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Behavioral effects of antidepressant treatments in mice: a focus on BDNF

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ACADEMIC DISSERTATION

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Abstract

Major depressive disorder (MDD) affects millions of people every year and produces significant human suffering and economic burden to society. The symptomatology of MDD is heterogeneous and multidimensional, and only two core symptoms, depressed mood and anhedonia, are frequently shared by patients. Consequently, modeling of MDD is challenging, and only depression-related phenomena, not depressed mood itself, can be examined in animals. MDD is commonly treated with antidepressant drugs (or antidepressants, ADs). However, monoamine-based ADs act in a delayed-onset manner and often exhibit only moderate clinical efficacy. Electroconvulsive therapy (ECT) remains the treatment of choice for treatment-resistant depression (TRD) and for cases for which a rapid clinical response is required. Given the practical and ethical limitations of ECT, the development of fast-acting ADs is needed. Importantly, the NMDA receptor antagonist ketamine has been shown to produce rapid and long-lasting AD effects in TRD patients.

Changes in the levels and signaling of neurotrophin brain-derived neurotrophic factor (BDNF) have been associated with the etiology of MDD. However, studies of genetically modified mice expressing altered levels of BDNF have not provided a solid link between BDNF deficiency and depression-related behavior. By contrast, emerging evidence indicates that the effects of ADs are mediated by BDNF and its tropomyosin-related kinase B receptor, TrkB. ADs enhance BDNF-TrkB signaling and thereby facilitate neuronal plasticity in the brain. Recent evidence indicates that these changes in plasticity lead to the restoration of juvenile-type plasticity in the adult rodent cortex, which allows environment-driven reorganization of brain networks. Based on these data, the network theory of AD action was formulated. However, it is unclear if this concept can be generalized to diverse neuronal networks.

The main aims of this thesis were to investigate the importance of TrkB signaling in the anxiety- and depression-like behavioral phenotype in mice, to examine the role of BDNF-TrkB signaling in the antidepressant-like effects of glutamatergic drugs in mice, to study the network theory of ADs in a mouse fear extinction paradigm and to investigate the behavioral effects of adult fluoxetine treatment in mice exposed to fluoxetine early in life.

When examining TrkB signaling-deficient mice (TrkB.T1), we observed that young and aged TrkB.T1 mice exhibited alterations in their exploration and emotional behavior and increased behavioral despair. These findings suggest that altered TrkB signaling leads to depression-like behavior, and thus, TrkB.T1 mice may be used as a genetic model of depression.

We next studied selected glutamatergic drugs in behavioral despair models and determined that, similar to their effects in humans, ketamine and the AMPA receptor potentiator LY 451646 produce an antidepressant-like effect in mice. In contrast to classical ADs, these drugs were also effective in BDNF heterozygote knock-out mice. Furthermore, neither of these drugs influenced BDNF protein or Trk-phosphorylation levels in wild-type or BDNF-deficient mice. These data suggest that the antidepressant-like effects of ketamine may be independent of BDNF-TrkB signaling.

Disturbances in the serotonergic system during early development may cause permanent behavioral effect in adult animals. In our study, early life exposure to fluoxetine, an AD that enhances serotonergic transmission, led to specific and persistent behavioral changes in adult animals. Intriguingly, adult fluoxetine treatment normalized some of these changes. We therefore examined whether fluoxetine can enable plastic changes in fear circuits in mice in conjunction with an environmental stimulus. We observed that the combination of fear exposure and fluoxetine treatment produced permanent fear extinction in the classical fear conditioning paradigm in mice.

Importantly, neither fluoxetine nor extinction alone produced permanent fear erasure. This finding supports the network theory of AD action and clinical observations demonstrating the superiority of the combination of drug administration and psychotherapy for the treatment of post-traumatic stress disorder and depression.

In conclusion, these data strengthen the connection between BDNF-TrkB signaling and the antidepressant-like effects of classical ADs and support the network hypothesis of AD action. In addition, these results also suggest that there may be fast-acting AD treatments with a mechanism of action that is independent of BDNF-TrkB signaling.

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Yours sincerely,

Jesse

To my family and friends

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Abbreviations and symbols

5-HT	Serotonin
AD	Antidepressant
ADF	Adult fluoxetine treatment
AMPA	Alfa-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
BDNF	Brain-derived neurotrophic factor
CamK	α-calcium/calmodulin-dependent protein kinase II
CMS	Chronic mild stress
CRF	Corticotropin-releasing factor
CS	Conditioned stimulus
CUS	Chronic unpredictable stress
DALY	Disability-adjusted life years
DG	Dentate gyrus
ECT	Electroconvulsive therapy
EPM	Elevated plus -maze test
FC	Fear conditioning
flBDNF	Floxed BDNF mice
FST	Forced swimming test
GFAP	Human glial fibrillary acidic protein
HC	Hippocampus
HPA	Hypothalamic-pituitary-adrenal axis
KO	Gene knockout
LD	Light/dark box test
LH	Learned helplessness
MAOI	Monoamine oxidase inhibitor
MAS	Mouse affective syndrome
MDD	Major depressive disorder
MB	Marble burying test
mRNA	Messenger ribonucleic acid
MS	Maternal separation
NaSSA	Noradrenergic and specific serotonergic antidepressant
NMDA	*N*-Methyl-*D*-aspartate
NOR	Novel object recognition test
NRI	Norepinephrine reuptake inhibitors
NSF	Novelty suppressed feeding
NT	Neurotrophin
OB	Olfactory bulbectomy
OF	Open field test
PNF	Postnatal fluoxetine treatment
SD	Social defeat
SEM	Standard error of mean
SERT/5-HTT	Serotonin transporter
SNP	Single nucleotide polymorphism

SNRI	Serotonin–norepinephrine reuptake inhibitor
SSRI	Serotonin selective re-uptake inhibitor
TCA	Tricyclic antidepressant
TMS	Transcranial magnetic stimulation
TRD	Treatment resistant depression
Trk	Tropomyosin-related kinase
TrkB	Tropomyosin-related kinase B
TrkB.T1	Dominant negative form of the TrkB receptor
TST	Tail suspension test
US	Unconditioned stimulus
VCX	Visual cortex
WT	Wild-type
YLD	Years lived with disability

List of original publications

This thesis was based on the following publications, which are referred to in the text by the corresponding Roman numerals:

I **Lindholm J.**, Kemppainen S., Koivisto H., Stavén S., Vesa L., Rantamäki T., Tanila H., Castrén E., 2013. TrkB signaling deficient mice show reduced interest to explore novelty - a new model of depression? Submitted.

II **Lindholm J.,** Autio H., Vesa L., Antila H., Lindemann L., Hoener M.C., Skolnick P., Rantamäki T., Castrén E., 2012. The antidepressant-like effects of glutamatergic drugs ketamine and AMPA receptor potentiator LY 451646 are preserved in $BDNF^{(+/-)}$ heterozygous null mice. Neuropharmacology. 62:391-397.

III Karpova N., **Lindholm J.,** Pruunsild P., Timmusk T., Castrén E. 2009. Long-lasting behavioral and molecular alterations induced by early postnatal fluoxetine exposure are restored by chronic fluoxetine treatment in adult mice. European Neuropsychopharmacology. 19:97-108.

IV Karpova N.N., Pickenhagen A., **Lindholm J.**, Tiraboschi E., Kulesskaya N., Agústsdóttir A., Antila H., Popova D., Akamine Y., Bahi A., Sullivan R., Hen R., Drew L.J., Castrén E. 2011. Fear erasure in mice requires synergy between antidepressant drugs and extinction training. Science. 334:1731-1734.

1 Introduction

Major depressive disorder (MDD) is a substantial burden on global health; approximately 7% of the population in Western societies is affected by the disorder every year, and the lifetime prevalence is nearly 20% (Kessler et al., 2003). MDD is responsible for 5.6% of premature deaths in countries with high incomes and 3.4% globally (Lopez et al., 2006). Furthermore, MDD is the leading cause of years lived with disability (YLD). The costs of MDD are a substantial burden on the world economy; the annual costs of depression in Europe alone exceed 100 billion euros. Furthermore, MDD has devastating effects on the lives of patients and their families.

MDD has high comorbidity with anxiety; approximately 60% of depressed patients suffer various symptoms of anxiety (Garcia-Toro et al., 2013; Kessler et al., 2003). Both MDD and anxiety are commonly treated with monoamine-based antidepressant drugs (ADs) such as the serotonin selective re-uptake inhibitor (SSRI) fluoxetine or the monoamine oxidase inhibitor (MAOI) moclobemide. However, many MDD patients (20-30%) respond poorly to current medication or suffer relapse after drug discontinuation (Brunoni et al., 2010; Fava, 2003). Furthermore, there is a delay of several weeks before ADs relieve the symptoms of MDD (Hirschfeld, 2000).

Neurotrophin brain-derived neurotrophic factor (BDNF) and its tropomyosin-related kinase B receptor (TrkB) have been connected to the mechanism of action of ADs and to the pathophysiology of MDD (Castrén et al., 2007; Duman and Monteggia, 2006). MDD is associated with decreased BDNF levels, which are thought to underlie reduced neuronal plasticity, neuronal atrophy and even loss of synaptic connections. In contrast, ADs enhance BDNF-TrkB signaling in animal models, normalize BDNF levels in MDD patients and produce several neuroplastic changes in the brain. Furthermore, direct administration of BDNF into the rat hippocampus produces antidepressant-like behavioral effects. Genetically modified mice with altered levels or signaling of BDNF have also demonstrated the key role of BDNF-TrkB signaling in the actions of ADs, but these mouse models have not provided any solid link between BDNF deficiency and depression-related behavior (Castrén & Rantamäki, 2010).

The main clinical problems associated with the use of monoamine-based ADs are their poor efficacy and delayed onset of action. Recent experimental evidence has provided insight into the neurobiological mechanism behind both of these phenomena. Specifically, ADs slowly reopen developmental-type plasticity in the adult rat visual cortex (Maya Vetencourt et al., 2008). This heightened plasticity enables environment-driven synapse reorganization within the visual cortex. Thus, for optimal results, ADs should be combined with adequate functional therapy. Indeed, many MDD patients appear to respond well when simultaneously treated with ADs and psychotherapy (Pampallona et al., 2004; Oestergaard & Moldrup, 2011). However, this concept has only been

studied in the adult rat visual cortex, and further studies are needed to generalize this finding to other neuronal circuits and systems.

The delayed onset of action of current MDD therapies remains the most significant problem for patients at high risk of committing suicide. Electroconvulsive therapy (ECT) continues to be the treatment of choice for these patients. However, ECT may cause side effects, such as memory problems, cardiovascular changes, nausea, headache and muscle aches (Benbow, 2005). Thus, safer fast-acting ADs are needed. The rapid antidepressant effects of the NMDA receptor antagonist ketamine have recently received considerable attention in the scientific community. Interestingly, changes in BDNF-TrkB signaling have also been implicated in the antidepressant effects of ketamine. However, the mechanisms of action underlying the antidepressant effects of ECT and ketamine are unknown.

2 Review of the literature

2.1 Major depressive disorder

Affective disorders can be divided into mania, bipolar affective disorders and unipolar depressive disorders (WHO, 2010). This review of the literature concentrates on unipolar depressive disorder, which is also known as major depressive disorder (MDD).

Globally, MDD is one of largest burdens for health, particularly in high-income countries (Wittchen et al., 2011) because depression is a leading cause of disability for both women and men (Lopez et al., 2006). However, women are 50% more likely to suffer from depression than men. MDD greatly affects patients, their relatives and society. The overall burden of MDD can be divided into human and economic burdens.

Human suffering and changes in quality of life can be measured with the parameters YLD (years lived with disability) and DALYs (disability-adjusted life years), which are used to measure the overall burden of disease (one DALY represents one lost year of "healthy" life) (Simon, 2003). Neuropsychiatric conditions are responsible for 37% of YLD, and MDD is the leading cause of YLD; depression causes 9.1% of total YLD in low- and middle-income countries and 11.8% in high-income countries (Lopez et al., 2006). Furthermore, MDD is the seventh leading cause of DALYs globally (3.4%). In high-income countries, depression is the third leading cause of DALYs (5.6%); only ischemic heart diseases (8.3%) and cerebrovascular diseases (6.3%) cause more DALYs than MDD. Similarly, in European countries, MDD is the third leading cause of DALYs (6.0%) after ischemic heart disease (10.1%) and cerebrovascular disease (6.8%) (Olesen & Leonardi, 2003).

