Section 1
Chapter

1

Basic science

Neuropharmacology of mental illness

Paul A. Fredrickson and Elliott Richelson

The interplay of mental illness and sleep is multifaceted. Most psychiatric disorders produce sleep complaints. Behavioral health clinicians routinely inquire about a patient's sleep during diagnostic assessment and to monitor treatment response. Mental illness treatment that does not address sleep problems may lead to relapse. Persistence of insomnia is a negative prognostic sign, particularly for depression.

Insomnia is the most common sleep complaint. Chronic insomnia is prevalent in about 10% of the United States' population. Only about one-fifth of these sufferers receive prescription hypnotics. Prescribing practices have changed in recent decades. From 1979 to 1999, survey data showed a decline in traditional hypnotics by 50%. Over that same span, the use of antidepressant drugs to treat insomnia increased more than two-fold [1].

Many people self-medicate for sleep problems. Over-the-counter remedies are primarily antihistaminic agents. Alcohol is also a common remedy, but, for reasons of limited efficacy and abuse potential, its use should be discouraged. Cognitive behavioral therapies, which are outside the scope of this chapter, are an effective alternative for many with chronic insomnia.

The focus of this chapter will be on the prescription drugs used to treat psychiatric disorders that either have beneficial or adverse effects on sleep. Some of these drugs are commonly prescribed off-label for insomnia. Others produce insomnia, excessive daytime sleepiness, or parasomnias.

Antidepressant therapies

Current treatments for depression are largely based on concepts arising from monoamine oxidase inhibitor (MAOI) and tricyclic antidepressant (TCA) drugs discovered decades ago. These drugs affect monoamines (serotonin, norepinephrine [NE], dopamine [DA]), particularly serotonin and norepinephrine reuptake. Monoamine transporter reuptake blockade is also the basis for newer, selective reuptake inhibitors that selectively either block the serotonin (e.g., serotonin specific reuptake inhibitors, or SSRIs, such as fluoxetine) or norepinephrine transporters (e.g., reboxetine). The major advantage of these drugs is they produce fewer side effects than older-generation antidepressants, largely by virtue of their weak or absent effects on many receptors for neurotransmitters. However, it is debatable whether the newer drugs are as effective as the older ones. Some new-generation antidepressants block both serotonin and NE transporters (venlafaxine, duloxetine). Dual (serotonin/NE) or triple (5-HT, NE, DA) transporter inhibitors may be more efficacious than single reuptake inhibitors, without the side effects of older-generation drugs [2, 3].

Non-therapeutic side effects of antidepressant medications arise when they affect additional neurotransmitter systems. For example, muscarinic receptor blockade causes dry mouth; histamine H_1 receptor blockade produces sedation; and α_1-adrenergic receptor blockade results in hypotension. Such adverse effects are more common with TCAs and MAOIs than newer, more selective agents [4–6].

Interestingly, many of the same systems targeted by psychiatric drugs are integral to control of sleep and wakefulness. Acetylcholine (ACh) projections from the basal forebrain; histamine from the tuberomammillary nucleus (TMN); norepinephrine from the locus coeruleus; dopamine from the substantia nigra/ventral tegmental area; and serotonin from the raphe nuclei promote wakefulness. Thus, it is not surprising that sleep disorders and affective

1

syndromes coexist, or that treatment of depression may affect sleep. Blockade of these neurotransmitter effects may produce unwanted side effects such as sedation, but also have potential to treat insomnia complaints [7].

So-called "vegetative symptoms" of depression (sleep, appetite, libido) imply hypothalamic dysfunction. Although research on hypothalamic peptides and depression has not yet reached consensus, this is an intriguing area of study, particularly for the intersection of sleep and mental illness. Although a comprehensive review is beyond the scope of this chapter, one peptide of special interest is hypocretin (also known as orexin). Hypocretin is present in two forms: hypocretin 1 and 2 are 33 and 28 amino acid peptides respectively. Hypocretins are produced in the lateral hypothalamus, with projections to multiple brain areas. Loss of hypocretin neurons is found in narcolepsy. Its alerting effects are important in order to maintain a stable state of wakefulness and to prevent inadvertent switching between sleep and wake brain systems. Blockade of hypocretin effects has recently been explored as a treatment for insomnia. However, low activity of hypocretin neurons during rest means that hypocretin antagonists are likely to be most sedating during waking, active hours. This may limit utility for management of insomnia [8].

