetic variant, in the FKBP5 gene (which encodes a co-chaperone of heat shock protein 90; HSP90) that results in higher affinity of glucocorticoid receptors for cortisol (Binder et al., 2004). These individuals respond much faster to antidepressants and have a higher recurrence of depressive episodes than individuals without this mutation.

Brain-derived neurotrophic factor (BDNF) may have a central role in the effectiveness of antidepressants, but there is no firm evidence of an association of its alleles with major depressive disorder (Hong et al., 2003; Tsai et al., 2003; Schumacher et al., 2005). Certain polymorphisms in the gene promoter of the monoamine oxidase A enzyme (involved in the metabolism of catecholamines and the target of one group of antidepressants, the monoamine oxidase inhibitors) are found in subgroups of patients with major depression or anxiety (Schulze et al., 2000; Du et al., 2004).

A polymorphism in the catechol-O-methyltransferase (COMT; another catecholamine metabolizing enzyme) is associated with treatment response to mirtazepine, but not to paroxetine in major depression (Szegedi et al., 2005).

A single nucleotide polymorphism in the gene of the human tryptophan hydroxylase-2 enzyme (involved in the synthesis of serotonin), with roughly 80% loss of function, is associated with major depression, but not with bipolar illness (Zhang et al., 2005).

A recent search for pharmacokinetic effects of cytochromes *CYP2D6* and *CYP2C19* alleles suggested that, for 14 of 20 investigated antidepressants, at least a doubling of the dose would be needed in extensive metabolizers compared with poor metabolizers. This variation in effects does strengthen the argument for antidepressant plasma monitoring in depression resistant to treatment (Kirchheiner et al., 2001).

No other genes have yet been convincingly linked to major depression (Ebmeier et al., 2006).

In regard to bipolar disorder, molecular genetic studies have reported many linkage loci and candidate genes. However, none of these findings have been consistently replicated. Meta-analyses of linkage studies have also reported conflicting results. Among recently reported candidate genes, BDNF, AKT1, XBP1, G72, GRK3,

GRIN2A, HTR4, IMPA2, GABRA1 and GABRA5 may have some importance (Sklar et al., 2002; Hong et al., 2003; for a review, see Kato et al., 2005).

Among the polymorphisms of monoamine-related genes, some were found to cause functional alteration and to be associated with bipolar disorder in two or more studies. These include monoamine oxidase A (MAO-A; Lim et al., 1995; Rubinsztein et al., 1996; Preisig et al., 2000), serotonin transporter (5-HTT; Collier et al., 1996a, b; Oruc et al., 1997; Furlong et al., 1998) and serotonin 2C receptor (5-HT_{2C}; Oruc et al., 1997; Lerer et al., 2001). Catechol-O-methyltransferase (COMT) is also included in such genes, although association was found only for ultra-ultra-rapid-cycling bipolar disorder (Kirov et al., 1998; Papolos et al., 1998).

Functional genomics using microarray technology has been used to identify more than 300 genes in animal and human brain affected by antidepressant drug treatments (Sibille and Hen, 2001; Yamada and Higuchi, 2002; Sibille et al., 2004) and this may offer insights for novel therapeutic targets.

Environmental contribution, 50–60%, also remains poorly defined, with suggestions that prenatal factors, early severe childhood emotional trauma, chronic physical illness and even viral infections might be involved (Wong and Licinio, 2001).

Severe obesity may be a causative factor for depression, according to a study that examined depression before and after surgically induced weight loss. Beck Depression Inventory (BDI) questionnaires were completed before and at yearly intervals after gastric-restrictive weight-loss surgery. Results showed that severely obese subjects, especially women with poor body image, were at high risk for depression, while weight loss was associated with reduced BDI scores. The findings also showed that severe obesity might cause or aggravate depression (Dixon et al., 2003). The future identification of genes that confer risk for depression in humans and understanding how specific types of environmental factors interact synergistically with genetic vulnerability will constitute a considerable leap forward in the field of depression. It will be possible to develop more valid animal models of human depression. Important advances will also require the development of evermore penetrating brain imaging methodologies to enable the detection of molecular and cellular biomarkers in living patients.

1.1.3 Pathogenesis of mood disorders

1.1.3.1 The monoamine hypothesis of depression

Historically, the focus in neurobiological studies of mood disorders has been the monoaminergic neurotransmitter systems, which are extensively distributed throughout the network of limbic, striatal and prefrontal cortical neuronal circuits thought to support the behavioral and visceral manifestations of mood disorders (Fig. 5).