The economic burden of MDD is high; it has been estimated that the annual cost of depression in Europe alone is 92-118 billion euros (Andlin-Sobocki et al., 2005; Gustavsson et al., 2011; Olesen et al., 2012). Approximately 36-40% of this cost is direct costs, including hospitalization, medical care and medication; the remaining costs are indirect, such as loss of productivity (Gustavsson et al., 2011).

Many depressed patients have suicidal thoughts, and approximately 30% have attempted suicide (Pawlak et al., 2013). It is estimated that 4-15% of depressed patients die of suicide (Bostwick & Pankratz, 2000; Guze & Robins, 1970). For approximately 9 of 10 suicide victims, some type of psychiatric disorder is an underlying cause, and 2 of 3 of suicide victims have been diagnosed with depression (Cavanagh et al., 2003; Henriksson et al., 1993). Thus, depression is the most significant risk factor for lifetime suicide (attempted) (Bernal et al., 2007); male gender and high alcohol consumption are other high-risk factors for suicide (Hawton et al., 2013; WHO, 2001). In addition, comorbidity with other psychiatric disorders, such as substance abuse and anxiety, increases suicide risk (WHO, 2001).

2.1.1 Epidemiology of depression

Depression is a common disorder that is widely distributed through society in all ages and socio-economic classes in the general population. However, depression is more common in females than males, in young adults than elderly people and in less-educated and lower-income populations (Kessler et al., 2003). The lifetime prevalence of depression in the general population varies between 1 to 19%, depending on country and culture (Kessler et al., 2003; 2005; Kessler & Bromet, 2013). In the USA, the lifetime prevalence of depression is 16-19%, and the 12 month prevalence is 6.6%; in Taiwan, the lifetime prevalence is 1.5%, and the 12 month prevalence is 0.8% (Doris et al., 1999; Kessler et al., 2003; 2005; Kessler & Bromet, 2013). Globally, the lifetime prevalence of depression is 14.6% in high-income and 11.1% in low- and middle-income countries, with respective 12-month prevalences of 5.5% and 5.9% (Kessler & Bromet, 2013).

2.1.2 Symptoms and diagnosis of depression

The first descriptions of depression (lat. *melancholy*) in the literature are from ancient times (Davison, 2006). Since then, there have been many symptomatic definitions of depression, including depressed mood, lack of motivation, changes in appetite and weight and suicidal thoughts. In 1948, the World Health Organization (WHO) published the first diagnostic criteria for depression in the Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD-6) (WHO, 1948). Four years later, the American Psychiatric Association (APA) added similar criteria for depression to the Diagnostic and Statistical Manual: Mental Disorders, First edition (DSM-I) (APA, 1952). Although our understanding of depression, its diversity and treatment has evolved during the last six decades, the neurobiological basis of depression remains unclear.

There are two main diagnostic criteria for depression: ICD-10 and DSM-IV (WHO, 1994; APA, 1994). Both criteria define depression in a similar way. According to the definitions, a depressive episode should last for at least two weeks and include two of the following criteria: 1) depressed mood most of the time, 2) loss of interest or pleasure (anhedonia) in activities that are normally pleasurable and 3) decreased energy (Table 1). Several additional symptoms must be present for diagnosis, such as suicidal thoughts, loss of confidence or self-esteem, sleep disturbances or changes in appetite (Gruenberg et al., 2005; Pedersen et al., 2001; Remick, 2002). The severity of depression, which is classified as mild, moderate or severe depression, depends on the number and clinical severity of these symptoms. Exclusion criteria are manic or hypomanic episodes and substance abuse-induced depression.

MDD has high comorbidity with several psychiatric disorders and other diseases. Nearly 3 of 4 (72.1%) MDD patients have a lifetime prevalence of some other DSM-IV disorder (Kessler et al., 2003).

Lifetime comorbidity with anxiety is approximately 50-60%, with substance use is 24-27% and with impulse control disorder is 30% (Fava et al., 1997; Kessler et al., 2003; Kupfer & Frank, 2003). Furthermore, the 12 month comorbidities with the aforementioned neuropsychiatric conditions are 57.5%, 8.5% and 16.6%, respectively (Kessler et al., 2003).

Table 1. Diagnostic criteria for major depressive disorder

	DSM IV	ICD-10
Duration of symptoms	Depressed mood most of day for at least 2 weeks	Depressed mood at least 2 weeks
Symtoms	1. Depressed mood most of the day 2. Loss of interest and enjoyment, anhedonia 3. Loss of energy or fatigue nearly every day 4. Feelings of worthlessness or excessive or inappropriate guilt 5. Suicidal thoughts or attemps, or thoughts of death 6. Reduced concentration and attention 7. Psychomotor agitation or retardation 8. Insomnia or hypersomnia nearly every day 9. Altered appetite with weight changes (>5%)	1. Depressed mood most of the day 2. Loss of interest and enjoyment, anhedonia 3. Loss of energy or fatigue most of the time 4. Unfounded ideas of guilty and unworthniness 5. Repetitive suicidal thoughts or attemps, or thoughts of death 6. Reduced concentration and attention 7. Psychomotor agitation or retardation 8. Disturbed sleep 9. Altered appetite with weight changes 10. Reduced self-esteem and self-confidence
Diagnosis	Five or more symptoms, which should include 1 or 2.	At least 2 symptoms from 1-3 and some from 4-10. There should be at least 4 symptoms for diagnosis mild depression 4-5 symptoms, moderate depression 6-7 symptoms and severe depression 8-10 symptoms (including all 1-3).

References: APA, 1994; Gruenberg et al., 2005; Pedersen et al., 2001; Remick, 2002; WHO, 1994

2.1.3 Treatments for depression

Treatments for depression vary depending on the symptoms and severity of depression. Standard treatments include pharmacotherapy (Table 2), psychotherapy and their combination. Moreover, severe and drug-resistant depression is commonly treated with ECT or transcranial magnetic stimulation (TMS).

Table 2. Medical treatments of depression

Class of antidepressants	AD
Selective serotonin reuptake inhibitors (SSRI)	Citalopram
	Escitalopram
	Paroxetine
	Fluoxetine
	Fluvoxamine
	Sertraline
Tricyclic antidepressants (TCA), tertiary	Amitriptyline
	Clomipramine
	Doxepin
	Imipramine
TCAs, secondary	Desipramine
	Nortriptyline
Norepinephrine reuptake inhibitors (NRI)	Reboxetine
Serotonin–norepinephrine reuptake inhibitors (SNRI)	Duloxetine
	Venlafaxine
Monoamine oxidase A inhibitor (MAOI)	Moclobemide
Noradrenergic and specific serotonergic ADs (NaSSA)	Mianserin
	Mirtazapine
Norepinephrine-dopamine reuptake inhibitors	Bupropion
Selective serotonin reuptake enhancers	Tianeptine
MT1 and MT2 agonist, 5-HT2 antagonist	Agomelatine
Serotonin antagonist and reuptake inhibitors	Etoperidone
	Nefazodone
	Trazodone

References: Lam et al., 2009

Pharmacotherapy is usually started as a monotherapy with serotonin selective reuptake inhibitors (SSRIs) such as citalopram, fluoxetine or sertraline (Depont et al., 2003; Ellis et al., 2004). Other possible choices include serotonin-norepinephrine reuptake inhibitors (SNRI; e.g., venlafaxine and duloxetine), selective norepinephrine reuptake inhibitors (NRI; e.g., reboxetine), noradrenergic and specific serotonergic antidepressants (NaSSA; e.g., mianserine and mirtazapine), monoamine oxidase A inhibitors (MAOI-A; e.g., moclobemide) and tricyclic antidepressants (TCA; e.g., imipramine,

desipramine and amitriptyline). Polypharmacotherapy is usually not beneficial; rather, the replacement of inefficacious ADs with other pharmacological classes of ADs may help.

Psychotherapy is an effective treatment for mild and moderate depression (Ellis et al., 2004). However, the combination of psycho- and pharmaco-therapy usually yields more pronounced clinical effects than either treatment alone (Pampallona et al., 2004; Oestergaard & Moldrup, 2011). In more severe cases, when several pharmacotherapies combined with psychotherapy fail to relieve symptoms of depression, the use of ECT or TMS is considered (Brunoni et al., 2010; Ellis et al., 2004; Nemeroff, 2007).

A significant number of depressed patients do not respond adequately to ADs. This medical condition, called treatment-resistant depression (TRD), affects approximately 30% of depressed patients (Olchanski et al., 2013). The widely used definition of TRD requires an unsuccessful response to an adequate course of treatment (Nemeroff, 2007). However, the definition of inadequate response has been broadly discussed in the field. Thus, Thase and Rush (1997) introduced a 5 step staging system for AD resistance, starting with the failure of at least one adequate trial of one major class of ADs (stage 1) and ending with the failure of four adequate trials of different AD classes and failure of a course of ECT (stage 5). The cost of TRD patients is a high economic burden for society and is approximately 90% higher than the cost for a non-TRD patient (Olchanski et al., 2013).

2.2 Antidepressant drugs

Different causes of depression have been proposed since ancient times, when depression was hypothesized to be caused by an imbalance of bodily fluids (Davison, 2006). At the beginning of the 20th century, it was shown that reserpine, an antihypertensive and monoamine-depleting drug, causes depression-like symptoms in chronic hypertensive patients (a finding that has since been questioned) (Baumeister et al., 2003). In the 1950s, iproniazid, a close derivative of the tuberculosis drug isoniazid, was serendipitously found to recover the depressed mood of tuberculosis patients with depressive symptoms. Around the same time, imipramine, a tricyclic agent similar in structure to the antipsychotic chlorpromazin, was shown to produce antidepressant effects in psychiatric patients (Kuhn, 1958). Iproniazid and imipramine were subsequently shown to increase extracellular levels of the neurotransmitters serotonin (5-HT) and/or norepinephrine in the brain. These drugs inhibit either the monoamine-catabolizing enzyme monoamine oxidase (MAO) or the re-uptake mechanism of monoamines, leading to an increase in serotonin and norepinephrine levels in the synaptic cleft and enhanced serotonergic or/and noradrenergic transmission in the brain (Ashcroft et al., 1972; Coppen, 1967; Schildkraut, 1965). Based on these observations, the monoamine hypothesis of depression was introduced (Schildkraut, 1965), and all currently clinically used ADs influence the

monoaminergic system of the brain. However, even though biochemical responses and pharmacological side effects appear within hours after drug administration, the clinical symptoms of depression are relieved only after a delay of several weeks. This contradiction has been contemplated by investigators and clinicians for decades (Hindmarch, 2002). During the last two decades, knowledge about depression and its etiology has evolved, and novel theories have been introduced. The neurotrophin hypothesis of depression, which is currently one of the strongest theories of depression, will be discussed in greater detail.

2.3 Neurotrophins, depression and antidepressant action

2.3.1 Neurobiology of neurotrophins

Neurotrophins (NTs) consist of a small family of neurotrophic factors that include nerve growth factor (NGF), BDNF, neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4). NTs control the differentiation and survival of neurons during early development (Huang & Reichardt, 2001). Later in adulthood, NTs regulate synaptic function and plasticity and neuronal survival (Huang & Reichardt , 2001). NTs act through specific high-affinity tropomyosin-related kinase (Trk) receptors: TrkA for NGF; TrkB for BDNF, NT-3 and NT-4/5 and TrkC for NT-3. All NTs bind preferentially as pro-forms to the low-affinity $p75^{NRT}$ receptor, which is related to controlled cell death, also known as apoptosis (Lu et al., 2005).

Among these NTs, the role of BDNF and its signaling cascade through the TrkB receptor in regulating activity-dependent neuronal and network plasticity in the developing and adult central nervous system has been increasingly recognized (Park & Poo, 2013; Poo, 2001; Thoenen, 1995). Neuronal activity regulates the production and release of BDNF, which plays a critical role in their activity-dependent plasticity. BDNF acts as a dimer and binds to the extracellular portion of the TrkB receptor, leading to receptor dimerization. This change induces subsequent receptor transphosphorylation and the phosphorylation of other intracellular tyrosine residues (Y515 and 816) that regulate the activation of several signaling pathways, including the Ras-MAPK (mitogen-activated protein kinase), PI3k (phosphatidylinositol 3-kinase)-Akt (protein kinase B) and phospholipase Cγ (PLCγ) pathways (Minichiello at al., 2009). The activation of these pathways regulates neuronal transmission and plasticity and the survival, proliferation and differentiation of cells (Figure 1). Furthermore, TrkB signaling cascades can be activated in the absence of NTs, such as by adenosine agonists and zinc (Huang et al., 2008; Lee and Chao, 2001; Nagappan et al., 2008).