Antidepressant drugs to treat sleep disorders

Trazodone

The majority of depressed patients report difficulty initiating or maintaining sleep. Antidepressant drugs may help this symptom, make it worse, or have no effect. Stimulating antidepressants, such as bupropion and SSRIs, may produce insomnia or worsen a prior complaint. Trazodone is pharmacologically distinct from the SSRIs (see Figure 1.1). It is frequently prescribed to alleviate the sleep-disrupting effects of stimulating antidepressants. Effects on sleep are consistent with the drug's antagonism at α_1 and 5-HT$_2$ receptors (see Table 1.1). Several studies report subject improvement in sleep, and one double-blind crossover study showed increased sleep time, sleep efficiency, and slow-wave sleep in depressed insomnia patients treated with SSRIs [9].

A more controversial use for trazodone is as a hypnotic in patients without a diagnosis of depression.

Trazodone is now widely prescribed off-label for insomnia. Although risk vs. benefit has not been systematically assessed in general insomnia populations, its widespread use seems to derive in part from concerns regarding the safety and appropriateness of hypnotic drugs for long-term use.

Many patients recovering from alcohol or other addictions are prescribed trazodone because it is without abuse potential. Patients suffering from alcohol dependence often complain of insomnia during the initial weeks and months of abstinence. Concern about abuse potential of hypnotics may limit therapeutic intervention, thus running the risk of self-medication with alcohol. Not only do recovering alcoholic subjects report sleep initiation and maintenance problems, they also show objective evidence of lighter sleep. Polysomnographic studies show diminished slow-wave sleep. Because sleep architecture may not normalize for a year or more, long-term management of sleep problems is important. Trazodone seems promising in this population in that it promotes slow-wave sleep and improved sleep continuity, although its efficacy for this indication has not been rigorously studied [10].

Mirtazapine

Mirtazapine, a sedating antidepressant, is an antagonist (in order of its potency) of histamine H$_1$, 5-HT$_{2A}$, α_{2A}, and 5-HT$_3$ receptors. A recent report suggested mirtazapine may be effective for treatment of sleep apnea. However, a randomized placebo-controlled trial failed to confirm a beneficial effect on apnea [11]. Furthermore, because weight gain is common with mirtazapine, the drug should be prescribed with caution in this patient group. Similarly, off-label treatment of insomnia with mirtazapine should be approached with caution for this same reason.

Tricyclic antidepressants

The prototype drug in this class is amitriptyline. While no longer commonly prescribed as a first-line therapy for depression, many practitioners make use of amitriptyline for management of insomnia in preference to conventional hypnotic drugs. While safe from the standpoint of abuse potential, other adverse effects may limit tolerability and safety. Amitriptyline can produce unwanted daytime sedation, which may be a hazard when operating a motor vehicle. The majority of amitriptyline's sedative effects are due to its blockade of the histamine H$_1$ receptor.

2

Table 1.1 Receptor binding affinities of some psychotherapeutic drugs used to treat insomnia

Drug	Histamine H_1	Serotonin 5-HT$_{2A}$	Muscarinic acetylcholine	α_1-Adrenergic
Amitriptyline	91	3.4	5.6	3.7
Diphenhydramine	6.3	0.38	1.3	0.08
Doxepin	420	4.0	1.2	4.2
Mirtazapine	700	6.1	0.15	0.2
Quetiapine	5.2	3.2	0.1	12.3
Trazodone	0.29	13	0.00031	2.8

Notes: Affinity is $(1/K_d) \times 10^{-7}$, where K_d is the equilibrium dissociation constant in molarity. The higher the number, the more potent the binding. Data for antidepressants are from Richelson, Cusack, Richelson [4, 5, 21]; data for quetiapine are from Richelson [22], and data for diphenhydramine are from Mansbach [23].
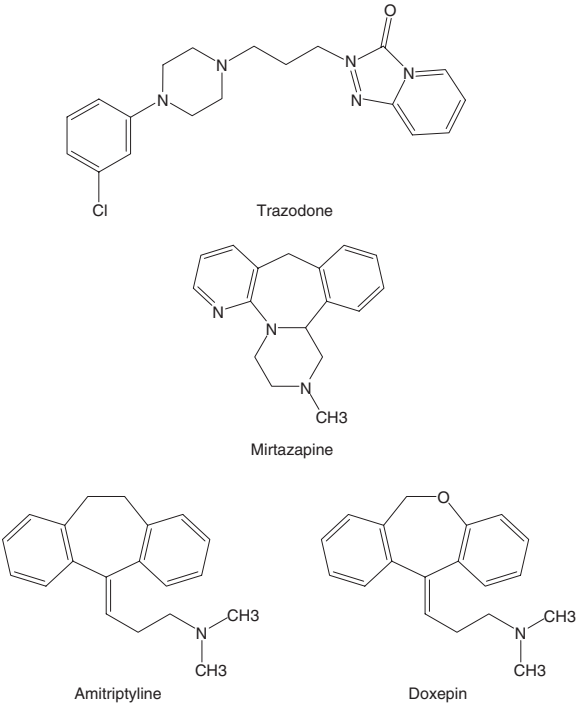

Figure 1.1 Structures of antidepressants used off-label as hypnotic drugs.