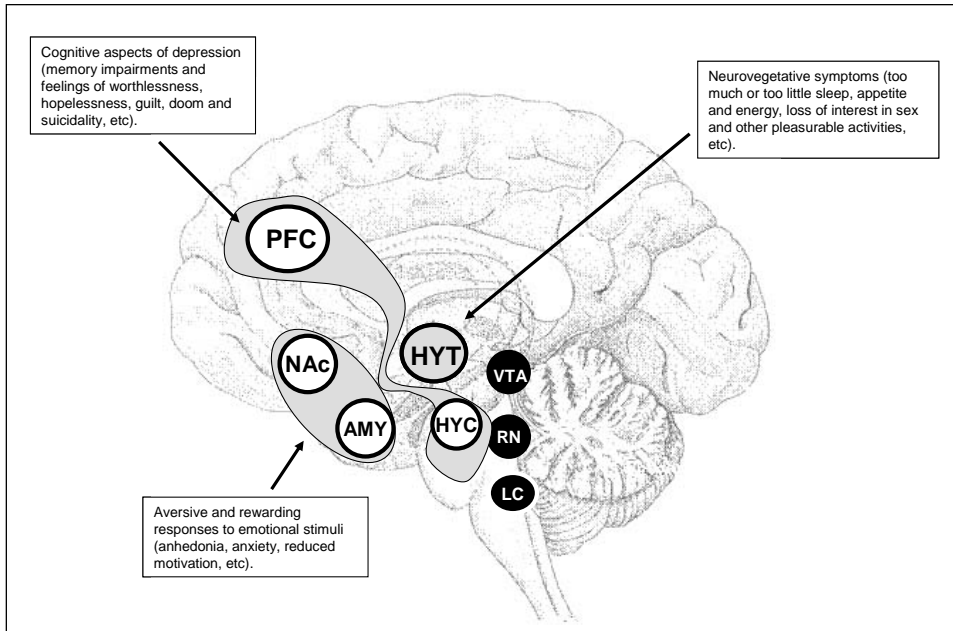
One of the first neurochemical theories of depression was the monoamine impairment hypothesis. According to this hypothesis, major depression results from a deficiency/imbalance of available monoamines (the indoleamine serotonin and catecholamines norepinephrine and dopamine) or subnormal monoamine receptors functioning in certain regions of the brain (for a review, see Iversen, 2005).

Some 50 years ago, the monoamine hypothesis of depression initiated a new era of research in “biological psychiatry”. The discovery of the first effective antidepressant drugs and the rapid advances in research, which led to the understanding of their mechanisms of action on monoamine systems in the brain, represented the start of the so-called “psychopharmacology revolution” which transformed the practice of psychiatry (for a review, see Iversen, 2005).

The findings first suggesting a link between brain monoamines and depression took place in the 1950s, when it was known that the drug reserpine, used for a while in the treatment of hypertension, tended to cause depression as an unwanted side-effect (Muller et al., 1955). Reserpine causes a profound depletion of the brain stores of serotonin and noradrenaline by blocking vesicular monoamine storage (Shore et al., 1955). Animals and a fraction of humans receiving reserpine develop depression. The marked depression of behavior seen in animals treated with reserpine could be reversed by administering the catecholamine precursor L-DOPA (Carlsson et al., 1957), which also reversed the depressive symptoms induced by reserpine in human subjects.

Moreover, the fortuitous discovery that the drug isoniazid, used for the treatment of tuberculosis exerted a mood-elevating effect (Crane, 1956; Kline, 1961) and the subsequent finding that this drug inhibited monoamine oxidase, one of the enzymes responsible for serotonin and

Fig. 5. Monoaminergic nuclei and principal projection areas in the human brain: support of the behavioral and visceral manifestations of mood disorders



Monoaminergic innervation is extensively distributed throughout the limbic, striatal and prefrontal cortical areas (not shown for simplicity). The ventral tegmental area (VTA) provides dopaminergic input to the nucleus accumbens (NAc), amygdala (AMY), prefrontal cortex (PFC) and other limbic structures. Noradrenaline, from the locus coeruleus (LC), and serotonin, from the raphe nuclei (RN), innervate (not shown) all of the regions shown in the panel. The brain areas illustrated in the panel operate as a series of highly interacting parallel circuits, from which researchers are beginning to formulate the neural circuitry involved in depression (adapted from Berton and Nestler, 2006). HYP: hypothalamus; HYC: hippocampus.

Noradrenaline breakdown (Zeller, 1952) reinforced the monoamine hypothesis of depression.

Further support for the hypothesis was obtained in the 1960s, when it was discovered that tricyclic antidepressants inhibit monoamine transporters (Hertting et al., 1961). This finding led to the first understanding of the mechanism of action of the first generation of antidepressants and triggered the first formulation of the “monoamine hypothesis of depression” as a deficiency of either noradrenaline (Bunney and Davis, 1965; Schildkraut, 1965) or serotonin in the brain (Ashcroft et al., 1966; Coppen et al., 1967; Lapin and Oxenkrug, 1969; see Fig. 6). Initially, the noradrenaline version of this hypothesis was preferred by American researchers and the serotonin deficiency concept found more favor in Europe.

In 1996, impaired serotonergic neurotransmission in the brains of untreated patients with moderate to severe depression was demonstrated *in vivo* for the first time (Mann et al., 1996).

During the past 40 years, following the formulation of the “monoamine hypothesis of depression”, there was a lot of research activity aimed at validating the hypothesis. The principal attempts to validate the monoamine hypothesis and results are summarized in Table 4.