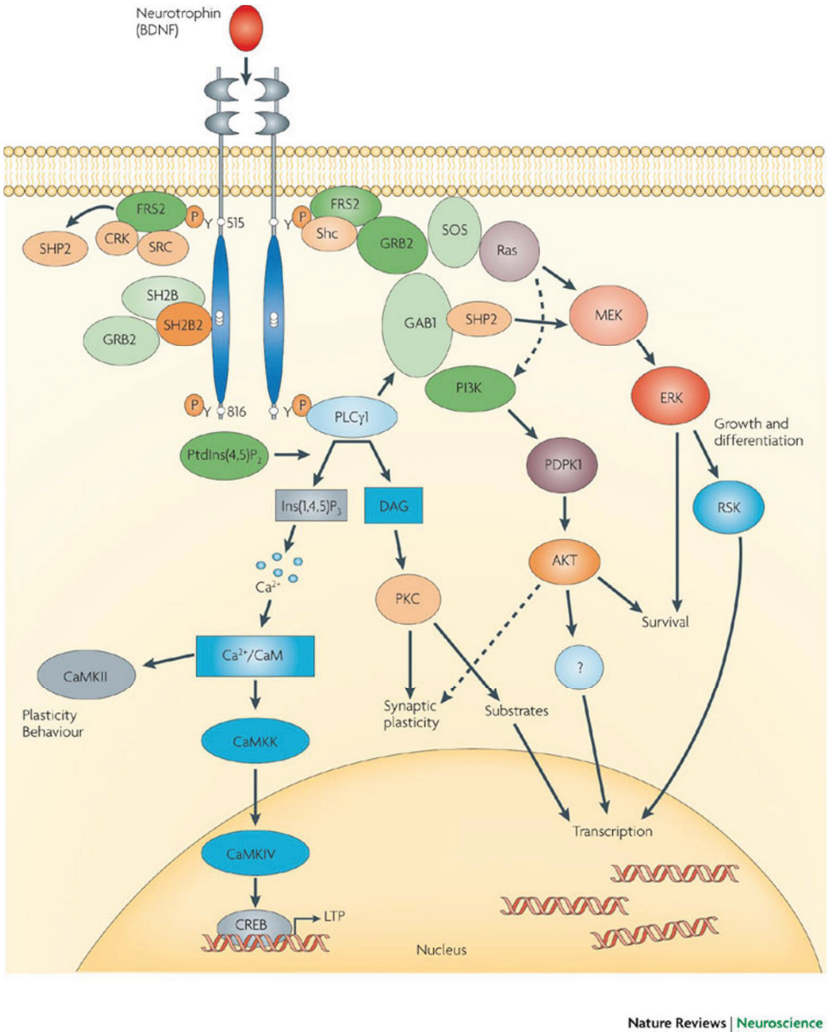

Figure 1. Major TrkB-signaling-activated pathways (adapted from Minichiello, 2009). The interaction between the TrkB receptor and neurotrophins activates three main intracellular signaling pathways. Phosphorylation and recruitment of adaptors to Y515 leads to the activation of the Ras–MAPK signaling cascade, which leads to neuronal differentiation and growth through MAPK/ERK kinase (MEK) and extracellular signal-regulated kinase (ERK) and to the activation of the phosphatidylinositol 3-kinase (PI3K) cascade, which promotes the survival and growth of neurons and other cells. The phosphorylation of Y816 activates phospholipase Cγ1 (PLCγ1), leading to the generation of inositol-1,4,5-trisphosphate [Ins(1,4,5)P3] and diacylglycerol (DAG). Whereas DAG stimulates protein kinase C (PKC) isoforms, Ins(1,4,5)P3 promotes the release of Ca2+ from internal stores and the subsequent activation of Ca2+/calmodulin (Ca2+/CaM)-dependent protein kinases (CaMKII, CaMKK and CaMKIV). All three signaling pathways also regulate gene transcription, and some may be involved in long-term potentiation (LTP). BDNF, brain-derived neurotrophic factor; FRS2, fibroblast growth factor receptor substrate 2; GRB2, growth factor receptor-bound protein 2; PDPK1, 3-phosphoinositide-dependent protein kinase 1; PtdIns(4,5)P2, phosphatidylinositol-4,5-bisphosphate; RSK, ribosomal protein S6 kinase; SHP2, SRC-homology phosphatase 2; SOS, son of sevenless. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Neuroscience (Minichiello, 2009), copyright (2009).

2.3.2 Neurotrophin hypothesis of depression and antidepressant action

The neurotrophin hypothesis of depression presumes that stress-induced reduction of BDNF signaling and neuronal plasticity causes atrophy and weakening of synaptic connections in specific brain areas, finally leading to altered information processing and mood disorders (Castrén, 2005; Duman et al., 1997; Duman & Monteggia, 2006). Furthermore, AD treatment enhances BDNF signaling and, in the long term, increases BDNF-mediated neuronal plasticity in the brain, facilitating patient recovery. Because BDNF-induced changes in plasticity take time to develop, the clinical relief of MDD symptoms is delayed. This hypothesis was introduced nearly two decades ago, based on findings that the mRNA and protein levels of BDNF in the rodent hippocampus (HC) correlate with depressive behaviors and anatomical changes in the HC induced by stress (Duman et al., 1997; Duman & Monteggia, 2006). Furthermore, Nibuya et al. (1995) observed that acute and chronic ECT treatment and chronic administration of ADs, including desipramine and sertraline, increased BDNF and TrkB mRNA levels in the rat HC.

Acute or chronic stress activates the hypothalamic-pituitary-adrenal (HPA) axis and increases the synthesis and release of glucocorticoids (cortisol in humans and corticosterone in rodents) and corticotropin-releasing factor (CRF). Severe long term stress and hypercortisolemia can induce damage and atrophy in neurons of the CA3 subregion of the HC and reduce neurogenesis in the adult hippocampal dentate gyrus (DG), brain areas related to learning, memory and mood disorders (Fuchs & Gould, 2000; Gould et al., 1997; Gould et al., 1998; McEwen, 2000; McKittrick et al., 2000). In humans, the hippocampal volume is decreased in patients suffering from MDD, most likely due to a decreased number of synaptic connections (Bremner et al., 2000; Sheline et al., 1996). Similar to stressed subjects, depressed patients have increased plasma cortisol levels and increased CRF levels in the cerebrospinal fluid (Burke et al., 2005; Merali et al., 2004). In animal studies, different stressors or corticoid injections decrease the expression of BDNF in the HC and prefrontal cortex, brain areas related to mood disorders. Altered BDNF expression levels can be reversed by both chronic ECT and AD treatment (Barrientos et al., 2003; Nibuya et al., 1995; Rasmusson et al., 2002; Roceri et al., 2002; Roceri et al., 2004). Furthermore, several clinical studies have shown that BDNF serum levels are decreased in depressed patients (Karege et al., 2005; Monteleone et al., 2008; Sen et al., 2008), and altered mRNA levels of BDNF and its TrkB receptor and lower BDNF plasma concentrations have been associated with suicidal subjects (Dwivedi et al., 2003; Kim et al., 2007). These findings demonstrate that stress and depression are correlated with altered BDNF-TrkB signaling.

The effects of increasing and decreasing BDNF levels in the brain have been widely studied in animals. Direct injection of BDNF into the DG or CA3 of the HC and midbrain leads to an antidepressant-like effect and enhancement of the antidepressant-like effect of paroxetine in rodent

models of depression-like behavior (Deltheil et al., 2008; Shirayama et al., 2002; Siuciak et al., 1997). The tyrosine kinase inhibitor K252a blocks this effect, suggesting that the antidepressant-like behavior of BDNF is dependent on TrkB activity (Shirayama et al., 2002). Surprisingly, peripheral administration of BDNF also appears to produce antidepressant-like effects similar to those induced by intracranial administration (Schmidt & Duman, 2010). In contrast, direct injection of BDNF into the ventral tegmental area causes depression-like behavior, while blocking BDNF signaling in the nucleus accumbens produces antidepressant-like behavior (Berton et al., 2006; Eisch et al., 2003). A global reduction of BDNF expression and protein levels in the brain has not produced clear depression- or anxiety-like phenotypes in transgenic mice and has yielded controversial results. A summary of transgenic mice with altered BDNF-TrkB-signaling is described below (see chapter 2.4.6). Although these findings indicate a key role for BDNF in the pathology of MDD, the potential connection between BDNF and MDD remains unclear because the results of studies in BDNF transgenic mice have not been conclusive.

A single nucleotide polymorphism (SNP) has been observed in the human *BDNF* gene, in which valine (Val) is substituted with methionine (Met) in codon 66 (Val66Met). This SNP is only observed in humans and is commonly expressed in the general population (Val/Met: 20-50%, Met/Met 3-20%) and is more common in Asian than Caucasian populations (Verhagen et al., 2008). Humans that are heterozygous for the Met allele display smaller hippocampal volumes and poor performance on hippocampal-dependent memory tasks. However, a connection between this SNP and clinical depression and anxiety is unclear (Gratacos et al., 2007; Verhagen et al., 2008). There is a potential association between Val66Met SNP and other mental disorders, such as substance abuse, eating disorders and schizophrenia (Gratacos et al., 2007). Chen et al. (2006) produced a mouse line with a knock-in of this SNP. However, like other transgenic mouse models of BDNF, these mice did not further clarify the connection between BDNF and the pathophysiology of depression (for a further review, see Chapter 2.4.6).

While the role of BDNF-TrkB signaling in the pathology of depression is unclear and controversial, the role of this signaling system in the effect of ADs is better characterized (Adachi et al., 2008; Castrén et al., 2007; Duman & Monteggia, 2006). Different classes of ADs can activate TrkB signaling after acute and long-term administration (Rantamäki et al., 2007; Saarelainen et al., 2003). Similarly, ADs, ECT and physical exercise have been shown to increase BNDF levels after several days of treatment in animal models and humans (Chen et al., 2001; Coppell et al., 2003; Duman et al., 2008; Marais et al., 2009; Nibuya et al., 1995; Russo-Neustadt et al., 1999; Zetterstrom et al., 1998). Importantly, stress-induced decreases in BDNF expression and serum protein levels can be restored by chronic AD treatment in both experimental animals and depressed patients (Duman & Monteggia, 2006; Nibuya et al., 1995; Sen et al., 2008). Furthermore, the presence of BDNF and the activation of

TrkB-related signaling are needed for antidepressant-like effects in rodents (Ibarguen-Vargas et al., 2008; Saarelainen et al., 2003). However, the regulation of BDNF expression by ADs is more complex because BDNF mRNA levels have been shown to decrease shortly after AD administration in some studies (Coppell et al., 2003; Kozisek et al., 2008). In addition, a direct injection of BDNF into the HC or overexpression of full-length TrkB in the brain of transgenic mice produces antidepressant-like behaviors (Koponen et al., 2005; Shirayama et al., 2002; Siuciak et al., 1997).

2.3.3 Network hypothesis of AD action

In clinical patients, ADs relieve the symptoms of depression after several weeks of treatment. It has been suggested that this delay is due to the need for the growth of neuronal connections, neurogenesis and plasticity (Castrén, 2004; 2005). ADs enhance neuronal plasticity at many levels of the nervous system *via* a mechanism that involves BDNF signaling (Krystal et al., 2009; Maya Vetencourt et al., 2008). Chronic but not acute administration of ADs or ECT enhances neurogenesis and the survival of newborn neurons in the DG of the adult HC, which seems to be important for antidepressant-like behavior in rodent models (Bergami et al., 2008; Dranovsky and Hen, 2006; Madsen et al., 2000; Malberg et al., 2000; Santarelli et al., 2003; Wu & Castrén, 2009). Furthermore, ADs have been shown to specifically enhance the turnover of new neurons in the HC rather than only increasing their proliferation (Sairanen et al., 2005). AD treatments can also increase synaptic connections in the brain and in areas other than those where neurogenesis occurs (Chen et al., 2008; Hajszan et al., 2005; O'Leary et al., 2009). Moreover, BDNF-TrkB signaling is required for these effects (O'Leary et al., 2009).