Doxepin is also sometimes prescribed for its sedating properties. It, too, is a potent H_1 receptor blocking drug. Due to its half-life of approximately 17 hours, daytime sedation may be a serious side effect. Dosing schedules for doxepin and the tricyclics as hypnotics have not been systematically reported in the literature. However, recent trials of very low dose doxepin (1 to 6 mg) show promise as a hypnotic [12]. Doxepin may be particularly effective for sleep-maintenance insomnia, a common problem in the elderly. Because brain histamine levels peak late in the night and in the morning hours, antihistaminic effects are apt to be most effective at those hours. Low doses in recent studies have not caused daytime sedation or weight gain.

Sleep disruption from antidepressant drugs

Insomnia: Many TCAs, MAOIs, most SSRIs, and bupropion can produce this complaint [13]. Patients with major depression have a high rate of insomnia. Antidepressant treatments, such as the SSRIs or bupropion, may initially worsen sleep, resulting in reduced compliance. Because sleep complaints are so commonly associated with depression, the impact of the drug on sleep may not be immediately recognized. However, if sleep complaints persist, or worsen, as other depressive symptoms improve, an adverse effect of the drug therapy may be inferred. Many clinicians choose trazodone as an adjunct, particularly with SSRIs and bupropion therapy.

Daytime sedation: This is an under-appreciated adverse effect in the opinion of the authors. Excessive daytime sleepiness, from any cause, is a source of motor vehicle accidents and may impair cognitive function in a variety of settings. Patients may not complain of this symptom, or may incorrectly attribute it to other causes such as normal aging. The clinician should remember that hypersomnia is a cardinal symptom of sleep apnea in snorers. Therefore, careful inquiry as to presence and course of this

symptom is important. Any patient with intrusive daytime sleepiness merits close attention until the source is identified and resolved. The physician should discuss potential hazards of operating a motor vehicle while taking sedating medication.

Parasomnias: This is a category of "things that go bump in the night." Common examples are sleep walking and night terrors, which are more frequently encountered in children than adults. An important parasomnia in older adults is REM behavior disorder (RBD). This fascinating condition occurs as a result of the loss of normal skeletal muscle atonia during rapid eye movement (REM) sleep. As a result, patients may display complex movements during REM sleep that match dream imagery. One of the author's (PAF) elderly patients, dreaming she was a young girl swinging from tree branches, vaulted from bed and awoke on the floor. Injuries to the sleeper or bed partner make this a serious target of therapy. REM behavior disorder is most common in elderly males, and is often associated with Lewy body dementia, multiple system atrophy, Parkinson's, and other synucleinopathies. However, RBD may also be triggered by antidepressant therapy, particularly SSRIs [14]. A careful history will help determine whether antidepressant therapy needs to be modified. Some patients will benefit from consultation with a sleep medicine physician, and a full sleep study may be indicated. If the antidepressant regimen cannot easily be modified, a trial of clonazepam or melatonin may provide sufficient control of the parasomnia.

Circadian rhythms

Circadian rhythm disturbance has been inferred in depression from the clinical symptom of diurnal variation in mood. Many patients describe their lowest point of the day as morning, with some improvement occurring as the day progresses. Antidepressant gene expression abnormalities are a potential target for antidepressant drug therapy, but as yet, little clinical data exist. Agomelatine and other melatonin receptor agonists show antidepressant activity in animal models [15].

Circadian rhythm disorders are also relevant to sleep complaints. Adolescents frequently exhibit a phase delay (preferred bedtime and time of arising is much later than societal norms). The elderly often have phase advance. Their early morning awakening complaints mimic the sleep complaint of major

depression, but they also have early bedtimes, consistent with their internal clock phase [16].

The atypical antipsychotics

Although developed for treatment of schizophrenia and other psychoses, new generation (also called atypical) antipsychotics are frequently prescribed as adjuncts for antidepressant therapy and for their anti-anxiety effects. Many clinicians prescribe these drugs for sleep complaints. Quetiapine appears to be a popular choice among this group. It has antagonistic effects at 5-HT_{2A} and histamine H_1 receptors that probably account for many of the benefits on sleep. Quetiapine has been reported as effective in primary insomnia, particularly sleep maintenance complaints [17]. However, definitive studies are lacking. Furthermore, potential adverse effects must be weighed against benefits. This is particularly true in the elderly demented.