Preclinical and clinical evidence has accumulated, mainly during the past two decades, indicating the involvement of the 5-HT system in the therapeutic action of antidepressant drugs (Artigas, 1993; Blier and De Montigny, 1994; Artigas et al., 1996a, b, 2006; Romero et al., 1996a, b, c). In particular, impairment of 5-HT synthesis leads to a transient reappearance of depressive symptoms in patients in remission obtained with various types of antidepressant drugs (Shopsin et al., 1975; Delgado et al., 1990). Conversely, tryptophan and lithium, two compounds that increase 5-HT function (Sharp et al., 1991, 1992) can potentiate the therapeutic effect of antidepressant drugs (De Montigny et al., 1983). Thus, there seems to be a clear association between the antidepressant response and enhanced 5-HT neurotransmission.

Although all currently approved antidepressant drugs appear to act through monoaminergic mechanisms, more than four decades of research have revealed some serious gaps and limitations in the monoamine hypothesis. For example, studies on noradrenaline spillover in plasma and cerebrospinal fluid documented increased noradrenaline output in depression (Veith et al., 1994; Wong et al., 2000). Moreover, the monoamine hypothesis does not fully explain why clinical effects only occur after chronic treatment for at least three weeks, whereas biochemical effects of antidepressants on monoamine systems occur within a few hours of treatment. Preclinical and clinical data support the idea that the acute effects of antidepressants are self-limited by a negative feedback involving the activation of somatodendritic serotonin and noradrenaline autoreceptors, which likely limits their clinical effects (Adell and Artigas, 1991; Artigas et al., 1996a, b, 2006; Romero et al., 1996a, b, c; Perez et al., 1997; Mateo et al., 2001; Adell et al., 2005), and suggest that during chronic treatment with antidepressants adaptive changes occur in pre- and postsynaptic receptors that are responsible for the therapeutic effect. Though theories that postulate

Table 4. Summary of the main lines of evidence implicating monoaminergic neurotransmission in depression (for a review, see Iversen, 2005).

Study approach	Evidence	Selected References
1. Direct measurements of monoamine function in human subjects: depressed vs control Monoamine metabolites in body fluids: 5-hydroxyindoleacetic acid (5-HIAA) from 5-HT, 3-methoxy-4-hydroxyphenyl glycol (MHPG) from NA, and homovanillic acid (HVA) from DA Turnover of monoamines in human brain: the probenecid method Post-mortem brain samples and living brain neuroimaging with PET	↓ 5-HIAA in CSF: 7 (+) and 8 (-) results	Post and Goodwin, 1976 (review); Gibbons and Davis, 1986; Brown and Linnola, 1990 Post and Goodwin, 1976 (review) Iversen, 2005 (review) Mann et al., 1986; Arango et al., 1990; Hrdina et al., 1993 Yatham et al., 2000 Stockmeier et al., 1998 Sargent et al., 2000
	↓ MHPG in CSF: 3 (+) and 3 (-) results	
	↓ HVA in CSF: 2 (+) and 7 (-) results	
	↓ 5-HIAA, MHPG or HVA in urine: (-) results	
	↓ Rates of 5-HIAA in CSF: 6 (+) and 3 (-) results	
	↓ Rates of HVA in CSF: 6 (+) and 2 (-) results	
	↓ 5-HT (whole brain, hypothalamus, amygdale)	
	↑ or no changes in 5-HT ₂ binding sites (frontal cortex)	
	↓ 5-HT ₂ binding sites (cortex): 2 (+) and 2 (-) results	
	↑ 5-HT _{1A} binding sites (prefrontal cortex, raphe nuclei)	
↓ 5-HT _{1A} binding sites (cortical regions). Reduction not altered by treatment with the selective serotonin reuptake inhibitor (SSRI), paroxetine		

	<p>↓ 5-HTT binding sites <i>in vivo</i> using [¹²³I]-β-citalopram</p> <p>↓ 5-HTT binding sites using [³H]-impramine (post-mortem cortex, hypothalamus, hippocampus)</p> <p>↓ 5-HTT binding sites using [³H]-citalopram (cortex, hypothalamus, hippocampus)</p> <p>Any changes in density of 5-HTT binding sites using [³H]-paroxetine (raphe, locus coeruleus)</p> <p>↓ NAT binding sites using [³H]-nisoxetine (locus coeruleus)</p> <p>↑ α₂ Adrenoceptor binding sites (frontal cortex, locus coeruleus, other regions)</p> <p>↑ MAO-A: 1 (+) and 1 (-) results</p> <p>↓ 5-HTT binding sites</p> <p>↑ 5-HTT binding sites in patients exhibiting clinical recovery in response to antidepressant drug treatment: 3 (+) and 3(-) results</p>	<p>Malison et al., 1998</p> <p>Stanley et al., 1982; Perry et al., 1983</p> <p>Leake et al., 1991</p> <p>Klimek et al., 1997</p> <p>Klimek et al., 1997</p> <p>Meana et al., 1992; Ordway et al., 2003</p> <p>Ordway et al., 1999; Du et al., 2002</p> <p>Briley et al., 1980; Paul et al., 1981; Langer and Galzin, 1988; Nemeroff et al., 1988</p> <p>Berrettini et al., 1982; Baron et al., 1986, 1987; Langer et al., 1987</p>
<p>Monoamine markers in blood platelets</p>	<p>↑ α₂ Adrenoceptor binding sites</p> <p>↓ α₂ Adrenoceptor binding sites in patients treated with antidepressant or electroconvulsive therapy (ECT)</p> <p>↑ β-Adrenoceptors in depressed patients and their downregulation in response to successful antidepressant treatment</p>	<p>García-Sevilla, 1989</p> <p>García-Sevilla et al., 1990</p> <p>Leonard et al., 1997 (review)</p>