The functional significance of AD-induced plasticity changes has been recently studied in more detail using visual cortex (VCX) plasticity as a model platform. During early development, environmental stimuli direct the formation of the neuronal network in the VCX, and after a critical period, a permanent neuronal network is formed in the VCX (Castrén & Rantamäki, 2010). The ability of ADs to increase synaptic plasticity *via* enhanced BDNF and TrkB signaling has attracted interest in this phenomenon. Maffei's and Castrén's groups studied whether chronic fluoxetine treatment could open a critical period-like state and enable environment-driven reformation of the neuronal network (Maya Vetencourt et al., 2008). In their studies, these authors found that fluoxetine and an enriched environment were able to re-open developmental-like plasticity in the adult rat visual cortex (Maya Vetencourt et al., 2008; Sale et al., 2007).

Closing one eye during a critical period of visual cortex development during early postnatal life leads to the ocular dominance of the open eye. After the critical period has closed, this dominance is permanent. However, chronic fluoxetine treatment can open a critical period-like state, which leads

to the enhanced plasticity-driven reformation of the neuronal network. Together with an environmental stimulus (opening the weak eye and closing the dominant eye), this reformation of the neuronal network recovers the vision of the weaker eye (Maya Vetencourt et al., 2008). Moreover, AD-induced plasticity and environmental stimuli are both required for permanent recovery of the vision of the poor eye. These results indicate that ADs can activate neuronal plasticity, which can lead to the functional reorganization of the neuronal network after the closure of the critical period, at least in the visual cortex of the rat (Castrén & Rantamäki, 2010). Based on these observations, the network theory of AD action has been formulated.

However, some aspects of this theory are not well substantiated. Apart from the visual cortex, the network theory of AD action has not been tested in other neurocircuits, and data supporting its generalization to humans are lacking. The translation of these results to humans is challenging because it is impossible to control the influence of the environment. In addition, many depressed patients benefit only from the pharmacotherapy and do not require psychotherapy for recovery. The AD effect might also be mediated *via* other mechanisms, as some treatments (e.g., ECT and ketamine) have fast-acting AD effects (Li et al., 2010a). Additional studies are needed to evaluate whether this concept can be generalized to diverse neuronal networks and to humans.

2.3.4 Searching for fast-acting antidepressants

The clinical effects of classical ADs appear only after several weeks of treatment, which can be explained by the network hypothesis of ADs, and often are inadequate against MDD. Furthermore, there may be other mechanisms, independent of those specified by the network hypothesis, by which plasticity and information processing are enhanced. For example, ECT, the most efficacious antidepressant, can improve depressed mood shortly after a single treatment. However, ECT has unwanted side effects, and the procedure is associated with ethical concerns. Thus, there is a need and potential for new fast-acting and orally administrable ADs.

There has been interest in drugs targeting brain glutamatergic signaling pathways as potential fast-acting AD candidates (Alt et al., 2006; Skolnick et al., 2009; Vollenweider & Kometer, 2010). Several clinical studies have demonstrated that a single intravenous infusion of the non-competitive NMDA receptor antagonist ketamine, a dissociative anesthetic, generates a rapid and long-lasting antidepressant effect at a subanesthetic dose (Berman et al., 2000; Machado-Vieira et al., 2009; Zarate et al., 2006). An antidepressant effect of ketamine has also been observed in TRD patients (aan het Rot et al., 2010; Diazgranados et al., 2010; Liebrenz et al., 2007). Similarly, the NR2B subtype NMDA receptor antagonist traxoprodil produced a robust antidepressant effect in patients for whom adequate SSRI treatment had failed (Preskorn et al., 2008).

The behavioral effects of ketamine have been widely investigated in rodents, in which ketamine produces rapid and long-lasting antidepressant-like effects at a wide range of subanesthetic doses and enhances the responses of classical ADs (Autry et al., 2011; Koike et al., 2011; Li et al., 2010a; Maeng et al., 2008; Popik et al., 2008; Reus et al., 2011). Similar to ketamine, other NMDA (N-methyl-D-aspartate) receptor antagonists have been shown to have antidepressant-like effects and to potentiate the effects of classical ADs in rodents (Rogoz et al., 2002). Because ketamine and other NMDA antagonists have severe side effects and the potential for abuse, other glutamate-based approaches, including the potentiation of AMPA (alfa-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, have increasingly become the focus of preclinical studies. Allosteric modulators of AMPA receptors (AMPA potentiators) exhibit antidepressant-like effects similar to the effects of imipramine and ketamine (Bai et al., 2001; Li et al., 2001; Li et al., 2003). Moreover, these AMPA potentiators enhanced the potency of classical antidepressants in preclinical tests of depression (Li et al., 2003). Similar to classical ADs, both NMDA antagonists and AMPA receptor potentiators have been shown to regulate BDNF expression both *in vivo* and *in vitro* (Autry et al., 2011; Garcia et al., 2008; Legutko et al., 2001; Reus et al., 2011). However, Réus et al. (2011) observed a dose-dependent effect of ketamine, in which the dose of ketamine was inversely proportional to BDNF expression levels in several brain areas. Similar effects have been observed with the AMPA potentiator LY 451646 (Mackowiak et al., 2002). Furthermore, a clinical study by Machado-Vieira et al. (2009) failed to identify an association between antidepressant response and serum BNDF levels in depressed patients.

2.4 Mouse behavior in antidepressant research

The discovery of the first ADs in the middle of the 20th century initiated a new era of depression research. The identification of their pharmacological mechanism of action necessitated the measurement of the efficacy of novel AD candidates. The first methods for screening antidepressant effects were developed for rats in the 1960-1970s and were later introduced to mice. Because depression is a complex disorder with a wide spectrum of symptoms and uncertain biological background, modeling depression in relatively simple rodent models is challenging and widely criticized. Symptoms of depression and their corresponding behavioral methods are described in Table 3. In addition, the most common behavioral endpoints in rodent depression studies are presented in Figure 2 and are described in the following sections.

Table 3. Modelling symptoms of depression in mice

Symptom of depression	Test
Depressed mood	Cannot be monitored
Loss of interest and enjoyment, anhedonia	Intracranial self-stimulation Sucrose preference Social withdrawal
Loss of energy or fatigue	Home cage activity Treadmill/runnig wheel activity Observation of nest building Observation of sexual behavior
Unfounded ideas of guilty and unworthniness	Cannot be monitored
Repetitive suicidal thoughts or attemps, or thoughts of death	Cannot be monitored
Reduced concentration and attention	Models of working and spatial memory: T-maze 8-arm radial maze Water maze
Psychomotor agitation or retardation	Locomotor activity Motor coordination
Disturbed sleep	Measurement of sleep architecture with electroencephalogy (EEG)
Altered appetite with weight changes	Food intake and body weight measurement
Reduced self-esteem and self-confidence	Cannot be monitored
References: Chourbaji et al. 2011, Cryan and Holmes 2005, Cryan and Mombereau 2004	

2.4.1 Validity of models

How can complex human diseases be modeled in other species? The validity of a disease model can be divided into three categories: face, construct and predictive validities (Belzung et al., 2001; Willner and Mitchell, 2002; Willner & Mitchell, 2006). Face validity measures the phenomenological similarity of a method and a selected symptom of a human disease, without requiring a deeper etiological basis. Predictive validity assesses the ability of the model to predict changes in the human subject based upon changes in the model. Construct validity goes even deeper in the analysis of human disease by measuring the etiological, pathological and symptomatological basis of a model. For a disease model, the most important of the three dimensions is the construct validity. However, for a depression model, construct validity is difficult to measure.

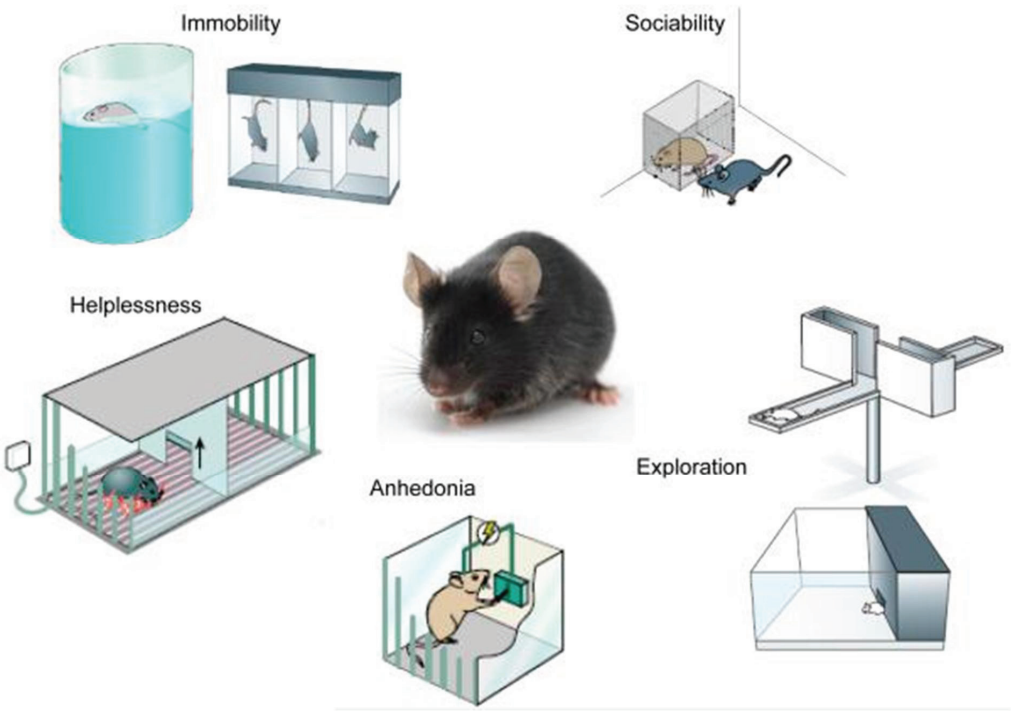

Figure 2. Common behavioral endpoints in rodent depression studies (adapted from Krishnan & Nestler, 2010). This figure diagrams certain widely utilized quantitative and automatable behavioral endpoints used in experiments with rats or mice as measures of depression-related behavior. They can be employed following chronic stress paradigms such as social defeat, to phenotype genetic mutant mice, to validate antidepressant treatments or as tools to localize genomic mediators of complex behaviors in QTL analyses (quantitative trait locus). The most popular endpoint is immobility, which is interpreted as a measure of behavioral despair or freezing in response to an inescapable stressor like forced swimming or tail suspension. The closely related helplessness can be inferred through the learned helplessness paradigm, where animals receive a series of inescapable electrical shocks in one compartment, and on subsequent testing days display a deficit in their motivation to avoid these shocks when a clear escape route is provided. Anhedonia in mice and rats can be measured in several ways, ranging from simple measures of sucrose preference (measuring the relative preference for a dilute solution of sucrose versus water), to preference for a high fat diet, to ICSS (intracranial self stimulation) where one directly measures motivation (lever pressing) to receive highly rewarding electrical stimulation. Reductions in exploratory behavior are often interpreted as elevations in anxiety, and can be quantified by measuring amounts of time spent in aversive portions of a field of exploration such as the open arms of the elevated plus maze (top) or the brightly illuminated portion of the light-dark box. One can also measure deficits in sociability, which may reflect impairments in natural reward or social anxiety. These assays have been employed in stress paradigms, mutant mouse models as well as models of secondary depression such as that seen, for example, with obesity, breast cancer or chronic interferon treatment. A common practice is to generate behavioral profiles by employing a broad battery of these tests following stress, genetic, or pharmacological manipulations, which can also include changes in weight and appetite, as well as deficits in self-grooming (deteriorations in fur coat). Reprinted with permission from the American Journal of Psychiatry, (Copyright ©2010). American Psychiatric Association.

Criteria for face validity. Face validity compares similarities between symptoms and signs of a disorder and the model (Belzung et al., 2001; Willner et al., 2002; Willner & Mitchell, 2006). Depression is expressed in various ways in MDD patients, and symptomology can differ from patient to patient. However, not all symptoms are equal; some main symptoms have higher weight than others. The most important symptoms of depression include decreased mood, feelings of worthlessness and thoughts of death or suicide and are impossible to model in animal experiments. However, many depressed patients usually lose their interest in daily satisfying activities, e.g., eating, drinking and engaging in sexual activity and social contact, that were previously pleasurable to the subject. This anhedonic behavior can be measured in rodents in a test based on sucrose preference. Furthermore, some patients experience changes in appetite and weight; both of these phenomena can be measured in mice with metabolic cages and scales, respectively. In addition, insomnia or disruptions in sleep can be modeled in mice directly by monitoring the characteristics of their sleep by electroencephalogram or indirectly by following mouse circadian rhythms. Moreover, fatigue and loss of energy can be measured in mice *via* decreased locomotor or running wheel activity or disruptions in nest building. These examples illustrate the diversity of symptoms of depression and the difficulty of modeling them. Furthermore, to induce behavioral changes in naïve animals, some instigator is needed; in AD research, stress is usually used to trigger abnormal behavior. In addition, to obtain adequate face validity for depression, tests and models should include responsiveness to common ADs, preferably under chronic administration.