Anti-anxiety and hypnotic agents: the GABAergic drugs

Just as antidepressant drug development has focused for decades on a narrow range of therapeutic targets, so, too, hypnotics, since the introduction of flurazepam, have primarily targeted GABA receptors, particularly the GABA_A subtype. As for the 5-HT_3 receptors, GABA_A receptors are pentameric ligand-gated ion channels (a pore is opened in the cell membrane when an agonist binds to the receptor, allowing flow of chloride ions into the cell). They comprise several types of subunits (alpha, beta, gamma) [18].

Prototype GABA_A agonists are the benzodiazepines (BZDs). This class of drug has antianxiety, sedative, anticonvulsant, and muscle-relaxing properties. Although only a few BZDs are approved by the US Food and Drug Administration (FDA) as sedative – hypnotic drugs (temazepam, triazolam, estazolam, quazepam), the differences between "sedative" and "anxiolytic" BZDs has more to do with dosing and marketing than pharmacologic distinctions.

Benzodiazepines bind allosterically (i.e., away from the site where the native ligand GABA binds), and increase the affinity of the receptor for the open state [19]. Most GABA_A receptors in the brain contain alpha 1, alpha 2, or alpha 3 subunits. GABA_A

4

receptors containing alpha 1 subunits are likely responsible for sedative actions of these drugs.

In the search for drugs with a more "pure" sedative effect, and less abuse potential, newer generation $GABA_A$ agonists have been developed (zolpidem, zaleplon, zopiclone, and eszopiclone). These drugs, particularly zolpidem and zaleplon, are specific in their affinity for $GABA_A$ receptors containing the alpha 1 subunit. They may, therefore, be less likely to have abuse potential and produce physical dependence. Indeed, experience in humans suggests they carry a low risk of physical dependence. In non-human primates, however, these drugs are self-administered, and discontinuation can lead to a withdrawal syndrome. Therefore, while they appear to be less likely to cause physical dependence, their suitability in populations susceptible to addiction is not established [20].

Summary

Clinical wisdom tells us psychotropic drugs are useful agents for the management of sleep complaints. We await confirmation from clinical trials for many of the compounds commonly used off-label to manage insomnia. A limited number of drugs are approved by the FDA in the United States for the management of insomnia, and most are GABAergic. New-generation GABAergic agents that are specific for the alpha 1 subunit do not have significant antianxiety effects as seen with the BZDs.

Concern about abuse potential has led many clinicians to question the suitability of BZD (and non-BZD GABAergic) hypnotics for long-term use, particularly in patients with a history of drug or alcohol problems. To some extent, prescribing practices reflect society's attitudes toward sleep problems vs. other complaints. Therefore, clinicians may feel more comfortable prescribing anxiolytic BZDs on a chronic basis for anxiety, but shy away from similar drugs for chronic insomnia complaints. Nevertheless, patients and clinicians require alternative therapies when traditional hypnotics fail, are not tolerated, or simply not preferred.

Psychotropic drugs may also have negative effects on sleep. Insomnia is common with SSRIs and bupropion. Less well appreciated is risk of RBD, thus far mostly reported with SSRIs.

Understanding the nature and source of the sleep complaint is key to successful therapy. Patients with anxiety contributing to difficulty falling asleep will benefit from anxiolytic drugs. Those with sleep maintenance complaints may do better with antidepressant or atypical antipsychotic therapy. Unfortunately, clinical trial data are sparse for effects of most psychotropic drugs on sleep complaints, particularly in patients with primary insomnia.

Further study of non-GABAergic drugs will broaden the therapeutic armamentarium for sleep complaints and help us understand the mechanisms of sleep complaints.

References

1. Roehrs T, Roth T. 'Hypnotic' prescription patterns in a large managed-care population. *Sleep Med.* 2004;**5**(5):463–6.

2. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. *Nat Rev Neurosci.* 2006;**7**(2):137–51.

3. Liang Y, Shaw AM, Boules M, *et al.* Antidepressant-like pharmacological profile of a novel triple reuptake inhibitor, (1S,2S)-3-(methylamino)-2-(naphthalen-2-yl)-1-phenylpropan-1-ol (PRC200-SS). *J Pharmacol Exp Ther.* 2008;**327**(2): 573–83.

4. Richelson E, Nelson A. Antagonism by antidepressants of neurotransmitter receptors of normal human brain in vitro. *J Pharmacol Exp Ther.* 1984;**230**(1):94–102.

5. Cusack B, Nelson A, Richelson E. Binding of antidepressants to human brain receptors: focus on newer generation compounds. *Psychopharmacology (Berl)*. 1994;**114**(4):559–65.