2. Manipulation of monoamines by administration of precursors or by depletion strategies: depressed vs control																					
Inhibition of monoamine synthesis	Inhibition of NA synthesis with α -methyl-para-tyrosine (AMT)	Depression and sedation in humans Reduction in behavior in primates Any exacerbation of symptoms in depressed patients not taking medication Clinical relapse in responders to NRIs and not in SSRIs responders	Sjoerdsma et al., 1965 Redmond et al., 1971a, b Miller et al., 1996a Miller et al., 1996b																		
	Inhibition of 5-HT synthesis with para-chlorophenylalanine (PCPA)	Reversion of the antidepressant actions of imipramine in depressed patients	Shopsin et al., 1975																		
	Tryptophan depletion	Recurrence of depression <table border="1" style="margin-left: 20px;"> <thead> <tr> <th>Subjects</th> <th>5-HT depletion</th> <th>NA depletion</th> </tr> </thead> <tbody> <tr> <td>Healthy controls</td> <td>-</td> <td>\pm</td> </tr> <tr> <td>Untreated depressed</td> <td>-</td> <td>-</td> </tr> <tr> <td>Recovered taking SSRI</td> <td>++++</td> <td>+</td> </tr> <tr> <td>Recovered taking NRI</td> <td>-</td> <td>++++</td> </tr> <tr> <td>Recovered taking NA/5-HT mixed drug</td> <td>++++</td> <td>++++</td> </tr> </tbody> </table>	Subjects	5-HT depletion	NA depletion	Healthy controls	-	\pm	Untreated depressed	-	-	Recovered taking SSRI	++++	+	Recovered taking NRI	-	++++	Recovered taking NA/5-HT mixed drug	++++	++++	Delgado et al., 1990, 1994, 1999; Benkelfat et al., 1994; Moreno et al., 1999; Delgado, 2004 (review)
	Subjects	5-HT depletion	NA depletion																		
	Healthy controls	-	\pm																		
Untreated depressed	-	-																			
Recovered taking SSRI	++++	+																			
Recovered taking NRI	-	++++																			
Recovered taking NA/5-HT mixed drug	++++	++++																			
5-HTP	Antidepressant effects in a subgroup of depressed patients	van Praag et al., 1983																			
Monoamine precursors	L-Tryptophan	Antidepressant effects: 3 (+) and 3 (-) results Potentiation of tranylcypromine (MAOI): several (+) results	Coppen et al., 1967, 1972; Carroll et al., 1970; Bunney et al., 1971; Dunner and Fieve, 1975; Jensen et al., 1975 Coppen et al., 1963; Pare et al., 1963; Glassman and Platman, 1969																		