Criteria for predictive validity. The predictive validity of a depression model is determined mainly by the responsiveness to AD treatment (Belzung & Griebel, 2001; Willner et al., 2002; Willner and Mitchell, 2006). A model should respond to commonly used ADs (true positive; SSRIs, TCA, MAOIs, SNRIs, ECT) and show negative results for clinically ineffective compounds (true negative). Furthermore, good predictive validity also requires the minimization of false-positive (compounds showing a response in the model but with no clinical effectiveness) and false-negative compounds (compounds showing no response in the model but with clinical effectiveness). In practice, it is impossible for a depression model to detect 100% of the clinically effective AD treatments because the clinical efficacy of some compounds is unclear. Moreover, there is great heterogeneity in the responses of MDD patients to AD treatments (Willner & Mitchell, 2006). It is generally accepted that a reliable depression model should respond to the chronic administration of several classes of ADs (SSRI, TCA, MAOI, SNRI) and ECT and should not respond to psychostimulants, anticholinergics, opiates or benzodiazepines (Willner & Mitchell, 2006). Responsiveness to the chronic administration of ADs increases the predictive validity of a depression model; good examples of depression models sensitive to chronic but not acute administration of ADs are chronic mild stress and olfactory bulbectomy tests (Harkin et al., 2003; Muscat et al., 1992; Papp et al., 1996; Strekalova et al., 2006).

Criteria for construct validity. Construct validity includes similarities both in symptoms and in the etiological basis of the disease (Belzung & Griebel, 2001; Willner et al., 2002; Willner & Mitchell, 2006). For good construct validity, a depression model should mimic the pathological state of MDD induced by the cause of the clinical disorder. However, variable factors (e.g., stressful life events, heredity and internal causes) can increase the vulnerability to MDD, with no single factor as a cause of depression (Tennant, 2002). Some biochemical and anatomical changes, such as disruption of the HPA axis and decreases in hippocampal volume and brain monoamine and BDNF levels, have been connected to the pathophysiology of depression. Moreover, the pathogenesis of depression is more likely due to an accumulation of a number of different risk factors. However, as long as the etiology of depression remains unclear, the construct validity of any model of depression is relatively poor.

2.4.2 Tests measuring antidepressant efficacy

Early methods for measuring antidepressant effects were mainly based on the monoamine hypothesis and thus measured monoamine dysfunction in the brain. One of these methods measures hypothermia induced by reserpine or apomorphine, which can be reversed with tricyclic ADs (Alpermann et al., 1992). These methods measure more pharmacological effects of ADs than modeling depression, and they have relatively good predictive validity when tested with classical ADs but poor or no face or construct validity.

In the 1960s-1970s, models of behavioral despair were developed. In these models, which were first designed with rats and subsequently modified for mice, rodents are exposed to an inescapable and unpredictable stress, which is said to model depression. In the learned helplessness (LH) model, mice are placed in a chamber from which they are unable to escape and exposed to inescapable and unpredictable shocks (Kudryavtseva et al., 1991). Repeated exposure to the shocks leads to the despair behavior, including vocalization and passivity and alteration of sleep-wake patterns. Later, when tested in escapable conditions, LH-treated mice fail to escape shock even when escape is possible; this response is considered behavioral despair. Chronic AD (imipramine) treatment reduced this behavior to the control animal level (Kudryavtseva et al., 1991).

Another method of inducing behavioral despair is the forced swimming test (FST), which was first introduced by Porsolt et al. (1977). In this model, mice are placed in a glass cylinder filled with water so that they cannot reach the bottom of the cylinder or climb out of it. Initially, the mice swim and attempt to climb out of the cylinder. After a few minutes, the mice begin to float and become immobile. Immobility of mice is considered a despair behavior, which can be reversed by acute administration of ADs (Petit-Demouliere et al., 2005; Porsolt et al., 1978). Another model of behavioral despair, the tail suspension test (TST) modified from the FST, was subsequently

introduced (Steru et al., 1985). Similar to the FST, mice are placed in an inescapable situation; in this test, the mice are hung by their tails from a hook, and the immobility time is measured. However, there are several differences between the FST and TST, apart from their notable similarities (Cryan et al., 2005). Unlike the FST, in the TST, mice are not required to have the ability to swim, which may be impaired in some genetically modified mice. Another advantage of the TST is that, contrary to the FST, it does not cause hypothermia in mice. There are also differences between the FST and the TST in their ability to differentiate classes of ADs (Cryan et al., 2005). Traditionally, it was presumed that SSRIs do not have antidepressant-like effects in the FST, but SSRIs have been shown to be effective in TST (Cryan et al., 2005). However, the selection of the mouse strain may strongly influence the effectiveness of ADs (Lucki et al., 2001; Petit-Demouliere et al., 2005). Furthermore, baseline immobility in both the FST and TST may vary widely depending on the genetic background of the strain (Bai et al., 2001). Measuring immobility or other behaviors subjectively is challenging and can be easily biased; thus, validated and automated recording systems are recommended for both the FST and TST (Crowley et al., 2004; Hayashi et al., 2011; Juszczak et al., 2008).

ADs and ECT decrease the immobility of mice in these tests. Furthermore, stressors, such as immobilization, foot shock and social defeat, increase the depression-like behavior of mice in the FST (Hebert et al., 1998). The FST and TST are also used to measure depression-like behavior after chronic mild stress in a model system. Although despair models reveal remarkable antidepressant effects, they have poor validity as depression models. One of their major disadvantages is that ADs, which work in depressed patients only after several weeks of treatment, have an acute effect in this model. Another substantial drawback of these methods is that the mice used in these tests are usually naïve, and behavioral despair is induced by short-term stress lasting only minutes. However, all ADs in clinical use exhibit antidepressant-like effects in these models. Thus, these tests do not accurately model depression but could be used to measure antidepressant efficacy.

2.4.3 Tests based on anhedonic behavior

Many depressed patients lose interest in daily satisfying elements (e.g., food, drink, sex and social contacts) that previously were pleasurable to the subject. This anhedonic behavior can be measured in rodents in a test based on sucrose preference (Forbes et al., 1996; Papp et al., 1996). Mice are naturally more interested in sweet solutions when given free choice between ordinary tap water and a sweet sucrose solution. Furthermore, when animals are stressed, they lose their interest in the sweet, pleasurable sucrose and consume equal volumes of water and sucrose solution (Forbes et al., 1996; Papp et al., 1996). Furthermore, as social interactions are typical for humans and mice,

social withdrawal can be considered together with other chronic stressors as a relevant trigger for this behavior.

Along with the sucrose preference test, other reward-based methods can be used to measure anhedonia. One example of these methods is the intra-cranial self-stimulation test, in which rodents are trained to self-stimulate with pleasurable electrical intracranial stimulation (Slattery et al., 2007). Chronic mild stress increases the threshold in this test, and chronic administration of ADs can recover these changes in the threshold (Fibiger & Phillips, 1981). However, this test is mostly used in addiction studies, and its use of it in AD studies may be not be appropriate.

2.4.4 Tests measuring emotional and fear behavior of mice

Because depression is often comorbid with anxiety in clinical patients and both disorders are treated with ADs (Borsini et al., 2002), it is relevant to also measure anxiety-like behaviors of mice. However, the symptoms of depression and anxiety are commonly mixed, and the interpretation of these models is difficult.

Classical methods to measure anxiolytic-like behavior are based on conflicts in mouse emotional behavior. Mice naturally explore novel areas, and when placed in a new, unsafe environment, they are faced with a conflict between their natural urge to explore new areas and threat from a menacing territory. When these mice are given an anxiolytic compound, they explore more in the threatening environment. Classical examples of this type of test are the open field (OF), the elevated plus maze (EPM) and the light/dark exploration (LD) tests. In the OF, the mouse is placed in an open, brightly illuminated round- or square-shaped area, and the exploration behavior of the mouse is observed (Walsh & Cummins, 1976). This test measures both anxiety-like behavior (time spent in the central portion of the area) and overall locomotion (total distance traveled and vertical activity) of the mice. In the EPM, the mouse is placed in a piece of equipment shaped like a cross, with two open arms and two arms with walls, that is elevated from the ground (Hogg, 1996; Montgomery, 1955). The mouse is placed in the middle of the maze, and the latency to the open arms, time spent in the open arms and the total arm entries are measured. The LD test is performed in a test box including two compartments, one of which has black walls and a cover and the other of which is brightly illuminated (Bourin & Hascoet, 2003). The mouse is positioned in the bright side of the apparatus, and the latency to the dark compartment, time spent in both compartments and transitions between compartments are measured. All of these tests are sensitive to anxiolytics, e.g., diazepam and alcohol. However, these tests are also sensitive to treatment-induced changes in mouse locomotion, e.g., amphetamine-induced hyperactivity causes false-positive results in this test. Thus, other methods are also used to measure anxiety-like behavior.

There are several tests that measure anxiety-like behavior independent from locomotor activity. In the novelty suppressed feeding (NSF) test, the mouse is subjected to fasting and is subsequently placed in an open, brightly lit area with a small piece of food in the middle (Dulawa & Hen, 2005; Hodes et al., 2010). Normally, the novel environment inhibits the mouse from eating the food. When treated with an anxiolytic compound (e.g., diazepam), the latency is decreased. Acute fluoxetine and TCA treatments do not have any effects, but chronic treatment produces anxiolytic effects in this model (Borsini et al., 2002; Dulawa & Hen, 2005; Hodes et al., 2010). Another simple and widely used test is the marble burying (MB) test, in which glass marbles are placed in the home cage of an individual mouse (De Boer & Koolhaas, 2003; Nicolas et al., 2006). Mice tend to cover all marbles, and anxiolytic compounds reduce this burying behavior. SSRIs, TCAs and many other ADs produce anxiolytic-like behavioral changes in this test when administered acutely and, for some ADs, when administered chronically (Borsini et al., 2002). However, this test has been criticized because it is more likely to measure stereotypical or compulsive behavior than anxiety-like behavior of mice (Thomas et al., 2009). Stressful handling (e.g., insertion of a rectal probe) induces hyperthermia in mice (Adriaan Bouwknecht et al., 2007). This increased body temperature can be normalized with anxiolytic drugs. However, ADs do not appear to have any effect on this model (Adriaan Bouwknecht et al., 2007; Borsini et al., 2002).

Mice are naturally interested in seeking novel environments and objects. This characteristic can be exploited to measure xenophobia and anxiety-like behavior in mice in the novel object recognition test (NOR) (Sik et al., 2003). Mice are introduced to two objects for a period of time. These objects are removed and replaced by two objects, a familiar object and a new object. Mice are normally more interested in the new object than the familiar one. Mice that spend less time investigating the novel object are considered to have anxiety-like behavior, whereas mice that spend more time with the new object exhibit anxiolytic behavior. However, this test is also used to measure the working memory of the animal, and interpretation of the test can be challenging. Clearly, complementary behavioral and biochemical data (e.g., cortisol levels) could strengthen the interpretation.

In threatening situations, mice have a natural tendency to either escape or freeze. In inescapable situations, mice are considered as freezing when they do not move other than breathing. In a classical model of fear conditioning (FC), mice are subjected to a conditioned stimulus (CS; e.g., sound) and, simultaneously, to an unpleasant, unconditioned stimulus (US; e.g., electric foot shock) (Myers & Davis, 2007). Subsequently, the mice freeze when presented with the CS. After the conditioning, anxiolytic compounds decrease the freezing time of mice. The effects of ADs in this model are variable; SSRIs have acute anxiolytic effects, whereas other classes of ADs have small or no effects on FC (Borsini et al., 2002). Similarly, chronic administration of ADs has varying anxiolytic

effects on freezing behavior (Borsini et al., 2002; Burghardt & Bauer, 2013). Furthermore, after the conditioning, the cued and contextual memory of mice can be measured. In the cue memory test, the mouse is placed in the novel context, and fear-response-related memory (freezing time during the CS) is assessed; the context-related anxiety-like memory can be measured in a familiar context in the absence of the CS.