6. Richelson E. Pharmacology of antidepressants. *Mayo Clin Proc.* 2001;**76**(5):511–27.

7. McCarley RW. Neurobiology of REM and NREM sleep. *Sleep Med.* 2007;**8**(4):302–30.

8. Brisbare-Roch C, Dingemanse J, Koberstein R, *et al.* Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat Med.* 2007;**13**(2):150–5.

9. Kaynak H, Kaynak D, Gozukirmizi E, Guilleminault C. The effects of trazodone on sleep in patients treated with stimulant antidepressants. *Sleep Med.* 2004;**5**(1):15–20.

10. Le Bon O, Murphy JR, Staner L, *et al.* Double-blind, placebo-controlled study of the efficacy of trazodone in alcohol post-withdrawal syndrome: polysomnographic and clinical evaluations. *J Clin Psychopharmacol.* 2003;**23**(4):377–83.

11. Marshall NS, Yee BJ, Desai AV, *et al.* Two randomized placebo-controlled trials to evaluate the efficacy and

tolerability of mirtazapine for the treatment of obstructive sleep apnea. *Sleep.* 2008;**31**(6):824–31.

12. Scharf M, Rogowski R, Hull S, *et al.* Efficacy and safety of doxepin 1 mg, 3 mg, and 6 mg in elderly patients with primary insomnia. *J Clin Psychiatry.* 2008;**69**(10):1557–64.

13. Mayers AG, Baldwin DS. Antidepressants and their effect on sleep. *Hum Psychopharmacol.* 2005;**20**(8):533–59.

14. Winkelman JW, James L. Serotonergic antidepressants are associated with REM sleep without atonia. *Sleep.* 2004;**27**(2):317–21.

15. Ghosh A, Hellewell JS. A review of the efficacy and tolerability of agomelatine in the treatment of major depression. *Expert Opin Investig Drugs.* 2007;**16**(12):1999–2004.

16. Barion A, Zee PC. A clinical approach to circadian rhythm sleep disorders. *Sleep Med.* 2007;**8**(6):566–77.

17. Wiegand MH, Landry F, Bruckner T, *et al.* Quetiapine in primary insomnia: a pilot study. *Psychopharmacology* (*Berl*). 2008;**196**(2):337–8.

18. Rudolph U, Mohler H. GABA-based therapeutic approaches: GABAA receptor subtype functions. *Curr Opin Pharmacol.* 2006;**6**(1):18–23.

19. Campo-Soria C, Chang Y, Weiss DS. Mechanism of action of benzodiazepines on GABAA receptors. *Br J Pharmacol.* 2006;**148**(7):984–90.

20. Licata SC, Rowlett JK. Abuse and dependence liability of benzodiazepine-type drugs: GABA(A) receptor modulation and beyond. *Pharmacol Biochem Behav.* 2008;**90**(1):74–89.

21. Richelson E. Interactions of antidepressants with neurotransmitter transporters and receptors and their clinical relevance. *J Clin Psychiatry.* 2003;**64**(Suppl 13):5–12.

22. Richelson E, Souder T. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. *Life Sci.* 2000;**68**(1):29–39.

23. Mansbach R. In vitro pharmacological profile of doxepin, a sleep-promoting histamine H1 antagonist [abstract]. *Eur Neuropsychopharmacol.* 2008;**18**(S4): S357.

Effects of antidepressants on gene expression

Antonio Drago, Diana De Ronchi, and Alessandro Serretti

Introduction

Depressive disorder is a common disease that affects one out of six people during their lifetime in the United States [1], and 18.4 million people per year in Europe [2]. Core symptoms include psychological suffering (for example depressed mood, altered cognition) and neurovegetative symptoms (for example sleep disturbances, variations in food intake). Besides the psychological suffering and the economic burden [2], depressive disorder enhances the risk of mortality through higher suicide rates, higher risk of type 2 diabetes, and higher incidence of coronaropathy [3]. Moreover, it dramatically impacts the prognoses of hosts of other relevant diseases [4]. Despite this relevance, the impressive impact on the community and the efforts produced by the scientific community so far, the disrupted mechanisms related to depressive phenomenology have not been clarified. Knowledge of depressive pathophysiology is rudimentary compared to other relevant chronic diseases such as diabetes: this imbalance may be related to the etiopathogenesis of depressive disorder itself, which is thought to rely on functional neuronal networks that are poorly characterized so far and, thereby, difficult to investigate [5]. Moreover, the techniques used to investigate the brain suffer from a list of limitations that weaken their quality: post mortem studies rely on the treatment of cerebral tissues that enhances the variability of laboratory settings, and imaging studies detect changes in neuronal activity using indirect markers of activation [6]. Even though these techniques have provided useful insights helping the formulation of the most relevant theories of depression (neuronal circuitry imbalance; downregulation of monoamine tone; neurotrophins and neurogenesis, neuroendocrine and neuroimmune interactions), all