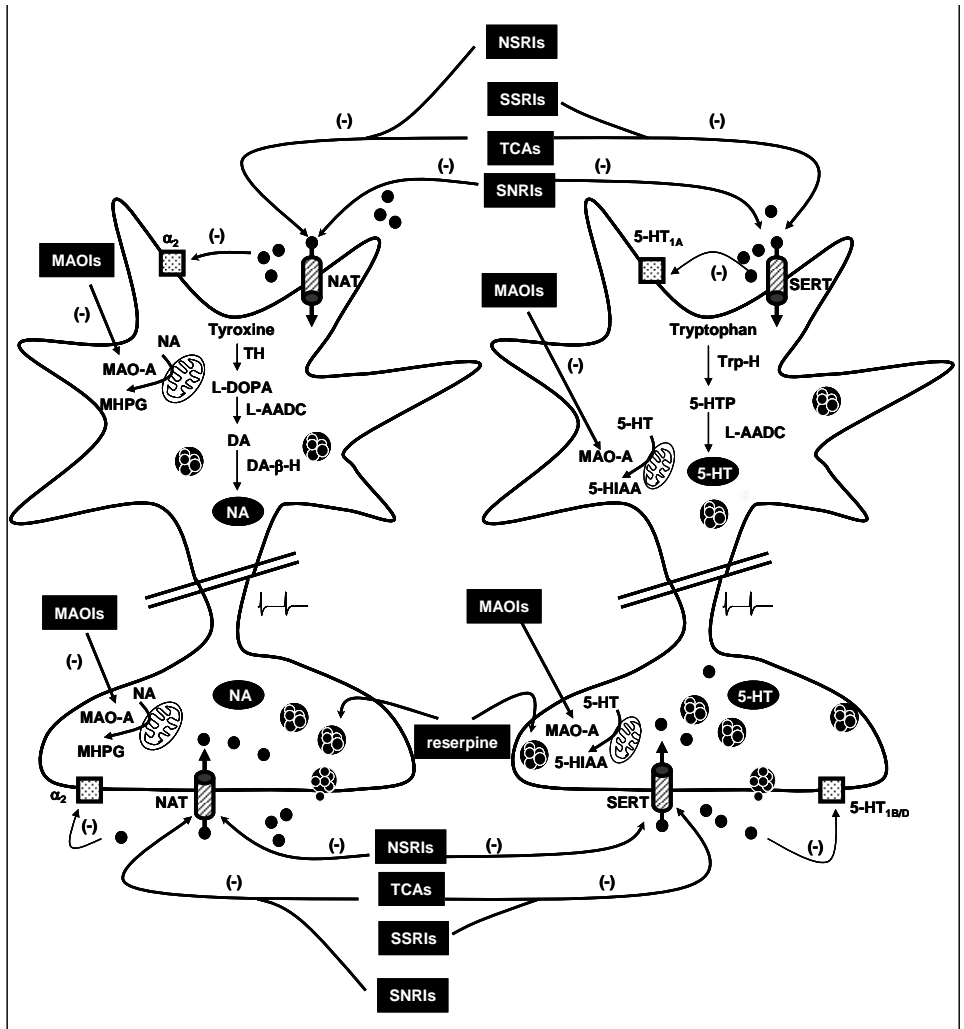
3. Neuroendocrine markers of monoamine function in depression	
Hypothalamus-pituitary-adrenal axis activity	<p>Hypercortisolaemia ("failure to suppress to dexamethasone", 40–50% endogenous depressed patients). Relation between hypercortisolaemia and increased density of α_2 adrenoceptors</p> <p>5-HT_{1A} and 5-HT_{2C} receptors stimulates cortisol and prolactin secretion in man</p> <p>Carroll et al., 1976; Checkley, 1980 (review); Arana et al., 1985 (review); Ressler and Nemeroff, 1999 (review)</p> <p>Meltzer and Maes, 1994</p>
4. Molecular and genetic approaches	
Molecular	<p>Chronic treatment with antidepressants upregulates CREB (cyclic AMP response element protein), BDNF (brain derived neurotrophic factor) and Bcl-2 protein. This may underlie the recent finding that chronic antidepressant treatment causes a proliferation of progenitor cells in the hippocampus</p> <p>Association of polymorphisms in MAO-A and the SERT in bipolar disorder</p> <p>Association of "short" variant of the SERT gene in depression</p> <p>Association of polymorphisms in tryptophan hydroxylase-2 enzyme (involved in the synthesis of serotonin) with major depression, but not with bipolar illness</p> <p>Nibuya et al., 1995, 1996; Duman et al., 1997 (review), Rajkowska, 2000a, b; Chen et al., 2001a, b; Vaidya and Durman, 2001 (review); Nestler et al., 2002 (review)</p> <p>Kato, 2001; Schulze et al., 2000; Du et al., 2004</p> <p>Caspi et al., 2003</p> <p>Zhang et al., 2005</p>
Genetic	

these long-term changes in receptor sensitivity have not been unequivocally proved (Siever and Davis, 1985), it is now believed that changes in brain gene expression that are elicited after chronic treatment might underlie the effects of antidepressants (Wong et al., 1996; for a review, see Wong and Licinio, 2001; Nestler et al., 2002; Carlson et al., 2006).

Although many attempts to find evidence for monoamine malfunction in depression proved largely ineffective, it is now accepted that monoamine neurotransmitter systems undoubtedly play an important role in modulating the expression of certain signs/symptoms of mood disorders. Inconsistent results obtained in some studies could be explained by the suggestion that the “monoamine hypothesis” applies only to a subgroup of depressed patients and there is no way of identifying patients in this hypothetical subgroup by clinical criteria (Irvensen, 2005). However, it is worth remembering that the “monoamine hypothesis” rests heavily on the finding that currently approved antidepressant drugs all appear to act through monoaminergic mechanisms – but it is less widely known why these drugs have a number of drawbacks, including delayed clinical response/remission (weeks to months) and, too frequently, incomplete or absent response/remission. A meta-analysis of clinical trials of antidepressants (Fava and Davidson, 1996) suggested that no beneficial response at all was seen in 19–34% of depressed patients treated with antidepressant drugs, whereas there was only a partial response in a further 12–15%. Thus, almost half of all patients treated with antidepressants fail to show a full response. Clearly, there is a great clinical need for the ongoing development of new therapies that are more effective, rapid-acting and easily tolerated than existing ones.

In summary, despite monoamine hypothesis being still controversial, it has guided antidepressant drug discovery in the past 50 years; and the process of discovery continues today (for a review, see Adell et al., 2005). Nevertheless, novel biological approaches beyond the “monoamine hypothesis” are expected to evoke paradigm shifts in the future of depression research.

Fig. 6. The monoamine hypothesis of depression: mechanism of antidepressant action.