Fear responses can be erased with extinction training, which includes repeated appearance of the CS in the absence of the US (LeDoux, 2000; Milad et al., 2006; Myers & Davis, 2007). After extinction training, the freezing of mice in the presence of the CS is equal to the freezing time before conditioning. However, the effect of extinction training is short lived, and the fear response to the CS is recovered after some time. This phenomenon is called spontaneous recovery. Fear response can also be evoked with repeated appearance of the CS after the fear extinction in the original context (fear renewal). Extinction and spontaneous recovery are performed in a different context than that in which the conditioning was performed to prevent an influence of the conditioning context. Interestingly, extinction training in juvenile mice induces permanent fear erasure (Gogolla et al., 2009).

2.4.5 Depression models based on stress

There is strong evidence that stressful life events and depression have a considerable connection; up to 80% of depressed patients have had a major negative life event (Mazure, 1998). Most studies have identified episodic stressors (stressors with a beginning and end) as the factor most strongly connected to depression (Hammen, 2005). In general, these stressors are usually related to interpersonal loss/separation or the threat of them, particularly in women (Paykel, 2003; Tennant, 2002). Moreover, the secretion of the stress-related hormone cortisol is increased by 50% in depressed patients (Checkley, 1996), emphasizing the association between stress and depression.

Chronic mild stress. To mimic the connection between stressful events and depression, the chronic mild stress (CMS) procedure was introduced. In CMS, rodents (mice or rats) are exposed to unpredictable, mild environmental stressors for several weeks (Forbes et al., 1996; O'Neil & Moore, 2003; Papp et al., 1996; Strekalova et al., 2004; Strekalova et al., 2011). These stress factors can be social or physiological and vary from species to species. For rats and mice, stressors may include the following: wet bedding, disrupted dark-light circle, water and food deprivation/restrictions, immobilization (positioned in a small tube) and tilting of the cage at an angle (Pothion et al., 2004; Smirnov et al., 2013). However, some stressors, including exposure to a rat (for mice) or suspension of mice by the tail, may not be considered mild stressors but are still widely used in stress models (Strekalova et al., 2004). Chronic exposure to these stressors leads to anhedonia and other

depression-related behaviors (e.g., increased immobility in the FST or alterations in aggression, grooming and sexual behavior) in mice (Enkel et al., 2010; Mineur et al., 2003; Willner, 2005), which can be reversed by AD treatment (Muscat et al., 1992; Papp et al., 1996; Strekalova et al., 2006). CMS also increases the activity of the HPA axis and hypersecretion of corticosterone (Vollmayr & Henn, 2003). There are some drawbacks and concerns about this model; the stressors employed vary greatly depending on the laboratory, and reproducibility between laboratories is poor. Furthermore, the interstrain variability of sensitivity to CMS is high (Ibarguen-Vargas et al., 2008; Mineur et al., 2003; Pothion et al., 2004). However, a few requirements are essential to ensure success in this model with mice, including the need for multiple changing stressors that occur in unpredictable orders and intervals. The validity of CMS is relatively good; mice exposed to stressors exhibit anhedonia, decreased activity and sexual behavior, altered sleep patterns and loss of weight (Vollmayr & Henn, 2003). Furthermore, all major AD treatments, including ECT, work in the CMS model when administered chronically, thus increasing the predictive validity of this model (Muscat et al., 1992; Papp et al., 1996; Strekalova et al., 2006). Some biochemical changes (e.g., alterations in norepinephrine and serotonin systems) and changes in the HPA axis similar to those observed in human depression are also observed in this model.

Olfactory bulbectomy. Smell is the most important sense for rodents because it mediates messaging by pheromones and carries information about the behavioral and physiological status of an animal, social recognition, breeding and aggression behaviors (Shepherd, 2006). The loss of the sense of smell isolates a rodent from its psychosocial milieu, causing stress and behavioral and biochemical changes in animals. This phenomenon was used to construct the olfactory bulbectomy (OB) model of depression. In this model, rodents (mice or rats) are subjected to bilateral lesions of the olfactory bulbs. This procedure leads to anosminia, or loss of the sense of smell, causing long-lasting behavioral changes related to depression-like behaviors. These changes include abnormal sleep patterns, agitation, weight loss and changes in hedonic behavior (Liebenauer & Slotnick, 1996; Song & Leonard, 2005). Furthermore, OB increases the exploration and hyperactivity of rodents in an OF test (Zueger et al., 2005). This change can be reversed by chronic but not acute AD treatment (Freitas et al., 2013; Machado et al., 2012). OB appears to have good predictive validity because all classes of therapeutically active ADs yield positive results in this model when administered chronically (Harkin et al., 2003). In contrast, psychotropic drugs, which lack antidepressant activity, fail to reverse these behavioral changes. OB induces alterations in neurochemical, neuroendocrinological and neuroimmunological indicators that also can be reversed by chronic AD treatment (Roche et al., 2007).

Early life stress. Rodents and humans are social animals, and separation from other members of their species causes behavioral changes, such as depression in humans. In particular, adverse early

life experiences and stress significantly increase susceptibility to mental diseases, including depression (Newport et al., 2002). To model dysfunctional parenting, pups are separated from their mother for designated periods of time, usually during the first 2-3 postnatal weeks. Repeated deprivation of their mother decreases the maternal care of the pups, which is critical for mental and physical development. Thus, maternal separation (MS) increases anxiety and depression-like behavior in rodents (Millstein & Holmes, 2007). Neonatal stress induced by MS also causes changes in the HPA axis activity, leading to elevated corticosterone levels and decreases in hippocampal BDNF and neurogenesis in mice (Kikusui et al., 2009). In addition, AD treatment during the early postnatal period has been shown to produce long-lasting changes in depression- and anxiety-like behavior, which can be used to model depression (Ansorge et al., 2004; Vogel et al., 1990).

Social stress. Stress can be induced not only by social separation but also by subjecting animals to chronic social defeat (SD). The SD model includes daily exposure to a novel, physically superior aggressor for a defined period of time. This exposure leads to avoidance of social interaction, anhedonic behavior and increased anxiety-like behavior in the defeated mice (Buwalda et al., 2005; Strekalova et al., 2004; Tsankova et al., 2006). Furthermore, SD increases plasma corticosterone levels and decreases hippocampal BDNF levels. Similar to OB, the changes caused by SD can be reversed by chronic but not acute administration of ADs (Tsankova et al., 2006). The social interaction of mice can be measured in a resident-intruder test, in which a mouse (resident) is placed in a cage and can explore, thus familiarizing itself with the cage. Once the mouse becomes familiar with the cage, an intruder mouse is introduced, and aggressive and social contacts are counted.

2.4.6 Genetic mouse models of depression

Since the production of the first genetically modified mice, there has been a rapid increase in the number of transgenic mice established to model depression. Research on genetic models of depression has focused on monoamines and their metabolism, particularly serotonin (Fernandez & Gaspar, 2012; Gardier et al., 2009; Gardier, 2009). Other approaches have also been employed; for example, glucocorticoid receptor-impaired mice exhibit antidepressant-like behavior, whereas endocannabinoid CB1 receptor-deficient mice exhibit increased anxiety- and depression-like behavior (Cryan et al., 2002; Valverde and Torrens, 2012). Furthermore, mice overexpressing the CB2 receptor have a depression-resistant endophenotype (Garcia-Gutierrez et al., 2010). The introduction of the neurotrophin hypothesis of depression led to an increased number of transgenic mice related to NTs and particularly to BDNF. However, as long as the etiology of depression remains uncertain, a conclusive genetic model cannot be introduced. (Cryan et al., 2002; Gardier et al., 2009)

The serotonin transporter (SERT/5-HTT) plays a key role in the function of serotonergic neurons. SERT terminates synaptic transmission of serotonin by re-uptaking serotonin into the presynaptic neuron (Daws, 2009). Inhibition of SERT with an SSRI (e.g., fluoxetine) increases the concentration of serotonin in the synaptic cleft, leading to increased neuronal transmission. For decades, this pathway has been considered to play a key role in the mechanism of action of ADs. Total deletion of SERT in transgenic mice leads to a depression- and anxiety-like phenotype and sleep disturbances; these mice exhibit increased immobility time in the FST and TST; increased anxiety-like behavior in the EPM-, LD- and OF-tests; decreased locomotor activity and increased REM sleep (Alexandre et al., 2006; Holmes et al., 2003b). Similar changes in behavior are observed when wild-type (WT) or $SERT^{(+/-)}$ mice are exposed to SSRIs (e.g., fluoxetine or citalopram) during the early postnatal state (Ansorge et al., 2004). This contradictory effect of early life exposure is hypothesized to be due to disrupted activation of the presynaptic autoreceptor of serotonin, the 5-HT1A-receptor; administration of the highly specific 5-HT1A receptor antagonist WAY100635 in the early postnatal period and in adulthood reverses the behavioral changes induced by genetic SERT depletion (Alexandre et al., 2006; Holmes et al., 2003c). Furthermore, SERT plays an important role in the mechanism of antidepressant action of SSRIs in the FST and TST; the antidepressant effects of the SSRI fluoxetine but not the noradrenergics imipramine or desipramine are blunted in SERT knockout (KO) mice (Holmes et al., 2002). However, there appears to be some changes in the behavioral phenotype of SERT-KO mice depending on their genetic background (Holmes et al., 2002; Holmes et al., 2003a).

Because the 5-HT1A receptor is involved in the antidepressant-like effects of SSRIs, 5-HT1A-KO mice have been examined in tests measuring anxiety- and depression-like behavior. These mice displayed a paradoxical behavioral phenotype characterized by increased avoidance of open and threatening areas in the EPM and OF and decreased immobility time in the FST, indicating increased anxiety- and decreased depression-like behavior (Heisler et al., 1998; Parks et al., 1998). Moreover, many mice with modifications in genes related to the synthesis, release and re-uptake of neurotransmitters have been created, but most lack clear depression- or antidepressant-like behavioral phenotypes (Fernandez & Gaspar, 2012).

After the introduction of the neurotrophin hypothesis of depression in the 1990s, the focus of depression research turned to the NTs and, in particular, to BDNF. Several transgenic mice have been created with a modulation in BDNF expression. At the beginning of the 1990s, Ernfors et al. (1994) created a mutant mouse lacking BDNF in the brain, a constitutive BDNF KO. Mice lacking both BDNF alleles have severe health problems and die during the first weeks of life (Ernfors et al., 1994). However, heterozygous BDNF KO ($BDNF^{(+/-)}$) mice are vital, appear normal and display BDNF mRNA and protein levels in the brain that are reduced by half when compared to their littermate WT

controls (Ibarguen-Vargas et al., 2009; Korte et al., 1995; MacQueen et al., 2001). BDNF$^{(+/-)}$ mice have been widely studied, and their behavioral phenotype has been quantified, although results vary among laboratories (Table 4). These mice have been shown to have increased appetite and gain weight easily (Chen et al., 2006; Kernie et al., 2000; Lyons et al., 1999). They have normal visual, auditory and nociceptive senses but have some impairment in the olfactory system (Bath et al., 2008; Liu et al., 2004). Most of the studies found no differences in locomotor activity in OF or novel cage tests when compared with the control group (Chen et al., 2006; Chourbaji et al., 2004; Ibarguen-Vargas et al., 2009; Li et al., 2010b; Lyons et al., 1999; MacQueen et al., 2001; Psotta et al., 2013). However, Kernie et al. (2000) observed a significant reduction in the activity of these mice. BDNF$^{(+/-)}$ mice have impaired contextual memory in the Morris water maze and FC test, but their cued memory is intact (Chen et al., 2006; Linnarsson et al., 1997; Liu et al., 2004); however, not all studies are in agreement on this issue (Chourbaji et al., 2004). These mice also appear to have impaired fear extinction behavior in the FC paradigm (Psotta et al., 2013). The anxiety- and depression-like behaviors of BDNF$^{(+/-)}$ mice appear to be more complex. Some experiments have revealed no changes in anxiety-like behavior (Chourbaji et al., 2004; Ibarguen-Vargas et al., 2009), whereas others have observed increased aggressiveness and anxiety-like behavior (Chen et al., 2006; Li et al., 2010b; Lyons et al., 1999). Furthermore, these mice do not appear to have depression-like behavior in the FST or TST test (Chourbaji et al., 2004; Duman et al., 2007; Ibarguen-Vargas et al., 2009; MacQueen et al., 2001; Saarelainen et al., 2003), but they have increased latencies to escape in LH (MacQueen et al., 2001). In contrast, stressful experiences appear to trigger anxiety- and depression-like behavior in BDNF$^{(+/-)}$ mice (Carola & Gross, 2010; Duman et al., 2007). Moreover, the effects of ADs are blocked in these mice when tested in the FST or TST (Ibarguen-Vargas et al., 2009; Saarelainen et al., 2003). In conclusion, BDNF$^{(+/-)}$ mice gain more weight, are more aggressive and learn and remember less well than their littermate WT controls. These animals do not exhibit clear anxiety- or depression-like behaviors but are more vulnerable to stress. Furthermore, the effects of ADs are blocked in these mice.