these models appear to be inadequate when facing the depressive phenotypes [5]. Finally, the incomplete knowledge of depressive disorder limits the characterization of its etiology to a description of risk factors [4, 7] impacted by the genetic background and reactivity by means that are poorly understood and inconsistently demonstrated [8], and the studies' designs rely on the definition of the disorder that is based on phenomenology and is constantly changing through years: there is a possibility that what is defined as depressive disorder in studies actually encompasses different pathophysiological processes still poorly understood. Consistently, the efficacy of antidepressant drug treatments, which are designed and based on the monaminergic theory of depression, is limited: up to 30% of depressed patients treated with antidepressant drugs do not reach remission [9]. More efforts are to be devoted in order to further investigate the field. One interesting and promising point of observation that could reveal some aspects of depressive disruptions and of the pharmacodynamics of antidepressant effects is the investigation of DNA reactivity to antidepressant treatment. While it is self-evident that this approach could give some interesting breakthroughs in pharmacodynamics, the boundary between antidepressant drug-triggered DNA reactivity and depressive phenotypes may be less obvious. Nevertheless, it must be underlined that antidepressants work in the majority of cases, and that even though their efficacy can vary greatly in one subject there are marginal differences between the different classes of antidepressants in large population samples. A common mechanism of action is to be hypothesized and it may – *ex adiuvantibus* – be related to the pathophysiology of depressive disorders. This accepted, the investigation

---

of the DNA adaptations after antidepressant challenge may provide some more details to the still unresolved mosaic of evidence that focuses on the depressive phenotype. In the present chapter the most up-to-date knowledge on the impact of antidepressant treatments on transcription and translation events will be described.

The main role of human DNA is to store information and to perpetuate it through time. To achieve this result, DNA encodes sufficient information to form cells, tissues, and an organism, guaranteeing the molecular means for adaptation, viability, and reactivity of all of them. In order to adapt to the stimuli coming from the outside, DNA codes for, and is reactive to, a wide array of molecular feedback looped mechanisms, second messengers cascades, and complicated membrane–cytosol–nuclei crosstalking, which starts at the surfaces of cells and ends at the cores of the nuclei, and the other way round. Tissues are the tight orchestration of single cells influencing each other's DNA reactivity by the means of mechanisms that are still not completely known. Antidepressants impact DNA reactivity by acting as external stimuli. In the following discussion the most relevant theories related to the putative disruptions underlying depressive disorders will be commented on, along with antidepressant triggered modifications of the DNA expression profile.

Monoamines

The monoamine hypothesis of depression suggests that depressive disorder is caused by imbalanced monoaminergic tone in the brain. This hypothesis originates from early clinical observations [10, 11]. The first hints on which this theory was based came from two structurally unrelated drugs designed for other than psychiatric diseases that about 40 years ago turned out to have potent antidepressant effect in humans, and were later shown to enhance central serotonin and noradrenalin transmission. During the same period it was found that reserpine, which acts by depleting monoamine stores, determines a reduction in both blood pressure and depressive symptoms. Starting from this evidence, serotonin and noradrenalin were first identified as relevant putative candidates for the pathophysiological imbalances that underline depressive disorder. More recent antidepressant agents that show a more favorable therapeutic index still act on the reuptake of monoamines and are named upon this action: SSRIs (selective serotonin reuptake inhibitors) and SNRIs (serotonin and noradrenalin reuptake inhibitors) are examples of drugs that have been designed on the basis of the monoaminergic theory of depressive disorder, and whose aim is to obtain an acute increase in monoaminergic tone in the synaptic cleft by inhibiting the reuptake of monoamines. These drugs are nowadays the first-line treatment for depressive disorder. Even though the monoamine-based agents are potent antidepressants [9], and alterations in central monoamine function might contribute marginally to genetic vulnerability to depressive disorder [12], it is not possible to define the cause of depressive disorder as simply being an imbalance in monoaminergic tone. In fact, the monoamine oxidase inhibitors and SSRIs produce immediate increases in monoamine transmission, which come before the effect on mood. Conversely, experimental depletion of monoamines can trigger a mild reduction in mood in unmedicated depressed patients, but does not alter mood in healthy controls [13]. With regard to the expression of genes triggered by antidepressant drugs designed on the monoaminergic theory, several lines of evidence report that antidepressants diminish the concentration of the noradrenalin or serotonin transporters on the surface of cells, but, interestingly, this event is not associated with altered mRNA expression of the genes that code for the serotonin transporter and for other serotonin receptors that have been put forward as possible mediators of the antidepressant effect (5-HT1A, 5-HT1B, 5-HT1C, and 5-HT2) [14–19]. The diminished concentration of serotonin transporter on the surface of cells is then to be associated with another event within the cell: this may be its inclusion into cytoplasmatic vesicles. The expression rate of the mRNA that codes for the VMAT2, an integral membrane protein that acts to transport monoamines – particularly neurotransmitters such as dopamine, norepinephrine, serotonin, and histamine – from cellular cytosol into synaptic vesicles, was reported to be diminished after short- and long-term treatment with fluoxetine [20], even though lack of effect on VMAT2 was detected after paroxetine or reserpine treatment [21]. The evidence so far is quite striking: the antidepressant treatment targeted on the serotonin system does not seem to play a major role in the genetic regulation of many of the most relevant mediators of serotonergic function. It must be said that at least one exception exists: tryptophan