Antidepressants are a heterogeneous group of drugs that act primarily by increasing the availability of monoamines at the synaptic cleft by interfering with their inactivation mechanisms (reuptake or metabolism). Understanding their pharmacology has provided the means for the formulation of the monoamine hypothesis of depression. It has also broadened the approach for developing new drugs, such as the selective serotonin reuptake inhibitors that have less side-effects but are not more efficacious than previously available tricyclic drugs. Acute effects of antidepressants are self-limited by a negative feedback involving the activation of somatodendritic and terminal serotonin and noradrenaline autoreceptors (somatodendritic 5-HT_{1A} and α_2 -adrenergic receptors; terminal 5-HT_{1B/1D} and α_2 -adrenergic receptors), which likely limits their clinical effects (Adell and Artigas, 1991; Artigas et al., 1996a, b, 2006; Romero et al., 1996a, b, c; Perez et al., 1997; Mateo et al., 2001; Adell et al., 2005). NA: noradrenaline or norepinephrine; 5-HT: serotonin; MAOIs: monoamine oxidase inhibitors; TH: tyrosine hydroxylase; L-AADC: L-aromatic amino acid decarboxylase; DA- β -H: dopamin-beta-hydroxylase; MHPG: 3-methoxy-4-hydroxyphenylglycol (noradrenaline metabolite); Trp-H: tryptophan hydroxylase; 5-HIAA: 5-hydroxyindole acetic acid (serotonin metabolite); SERT: serotonin transporter; NAT: noradrenaline transporter; NSRIs: noradrenaline and serotonin reuptake inhibitors; SSRIs: selective serotonin reuptake inhibitors; SNRIs: selective noradrenaline reuptake inhibitors; TCAs: tricyclic antidepressants; 5-HT_{1A}: serotonin 1A receptor; 5-HT_{1B}: serotonin 1B receptor; 5-HT_{1D}: serotonin 1D receptor.

1.1.3.2 Neuroendocrine hypothesis of mood disorders: dysregulation of the hippocampus and hypothalamic–pituitary–adrenal axis

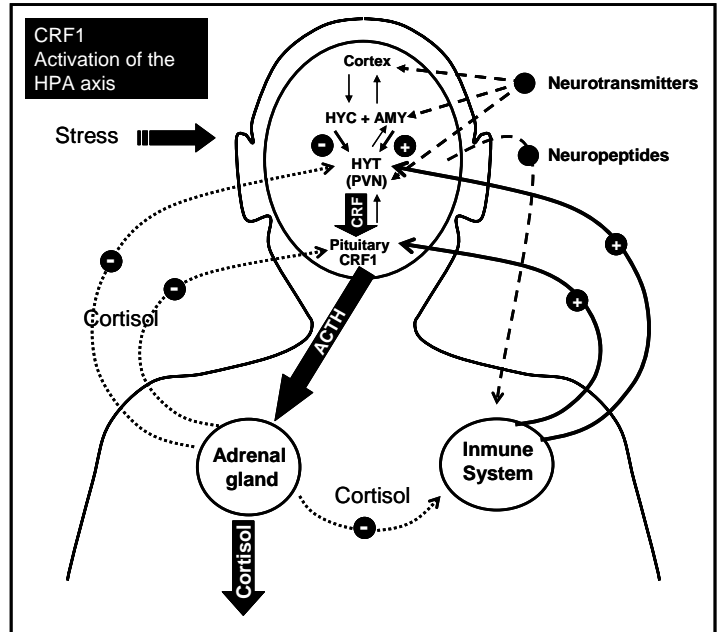
The observation that patients with Cushing's syndrome (hypercortisolemia) often experienced severe depression and anxiety, and the increased production and secretion of glucocorticoids such as cortisol in healthy people exposed to stress, in part contributed to the modern stress-diathesis hypothesis of depression in which excess secretion of cortisol is thought to play a significant pathophysiologic role in the etiology of depression and anxiety disorders (Nemeroff, 1996; Pariante and Miller, 2001; Barden, 2004; Gillespie and Nemeroff, 2005).

Currently, several novel approaches to the treatment of depression are being evaluated, based on the present understanding of the hypothalamic–pituitary–adrenal (HPA) axis dysregulation in mood disorders (Zobel et al., 2000; Belanoff et al., 2002).

A prominent mechanism by which the brain reacts to acute and chronic stress is activation of the HPA axis (Fig. 7). This axis is mainly controlled by the neuropeptide corticotrophin-releasing factor (CRF), secreted by neurones whose cell bodies are in the paraventricular nucleus (PVN) of the hypothalamus and other brain areas. CRF, acting in synergy with vasopressin, which is produced in either the same or distinct neurons of the paraventricular nucleus, stimulates the synthesis and release of pro-opiomelanocortin (POMC)-derived peptides (adrenocorticotropin hormone [ACTH], endorphins) from the anterior pituitary. ACTH then stimulates the synthesis and release of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal cortex. Glucocorticoid hormones terminate the stress response by negative feedback action at the level of the pituitary, hypothalamus and limbic brain areas, including the hippocampus (which exerts an inhibitory influence on hypothalamic CRF-containing neurons via a polysynaptic circuit), amygdala (which exerts a direct excitatory influence) and septum (Nestler et al., 2002; Barden, 2004; Berton and Nestler, 2006). This action is mediated by two identified types of corticosteroid receptors (Reul and de Kloet, 1985): the type I or mineralocorticoid receptor (MR) and the type II or glucocorticoid receptor (GR).

Multiple preclinical and clinical lines of evidence point to abnormalities of the axis in depression. Hyperactivity of the HPA axis is observed in approximately half of patients

Fig. 7. Regulation of the hypothalamic–pituitary–adrenal axis.