Because the behavioral phenotype of the constitutive BDNF$^{(+/-)}$-KO mouse is vague and new advances in gene technology have been introduced, additional genetically modified mice have been generated, that focus on the expression of BDNF (Table 5). Rios et al. (2001) produced two conditional BDNF KO mouse lines by crossing mice carrying the floxed BDNF (flBDNF) allele with another line expressing Cre recombinase under the direction of the α-calcium/calmodulin-dependent protein kinase II (CamK) promoter, which drives expression in post-mitotic neurons. Two mouse lines, CamK-cre93 and CamK-cre159, were created from the KO starting from postnatal day 21 and 15, respectively. Both of these lines displayed increased food intake, weight gain, anxiety-like behavior and hyperactivity (Rios et al., 2001). Similar to the study by Rios et al., Monteggia et al. (2007)

Table 4. Behavioral results of heterozygous BDNF-KO mice.

Behavioral tests	Result	Background	Reference
BW, LA and FI	Increased appetite and weight, and decreased locomotor activity in BDNF(+/-) mice, but not in heterozygous NT4/5, NT3, TrkC or TrkA knockout mice.	C57Bl/6 and 129Sv mixed F2 background	Kernie et al. 2000
Anhedonia, EPM, FST, LH, NOR, OF, PA, SP, staircase	No differences in most of tests. Longer escape latencies in LH.	C57Bl/6 and 129Sv mixed background	MacQueen et al. 2001
NSF, OF, RI, TST	No behavioral changes, effects of ADs blocked in heterozygous mice.	C57Bl/6 and 129Sv mixed background	Ibaarguen-Vargas et al. 2009
EZM, FC, FST, LD, novel cage, OF, RR	No behavioral changes.	C57Bl/6 and 129Sv mixed background	Chourbaji et al. 2004
EPM, OF	Increased sensitivity to pre/postnatal maternal environment (high or low maternal care)	C57Bl/6J and/or BALB/c	Carola et al. 2010
BW, FI, OF, RI	Increased appetite, weight and aggressiveness	C57Bl/6	Lyons et al. 1999
BW, EPM, FC, LA, NSF, OF, RI	Increased weight, aggressiveness, and anxiety-like behavior, impaired contextual but intact cue memory. No changes in locomotor activity.	C57Bl/6J	Chen et al. 2006
FST	No behavioral changes, effects of ADs blocked in heterozygous mice.	129Sv X BALB/c	Saarelainen et al. 2003
FC, pain, vision and auditory tests	No changes in senses or baseline freezing, impairment in contextual memory-, but not in cue memory test	C57Bl/6	Liu et al. 2004
FC, OF	No changes in locomotor activity, but adult animals have impaired fear extinction learning	C57Bl/6J	Psotta et al. 2013
Spontaneus olfactory discrimination	Impairment in olfactory system	C57Bl/6	Bath et al. 2008
FST	Depression-like behavior after stress or MEK inhibitor	C57Bl/6?	Duman et al. 2007
EPM, OF	Increased anxiety-like behavior, no changes in locomotor activity.	C57Bl/6	Li et al. 2010b
WM	Impaired learning and memory	129/J x BALB/c	Linnarsson et al. 1997

Abbreviations: Body weight (BW), elevated plus-maze (EPM), fear conditioning (FC), food intake (FI), forced swimming test (FST), elevated zero maze (EZM), locomotor activity (LA), light-dark box (LD), learned helplessness (LH), novel object recognition (NOR), novelty suppressed feeding (NSF), open field (OF), passive avoidance (PA), resident-intruder test (RI), RotaRod (RR), sucrose preference (SP), tail suspension test (TST), water maze (WM)

Table 5. Behavioral results of transgenic BDNF mice

Mutant mice	Behavioral tests	Result	Background	Reference
Inducible BDNF-KO in forebrain: adult KO	BW, FC, LA, RI, pain test	No changes in BW, LA, aggressiviness, cue memory or pain sensitivity, impaired contextual memory	mixed background: BL6/SJLxICR x ICR x Bl6/sv129	Monteggia et al. 2004
Inducible BDNF-KO in forebrain: early KO	BW, FC, FST, LA, RI	No changes in BW or aggressiviness, hyperactive phenotype, impaired contextual and cue memory	mixed background: BL6/SJLxICR x ICR x Bl6/sv129	Monteggia et al. 2004
Inducible BDNF-KO in forebrain	BW, FST, LA, NSF, OF, SP, TST	BDNF-KO increases vulnerability to chronic stress induced anxiogenic and anhedonic behaviors in female, but not in male mice.	mixed background: BL6/SJLxICR x ICR x Bl6/sv129	Autry et al. 2009
Conditional BDNF-KO: postnatal brain	BW, FI, LA, LD	Increased appetite, weight and anxiety-like behavior, hyperactivity	Mixed background	Rios et al. 2001
Conditional BDNF-KO	LA, EPM, FST, OF, SP	Hyperactivity in male mice, depression-like behavior in female mice. Effects of ADs blocked in both sex in FST.	-	Monteggia et al. 2007
BDNF (Val66Met) polymorphism	EPM, FC, LA, NSF, OF, RI	Increased weight, aggressiveness, and anxiety-like behavior, impaired contextual but intact cue memory. No changes in locomotor activity.	C57Bl/6J	Chen et al. 2006
BDNF (Val66Met) polymorphism	Spontaneus olfactory discrimination	Impairment in olfactory system	C57Bl/6	Bath et al. 2008
BDNF (Val66Met) polymorphism	EPM, FST, NSF, OF, SP, TM, WM	Depression- and anxiety-like behavior, poor spontaneus alteration only after stress. Rescuing effect of desipramine but not fluoxetine in FST.	-	Yu et a. 2012
BDNF (Val66Met) polymorphism	EPM, OF	Increased anxiety-like behavior, no changes in locomotor activity. Music rescues anxiogenic behavior.	C57Bl/6	Li et al. 2010b
BDNF overexpressing	WM	Heterozygous but not homozygous mice showed improved learning and memory	C57Bl/6J	Nakajo et al. 2008
BDNF overexpressing in exitatory neurons in forebrain	EPM, FST, OF	Anxiogenic- and antidepressant-like behavior. No changes in locomotor activity.	C57Bl/6	Govindarajan et al. 2006
BDNF overexpressing, hemizygous	ASR, EPM, FC, FST, LD, OF, PPI, RR, SA, TM, TST	Impaired working memory, but normal contextual and cue memory, impairments in ASR and PPI, anxiety-like behavior in LD but not in EPM. Normal motor and locomotor function, no changes in depression-like behavior.	C57Bl/6J	Papaleo et al. 2011

Abbreviations: Acustic startle response (ASR), body weight (BW),elevated plus-maze (EPM), fear conditioning (FC), food intake (FI), forced swimming test (FST), locomotor activity (LA), light-dark box (LD), novelty suppressed feeding (NSF), open field (OF), pre-pulse inhibition (PPI), resident-intruder test (RI), rotarod (RR), social approach (SA), sucrose preference (SP), tail suspension test (TST), T-maze (TM), water maze (WM)

generated two conditional BNDF KO mice lines. One line was constructed by crossing flBDNF mice with a line containing Cre-driven human glial fibrillary acidic protein (GFAP)-Cre, while the other was created by crossing flBDNF and CaMKII-Cre transgenic mice. GFAP-Cre mice express Cre recombinase in broad forebrain regions during late embryogenesis, whereas the CamKII-Cre mice express the Cre recombinase in similar regions during postnatal development (Monteggia et al., 2007). CaMKII-CrexflBDNF mice exhibit a significant reduction of BDNF mRNA levels in the HC and dorsal cerebral cortex, while GFAP-CrexflBDNF mice lack BDNF mRNA almost completely in the same brain areas. In behavioral analyses, Monteggia et al. (2007) observed that male conditional KOs in both lines had hyperactivity but normal depression-like behavior. By contrast, female mice had normal activity but increased depression-like behavior. Furthermore, the antidepressant effect of desipramine was blunted in both male and female conditional BDNF KO mice.

Another approach to create time-dependent KO mice is to use an inducible KO method. In this procedure, a pharmacological compound, e.g., tamoxifen or doxycycline, is used to either activate or block gene expression. Monteggia et al. (2004) produced trigenic mice in which BDNF expression was knocked out in the absence of doxycycline. In these mice, early KO of BDNF led to a hyperactive phenotype with impaired contextual and cue memory. In contrast, the adult KO had a milder phenotype, with some impairment in contextual memory. Autry et al. (2009) subsequently examined the influence of chronic unpredictable stress (CUS) on similar mice. These authors observed that female mice with induced BDNF KO were more vulnerable to CUS-induced anxiogenic and anhedonic behaviors; however, male mice did not exhibit changes in the same parameters.

Chen et al. (2006) created mice with a knock-in of the human Val66Met SNP of the BDNF gene. Similar to observations in humans, both hetero- (+/Met) and homozygous (Met/Met) Met-mice exhibited decreased total hippocampal volume and impaired contextual memory (Chen et al., 2006). However, their cue memory and locomotor activity were similar to the control animals. Met/Met mice had increased weight, aggressiveness and anxiety-like behavior. In addition, the effects of fluoxetine were blocked in OF and NSF tests. Since their establishment, these mice have been used in several experiments. Both +/Met and Met/Met mice have impairment of their olfactory system (Bath et al., 2008). Met/Met mice exhibit anxiogenic behavior that is rescuable with music exposure (Li et al., 2010b), and +/Met exhibit vulnerability to stress-induced anxiety- and depression-like behavior (Yu et al., 2012). This stress-induced depression-like behavior in the +/Met mice can be rescued with desipramine but not fluoxetine (Yu et al., 2012).

Direct infusion of BDNF to the DG of the HC causes antidepressant-like effects in rats (Shirayama et al., 2002). Thus, increasing BDNF expression in the brain genetically may lead to antidepressant-like behavior. Govindarajan et al. (2006) used mice overexpressing BDNF in excitatory neurons of the forebrain, including the hippocampus, cortex and amygdala, to test this hypothesis

(Huang et al., 1999). They observed an interesting behavioral phenotype of increased anxiety- and antidepressant-like behavior (Govindarajan et al., 2006). Furthermore, anxiogenic-like behavior was not caused by chronic immobilization stress. In contrast, another group found that these mice have anxiety-like behavior in the LD but not in the EPM (Papaleo et al., 2011). These authors did not observe changes in motor functions, contextual or cue fear memory or social or depression-like behavior. However, there were impairments in the working memory and auditory systems of these mice. The discrepancies between the results of these two independent experiments are likely attributable to sex differences because Govindarajan et al. used male mice and Papaleo et al. used female mice. Moreover, other studies have also demonstrated sex differences in genetically modified BDNF mice (Autry et al., 2009; Monteggia et al., 2007). Another line of mice overexpressing BDNF in the brain and other tissues exhibited enhanced performance in learning and memory tasks (Nakajo et al., 2008).

Studies of mice with altered BDNF expression have been unable to establish a clear connection between depression and BDNF; some of the studies have yielded controversial results or no behavioral changes. Although BDNF protein levels are reduced by half in $BDNF^{(+/-)}$ mice throughout their lifespan, their behavioral phenotype is mild and varies among studies. This finding may be explained by developmental compensatory mechanisms, variations in genetic background (Jacobson & Cryan, 2007), differences in sex (Dalla et al., 2010) or differences in practices among laboratories (Wahlsten et al., 2003). Furthermore, conditional KOs and mice overexpressing BDNF are subject to the same problems as $BDNF^{(+/-)}$ mice; behavioral results vary widely depending on the laboratory and location and time period of transgene expression. Thus, there is growing interest in ligand-independent genetic models of BDNF-TrkB-signaling (Table 6).