8

hydroxylase (TPH), which is the rate-limiting enzyme for the synthesis of serotonin. The expression level of the brain isoform of this enzyme was found to be affected by treatment with antidepressants [20, 22, 23]. Moreover, even though ineffective for these targets, antidepressants were found to affect the expression rates of the serotonin transporter, of serotonin receptor 2A and 1A mRNA in stressed animals and not in controls [23–26]: mRNA expression was enhanced in stressed animals before they were treated with antidepressants, but this difference was abolished after the treatment. This is a point of relevance: it could be derived that antidepressants are more effective, or at least act differently, on subjects prone to suffer anxiety or depressive episodes. Antidepressants could exert their activity within the brain in a different and specific way that is dependent on the background, both biological and psychological, of the subject. Beside this, there is evidence for a genetic regulation that is dependent on the serotonin system and that is mirrored by the expression rates of genes that do not code for strict serotonergic related products: monoaminergic receptors are not only expressed in cells that produce the mediator they are able to recognize, but they can be coded and exposed on the surface of cells that do not produce the corresponding ligand. In that case, the receptors are called heteroreceptors and their activity accounts for the complex net of interactions that bound the diverse monaminergic systems [27]. This may be related to the presence of altered mRNA expression of dopaminergic receptors after treatment with serotonergic drugs [28–31]. Finally, even though the expression level of some serotonergic receptors is not affected by the presence of antidepressants, the post-transcriptional events are actually impacted. In particular, the editing of the serotonin receptor 2C, which can be directed toward more or less functional isoforms of the receptor, is tuned toward the reduced function pole [32]. An impact on the editing of the AMPA/kainate receptors was found as well [33]. The evidence coming from these findings is still difficult to resolve into a consequential picture: the mutual relationships between the different neuronal nets and the relevance of specific sites of action of antidepressant within the central nervous system (CNS) tremendously increase the complexity of the field. Of note, the lack of association between antidepressants and the expression levels of serotonin-related receptor and transporter was detected when neurons were

investigated, while opposite findings were revealed in peripheral leukocytes, which showed lower baseline expression of the serotonin transporter gene. This difference was no more evident after treatment with antidepressants [34]. This opens the debate on how peripheral cells with specific differentiation may represent the biological reactions that pertain to the CNS. Even though some relevant variations of genes that belong to the serotonin system or to systems that are related to it have been shown to have their genetic expressive profile affected by antidepressant treatment, the monoaminergic theory of depression is probably not sufficient to cover the molecular disruptions that affect depressed individuals. There is some evidence that the most probable event that is related to the antidepressant efficacy of antidepressant treatments is based on neuroplastic changes that follow the acute effect of monoaminergic based antidepressants: this is more consistent with the time lag between therapy initiation and mood elevation that characterizes these drugs. Moreover, it is reasonable that the variation in the neuroplasticity of neurons, which is thought to be induced by antidepressants, takes some time to develop to an extent sufficient for a change in mood. Interestingly, the way antidepressants may achieve this goal is thought to be related to an impact on the transcriptional and translational activities within the CNS. Consistent with this, chronic treatment with antidepressants has been shown to upregulate the transcription factor CREB (cyclic-AMP-response element-binding protein), which is downstream of several serotonin and other stimulatory G-protein-coupled receptors, in the hippocampus, which is thought to be related to some aspects of the depressive phenotype, while the same cellular event is associated with depressive-like responses when triggered in the nucleus accumbens [3, 11, 35]. This further underlines the specificity of action of antidepressants in different parts of the brain. Consistent with this, the activity of a set of second messengers (protein kinase C (PKC)-delta, PKC-gamma, stress-activated protein kinase, cAMP-dependent protein kinase beta isoform, Janus protein kinase, and phosphofructokinase M) were all found to be downregulated after treatment with fluoxetine and citalopram in the whole brains of rats [36]. Moreover, treatment with citalopram and lithium (given separately) was found to be associated with an increased expression of the adenyl cyclase type 1 mRNA in the hippocampus, but not its

9

corresponding protein, while GTP-associated adenyl cyclase activity was found to be increased after treatment: this may indicate that the antidepressant treatment caused an enhanced adenyl cyclase/G protein coupling [37].