Corticotropin releasing factor (CRF)-containing neurons of the paraventricular nucleus (PVN) of the hypothalamus integrate information relevant to stress. CRF is released into the hypophyseal portal system and triggers the release of corticotrophin (ACTH) from the anterior pituitary via stimulation of CRF1 receptors. ACTH stimulates the secretion of glucocorticoid hormones (cortisol in humans or corticosterone in rodents) from the cortex of the adrenal gland. Increased glucocorticoid levels suppress hypothalamic CRF expression and pituitary ACTH release via negative feedback through hippocampal and hypothalamic glucocorticoid receptors. In this way, glucocorticoids (including synthetic forms such as dexamethasone) repress their own synthesis. The adrenal hypertrophy and consequential hypercortisolemia, associated with some cases of depression, induces a desensitisation of glucocorticoid receptors. This desensitisation of glucocorticoid receptors causes impaired negative feedback, increased activity of macrophages and increased release of proinflammatory cytokines by the immune system, that stimulate HPA axis and disturbed neuropeptide, noradrenaline and serotonin neurotransmission. At high levels, glucocorticoids also impair, and may even damage, the hippocampus, which could initiate and maintain this hypercortisolemic state. Adapted from Berton and Nestler (2006) and CNSforum.com.

with depression, as manifested by increased expression of CRF in the hypothalamus and prefrontal cortex and reduced CRF1 receptor mRNA (probably due to chronic hyperactivity of CRF), increased levels of CRF in the CSF, elevated plasma ACTH, increased cortisol production (urinary free cortisol), reduced feedback inhibition of the

axis by CRF and synthetic glucocorticoids (“dexamethasone nonsuppression”), reduced glucocorticoid receptors in hippocampus and increased pituitary and adrenal size (Carroll, 1968; Sapolsky, 2000; Pariante and Miller, 2001; Barden et al., 2004; Merali et al., 2004; Gillespie and Nemeroff, 2005; Korte et al., 2005). Normalization of the overactive HPA system occurs during successful antidepressant pharmacotherapy of depressive illness (Holsboer et al., 1982; Greden et al., 1983; de Kloet et al., 1988; Holsboer-Trachsler et al., 1991; Nemeroff, 1996; Arborelius et al., 1999; Holsboer, 2001). In fact, a failure to normalize HPA activity usually predicts a poor clinical outcome and is associated with early relapse of depression (Banki et al., 1992; Gillespie and Nemeroff, 2005), suggesting that elevated cerebrospinal fluid CRF may be a state marker for depressive vulnerability.

Furthermore, the HPA axis is also involved in neuroplasticity (see also section below). Sustained elevations of glucocorticoids, seen under conditions of prolonged and severe stress, may damage hippocampal CA3 neurons (reduction in dendritic branching, a loss of the highly specialized dendritic spines where the neurons receive their glutamatergic synaptic inputs, even possibly cell death; McEwen, 2000; Sapolsky, 2000; Nestler et al., 2002; Berton and Nestler, 2006). Stress and the resulting hypercortisolemia also reduce neurogenesis in adult hippocampal dentate gyrus (Fuchs and Gould, 2000).

Consistent with these human data are the observations that rodents separated from their mothers early in life show abnormalities in HPA axis function, which resemble those seen in some depressed humans (de Kloet et al., 1988; Francis and Meaney, 1999; Heim and Nemeroff, 2001). These abnormalities can persist into adulthood and be corrected by antidepressant treatments.

Excessive glucocorticoids could be a causative factor for the small reductions in hippocampal volume that have been reported in patients with depression or post-traumatic stress disorder, although this finding remains controversial and it is not known whether these reduced hippocampal volumes are a result of depression or an antecedent cause. It was reported that depression duration correlates with hippocampal volume loss in women with recurrent major depression (Sheline et al., 1999; Bremner et al., 2000; Manji et al., 2001).

In view of the above description, several novel approaches to the treatment of depression are being evaluated based

on the present understanding of HPA axis dysregulation in mood disorders (Zobel et al., 2000; Belanoff et al., 2002, see also Chapter 1.3). In this sense, intense attention is being given to antagonists of the CRF1 receptor, the major CRF receptor in brain, although agents directed against CRF2 receptors are also of interest (Arborelius et al., 1999; Holsboer, 2001). The CRF1 antagonist R121919 (Janssen) attenuates behavioral, neuroendocrine and autonomic responses to stress in primates (Habib et al., 2000); and it was effective in the treatment of depressed patients in a limited pilot study of 20 patients (Zobel et al., 2000). Unfortunately, this compound and numerous other CRF1 antagonists were dropped from subsequent studies due to hepatotoxicity and pharmacokinetic issues (Bosker et al., 2004). In the past few years, several pharmaceutical companies have been developing new selective CRF1 antagonists (Integrity database), such as NBI-34041 (GlaxoSmithKline/Neurocrine Biosciences), ONO-2333MS (Ono), SSR-125543A (Sanofi-Aventis) and TS-041 (Janssen/Taisho). These antagonists are currently undergoing clinical trials (Phase I), which should provide crucial information on efficacy, speed of action and safety, which will be necessary to determine whether this class of potential antidepressants has a faster clinical response than compounds based on reuptake blockade (for a review, see Adell et al., 2005).

At the moment, the major disappointment and frustration in this field is the failure to obtain clear proof of concept of the CRF1 antagonist mechanism as either anxiolytic or antidepressant in humans, despite decades of research.

However, clinical evidences suggest that depressive symptoms in patients with psychotic depression or Cushing syndrome might be rapidly ameliorated by glucocorticoid receptor antagonists (Gillespie and Nemeroff, 2005; Flores et al., 2006). The GR antagonist mifepristone (RU486), the use of which is associated with alterations of the HPA axis (Flores et al., 2006), is currently in Phase III clinical trials for psychotic major depression and might be the first nonmonoaminergic-based antidepressant on the market (Adell et al., 2005). Metyrapone, a glucocorticoid synthesis inhibitor, also shows some promise in treating depression when added to a standard antidepressant (Jahn et al., 2004; Berton and Nesler, 2006).

All of these compounds may be effective in treating depression through the interruption of reverberating neuroendocrine loops involving the HPA axis and several

areas of the brain (prefrontal cortex, amygdala, hippocampus, hypothalamus) that become excessively activated in response to stress, driven perhaps by hypersecretion of CRF (Gold et al., 2002; Gillespie and Nemeroff, 2005).

Although the majority of the literature has reported HPA overactivity in major depression, recent data provide evidence that ACTH, CRH and cortisol activity may only be elevated in some subtypes of major depression and that some depressed patients may actually have low HPA activity (Heim et al., 2000; Posener et al., 2000) Hence, both decreased and elevated HPA axis activity may be found in specific depressive subtypes. In many ways, this parallels the findings in catecholamine activity in depressed patients (for a review, see Schatzberg et al., 2002).

1.1.3.3 Chronobiological hypothesis of depression: depressed have a phase shift

Abnormalities in the circadian regulation of sleep, temperature and activity cycles have been described in major depression (Kupfer et al., 1982). Reduced latency to the onset of rapid-eye-movement (REM) sleep, reduced slow-wave sleep (stages 3, 4), as well as early-morning awakening led to the formulation of the hypothesis that depressed patients have a phase shift of the biological clock (for a review, see Lewy, 2002). Moreover, sleep deprivation and exposure to bright white light ameliorates depressive symptoms by correcting this phase shift. Sleep deprivation is an effective but short-lasting non-pharmacological treatment of depression and light therapy is an effective treatment for seasonal affective illness (Gerner et al., 1979). REM sleep deprivation is more effective than is total sleep deprivation (the effects last longer). In fact, antidepressant drugs suppress REM sleep and increase slow-wave sleep (for a review, see Mayers and Baldwin, 2005; Argyropoulos and Wilson, 2005; Lam, 2006).

Many patients with depression report their most serious symptoms in the morning, with some improvement as the day progresses. This might represent an exaggeration of the diurnal fluctuations in mood, motivation, energy level and responses to rewarding stimuli that are commonly seen in the healthy population. However, the molecular basis for these rhythms seen under normal and pathological conditions is poorly understood.

Most research on circadian rhythms focuses on the suprachiasmatic nucleus (SCN) of the hypothalamus, which is considered the master circadian pacemaker of the brain (Reppert and Weaver, 2002; Takahashi, 2004). An increasing number of reports shows that circadian genes (Clk, Per, Bmal, Cry, neuronal Pas domain protein 2 or NPAS2, glycogen synthase kinase 3 β or GSK3 β , etc.) may act outside the SCN, including limbic regions implicated in mood regulation (hippocampus, striatum, ventral tegmental area-nucleus accumbens pathway, etc.). Results of early preclinical studies support the hypothesis that abnormalities in circadian gene function could contribute to certain symptoms of depression and other mood disorders and suggest that these circadian transcription factors could be potential targets for possible new treatment drugs for depression (Li et al., 2002; Reppert and Weaver, 2002; Manji et al., 2003; Takahashi, 2004; McClung et al., 2005; Uz et al., 2005; Berton and Nestler, 2006; Manev and Uz, 2006).

1.1.3.4 Infectious hypothesis of mood disorders

The Borna-disease virus (BDV) hypothesis of depression was proposed in the 1990s, based on the fact that this infection causes disturbances in behavior and cognitive functions that can even lead to a fatal neurological disease. BDV is a neurotrophic, single-stranded enveloped RNA virus that persistently infects birds, rodents and primates (Lipkin et al., 1990). Several positive reports support the hypothesis: (1) presence of BDV antibodies and viral genomic transcripts in patients with recurrent depression (Rott et al., 1985; Bode, 1995; Bode et al., 1995), (2) isolation of infectious BDV from mononuclear cells of patients (Bode et al., 1996) and (3) detection of BDV antigen and RNA in human autopsy brain samples from patients with depression (de la Torre et al., 1996). However, there are also several negative reports that did not show a clear association of BDV with depression (Kim et al., 1999; Tsuji et al., 2000). Therefore, it is currently accepted that BDV infect a small, but significant portion of patients with depression; but only detailed, carefully controlled, prospective studies will determine whether there is a causal relation between some forms of depression and BDV (Wong and Licinio, 2001).