Neurotrophins

Critical molecules regulating signaling and neuroplasticity as potential long-term mediators of mood stabilization have been identified and they may play a relevant role in the response to antidepressant treatments. Together, they act as neurotrophic agents and they concur to the neuroplasticity of cells defined as the sum of diverse processes of vital importance through which the brain perceives, adapts to, and responds to a variety of internal and external stimuli. In terms of biological structures, neuroplasticity includes alterations of dendritic function, synaptic remodeling, long-term potentiation, axonal sprouting, neurite extension, synaptogenesis, and even neurogenesis. Some of the most critical molecules that are involved in these processes are: CREB, BDNF, Bcl-2, p53, and MAP kinases. Neurotrophic factors (such as BDNF) promote cell survival largely by suppressing intrinsic, cellular apoptotic machinery, rather than by inducing cell survival pathways [38]. Two intracellular signal transduction pathways are crucial in promoting neuronal survival – the mitogen activated protein (MAP) kinase cascade and the phosphotidylinositol-3 kinase (PI-3K)/Akt pathway [39, 40]. The activation of the MAP kinase pathway can inhibit apoptosis by inducing the phosphorylation of Bad and increasing the expression of Bcl-2, the latter effect likely involving the cAMP response element binding protein (CREB) [41, 42]. Indeed, this mechanism was confirmed by recent analyses by Chen and colleagues who reported that fluoxetine can enhance the expression of bcl-2 [43]. Phosphorylation of Bad occurs via activation of a downstream target of the MAP kinase cascade, ribosomal S-6 kinase (Rsk). Ribosomal S-6 kinase phosphorylates Bad and thereby promotes its inactivation. Activation of Rsk also mediates the actions of the MAP kinase cascade and neurotrophic factors on the expression of Bcl-2. Ribosomal S-6 kinase can phosphorylate the cAMP response element binding protein (CREB) and this leads to induction of Bcl-2 gene expression. Not only the neuronal death that these mechanisms are organized to prevent, but also a lack of neurogenesis, may

represent mechanisms by which neuroresilience is dampened. Studies have demonstrated that the greatest density of new cell growth is observed in the subventricular zone and the subgranular layer of the hippocampus, and decreased neurogenesis occurs during stress – both acute and chronic. This effect appears to be mediated by glucocorticoid receptors [44]: chronic psychosocial stress or corticosterone administration caused apical dendritic atrophy of hippocampal CA3 pyramidal neurons, which may be mediated by activation of the hypothalamic–pituitary–adrenal (HPA) axis [45, 46]. This context opened the way to the formulation of drug designs based on the antagonism of glucocorticoid receptors. Intriguingly, antidepressant treatments can upregulate the brain-derived neurotrophic factor (BDNF) signaling cascade after chronic administration through their impact on the cAMP–CREB cascade, which regulates the BDNF [47, 48], and thereby produce an antidepressant effect by increasing the expression of neurotrophic factors in the hippocampus [49]. Consistently, animal studies suggested that the impairment of the BDNF/TrkB system exposes animals to a blunted antidepressant response more than to a higher risk of developing depressive-like phenotypes [50–52]. Interestingly, it has been demonstrated that localization is central to the effect of the presence or absence of the BDNF: the ablation of the BDNF in the forebrain including the hippocampus results in a lack of sensitivity to antidepressant treatment, while ablation in the reward pathway ameliorated the adverse effects of social defeat, and if the BDNF system is interrupted in the dentate gyrus and the CA1 regions, a lack of antidepressant effect (desipramine and citalopram) is to be expected [51, 53–55]. There are some more insights into this field. For example, there is one report of striking evidence: a non-correspondence between changes in BDNF mRNA and protein expression induced by the antidepressant treatments and lithium was revealed by Jacobsen and Mork [56], which may indicate that the means by which antidepressants exert their action on the neurotrophic elements of neurons act indirectly. Otherwise, a sort of editing process could be responsible for this lack of association: it has been reported that duloxetine increases the expression of certain exons of the BDNF coding sequence (namely exons V, I, and III but not exon IV). Indeed, not only is the BDNF upregulated, but a particular isoform of it is produced within neurons under the influence