TrkB-KO mice suffer from serious developmental problems and die during the first three postnatal weeks (Klein et al., 1993). Therefore, conditional TrkB-KO mice are essential to examine the behavioral effects of TrkB receptor-deficient mice. Mice with forebrain-specific TrkB-KO ($TrkB^{CAMKII\text{-}CRE}$) are viable, have normal brain morphology, and lack TrkB receptors in the HC and forebrain neocortex (Minichiello et al., 1999). These mice have a hyperactive phenotype with impulsive behavior and increased mobility in the FST (Zörner et al., 2003). However, these animals do not appear to exhibit changes in the test for anxiety-like behavior. Another mouse line in which TrkB was deleted in adult progenitors exhibited decreased locomotor activity and increased anxiety-like behavior (Bergami et al., 2008). In contrast, TrkB.TK+ mice with enhanced TrkB signaling (overexpression of the full-length catalytic form of the TrkB receptor) have been shown to have reduced anxiety- and depression-like behavior and improved contextual and associative learning and memory without changes in locomotor activity or coordination (Koponen et al., 2004; Koponen et al., 2005). In contrast, transgenic mice (TrkB.T1) with decreased TrkB signaling (overexpression of the truncated dominant

negative form of the TrkB receptor) displayed inhibition of the antidepressant-like effect of imipramine in behavioral despair models (Saarelainen et al. 2003).

Furthermore, Chen et al. (2005) produced another knock-in mouse line with a point mutation in the TrkB receptor ($TrkB^{F616A}$). This mutation is not expected to influence the activity of the receptor, but when given a pharmacologically inert kinase inhibitor (1NMPP1), the activity of the TrkB receptor is inhibited. An advantage of these mice is that TrkB activity can be specifically and rapidly blocked without directly influencing the expression of TrkB. $TrkB^{F616A}$ mice have not yet been studied in large-scale behavioral studies.

Table 6. Behavioral results of transgenic TrkB mice

Mutant mice	Behavioral tests	Result	Background	Reference
Forebrain-Specific TrkB-Receptor Knockout	EM, EZM, FST, NOR, OF	Increased locomotor activity in OF and mobility in FST. No changes in anxiety-like behavior.	C57Bl/6N x 129/sv x CBA/J	Zörner et al. 2003
TrkB(+/-)	Spontaneus olfactory discrimination	Impairment in olfactory system	C57Bl/6	Bath et al. 2008
TrkB-KO in adult born neurons	EPM, OF	Decreased locomotor activity, increased anxiety-like behavior	C57Bl/6	Bergami et al. 2008
Overexpressing catalytic TrkB receptor (TrkB.TK+), used as heterozygous	FST	Antidepressant-like behavior	CD2F1 (BALB/c x DBA/2)	Koponen et al. 2005
Overexpressing catalytic TrkB receptor (TrkB.TK+), used as heterozygous	CTA, EPM, FC, HP, LD, OF, RR, WM, Y-maze	Anxiolytic-like behavior, enhanced contextual and associative learning and memory. No changes in locomotor activity or coordination.	CD2F1 (BALB/c x DBA/2)	Koponen et al. 2004
Overexpressing truncated TrkB receptor (TrkB.T1, dominant negative), used as heterozygous	FST	No behavioral changes, effects of ADs blocked in heterozygous mice.	CD2F1 (BALB/c x DBA/2)	Saarelainen et al. 2003
TrkBF616A, time controlled inhibition of TrkB receptor			C57Bl/6	

Abbreviations: conditioned taste aversion (CTA), emergency test (EM), elevated plus-maze (EPM), elevated zero maze (EZM), fear conditioning (FC), forced swimming test (FST), hotplate (HP), light-dark box (LD), novel object recognition (NOR), open field (OF), rotarod (RR), water maze (WM)

3 Aims of the study

The purpose of these studies was to achieve a better understanding of the phenomena related to depression and AD action and to identify new methods for depression/antidepressant research. In more detail, the objective was to study the behavioral effects of genetic and pharmacological modification of BDNF-TrkB-signaling in mice. The specific aims of this thesis were as follows:

I. To characterize the anxiety- and depression-like behavioral phenotype of TrkB-signaling deficient mice (TrkB.T1) and to compare the phenotype to that of heterozygous BDNF KO mice to better elucidate the importance of TrkB signaling in depression-related behavior.

II. To examine the effects of glutamatergic drugs, ketamine and the AMPA potentiator LY 451646 in animal models of behavioral despair and the role of BDNF signaling in their mechanism of action.

III. To investigate the influence of postnatal fluoxetine exposure and the potential rescuing effect of chronic fluoxetine treatment on the behavior of adult mice to examine the time-dependent dualistic characteristics of ADs.

IV. To study the role of fluoxetine-induced plasticity and BDNF in fear extinction in adult mice.

4 Experimental procedures

4.1 Animals

Mice were group-housed and maintained under a 12-h light–dark cycle (lights on from 6:00 am to 6:00 pm or 7:00 am to 7:00 pm), with food and water available *ad libitum*. Each group contained 6-20 mice for behavioral testing and 4-11 mice for biochemical analysis. Mice used for fear conditioning were housed individually for 7 days prior to fear conditioning.

All animal experiments were conducted in accordance with the Council of Europe (Directive 86/609), the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and Finnish guidelines. Efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The State Provincial Office of Southern or Eastern Finland approved the experimental protocols.

4.1.1 Animal genotypes

Adult C57Bl/6J WT mice were obtained from the Laboratory Animal Center of the University of Helsinki or purchased from Harlan (Venrey, The Netherlands).

Male B6.129S4-Bdnf^{tm1Jae}/J heterozygote mice (backcrossed to the C57Bl/6J background for more than 10 generations) (Ernfors et al., 1994) were obtained from Jackson Laboratory (Bar Harbor, ME, USA; Stock Number: 002266) and bred with C57Bl/6J female mice (Jackson Laboratory). BDNF mRNA and protein expression is reduced by half in these animals during their development and adulthood. To eliminate BDNF gene expression, a part of exon 5 of the BDNF gene was deleted and replaced by a neomycin cassette for ablation. Because homozygous BDNF KO mice die during their first week of life, heterozygous mice and their WT littermates were used for testing.

Generation of TrkB.T1 mice has been described previously by Saarelainen et al. (2000). These animals overexpress a truncated, dominant negative form of the TrkB receptor (TrkB.T1), which leads to decreased TrkB signaling activity. Because the transgene (N-terminally flag-tagged TrkB.T1 cDNA) was constructed under the Thy1 promoter, the TrkB.T1 receptor is expressed in postnatal neurons starting from postnatal week 1. Mice were originally bred and maintained in the CD2 (BALB/c x DBA/2) background but were backcrossed to the C57BL/6J background for more than 10 generations. WT C57BL/6J females for breeding were purchased from Harlan (Venrey, The Netherlands). Heterozygous mice and their WT littermates were used for testing.

The generation of the TrkB^{F616A} mice has been described earlier by Chen et al. (2005). These mice carry a single amino acid mutation near the ATP binding site of the receptor that makes the receptor susceptible to inhibition by a mutation-specific kinase inhibitor (1NMPP1). This mutation

and the use of 1NMPP1 allows for time-controlled inhibition of the TrkB receptor in this mouse. Mice were originally bred and maintained in the C57BL/6J background and maintained as an inbred strain, and homozygous females were bred with homozygous males. WT mice of the same origin were bred as a separate line and used as control animals for testing.

4.2 Drug treatments

4.2.1 Imipramine, ketamine and LY 451646 treatments

Animals received a single intraperitoneal injection of a vehicle (0.5% hydroxypropylmethylcellulose and 1% Tween80 in water), ketamine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany; 50 mg/kg), LY 451646 (N-[(2R)-2-(4'-Cyano[1,1'-biphenyl]-4-yl)propyl]-2-propanesulfonamide; Eli Lilly Greenfield Labs, Greenfield, IN, USA; 5 mg/kg) or imipramine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany; 30 mg/kg) in a volume of 10 ml/kg and were subjected to behavioral analysis either 35-45 min or 7 days after the injection. Doses were selected based on preliminary studies and the literature (Popik et al 2008; Bai et al 2001).

4.2.2 Fluoxetine treatments

Mice received fluoxetine (kindly provided by Dr. Jukka Sallinen, Orion Pharma, Helsinki, Finland) *via* drinking water in light-protected tubes. Solutions were prepared fresh daily. Fluoxetine was dissolved in tap water at a concentration of 0.08 mg/ml (approximately 10 mg/kg/day). The treatment continued through all behavioral sessions until sacrifice.

For postnatal fluoxetine administration (PNF), litters were randomly assigned to two groups: saline-injected (SAL, n=18 male pups) and fluoxetine-injected (FLX, n=24 male pups). Pups from each group were weighed and injected intraperitoneally daily (10:00 am–11:00 am) with either saline [1 × phosphate-buffered saline (PBS), 5 ml/kg] or fluoxetine (dissolved in 1 × PBS, 10 mg/kg, 5 ml/kg) starting at postnatal day 4 (P4) until P21 (Ansorge et al., 2004).

4.2.3 1NMPP1 kinase inhibitor

The $TrkB^{F616A}$ mice and their control animals were treated with the kinase inhibitor 1NMPP1 [1-(1,1-Dimethylethyl)-3-(1-naphthalenylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine; kindly provided by Professor Jari Yli-Kauhaluoma and Kirsi Harju, Division of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland] in their drinking water one week prior and during the behavioral testing (Bishop et al., 1999; Sallert et al., 2009). The concentration of 1NMPP1 in the drinking water was 5.0 µM.

4.3 Behavioral testing

The behavioral experiments were conducted in the order presented below, and the interval between subsequent tests was 2–3 days during the light phase. The experimenter was blind to the animal genotype and/or treatment. At the beginning of behavioral testing, the animals were 2–3 months (young adult) or 12 months (middle-aged) old.

4.3.1 Exploratory activity/Open field (I, II, III)

The spontaneous exploratory activity of mice was automatically monitored in a transparent, well-illuminated (~ 300 lx) cage equipped with two stacked frames of infrared photo detectors enabling separate monitoring of horizontal (XY-move time) and vertical activity (rearing). The young adult mice were tested using an activity monitor produced by MED Associates (cage dimensions, 28.5 cm × 28.5 cm x 20 cm; St. Albans, VT, USA), while middle-aged mice were tested with the TruScan® activity monitor (cage dimensions, 26 cm x 26 cm x 39 cm; Coulbourn Instruments, CO, USA). The test sessions for young adult mice were 30 min, while the sessions for middle-aged mice were 10 min. To avoid odor traces, the test cage was cleaned with 70% ethanol before each mouse.

For analysis of results, the cages were divided into two compartments: a compartment near the walls (7 cm from the walls) and a central area compartment. Interruptions of infrared photo beams were used to calculate the following parameters: the distance traveled (cm), time spent in compartments, immobility time and rearing.

4.3.2 Light/dark-box test (III)

The LD test was performed for 10 min in an acrylic cage (28.5 × 28.5 × 20 cm; MED Associates, St. Albans, VT, USA) divided into two equal size compartments: one with transparent walls that was open topped and brightly illuminated (~ 450 lx from a 40-W light bulb fixed 55 cm above the floor) and another compartment that was constructed from black plastic (passing infrared light) and covered by a lid. The two compartments were separated by a partition containing an opening (7 × 5 cm) in its center at floor level. The mouse was placed in the center of the light compartment facing away from the opening. The latency to enter the dark area, the time spent in the compartments, the total distance traveled, the immobility time and the number of entries into the dark compartment were measured over 10 min. Rearing time was also calculated. The testing apparatus was thoroughly cleaned after each animal using 70% ethanol.

4.3.3 Elevated Plus-maze test (I, III)

This test was performed in an elevated maze (40 cm above the floor) consisting of two open arms (30 cm x 5 cm), two enclosed arms (30 cm x 5 cm with 15-cm-high transparent or black-painted side and end walls) and a connecting central platform (5 cm x 5 cm). The mouse was placed on the central platform facing one of the enclosed arms, and the time spent in the open and closed arms and the number of total arm entries was observed for 5 minutes. A video camera positioned above the maze recorded the experiments (Ethovision XT 7, Noldus Information Technology, Wageningen, Netherlands). The total number of arm visits was taken as a measure of general activity, while the % time spent in the open arms was used as a measure of anxiety. Only mice with at least 5 arm entries were considered when calculating the latter parameter. Testing occurred in a dimly lit room. To avoid odor traces, the test cage was cleaned with 70% ethanol before each mouse.

4.3.4 Forced swimming test (I, II, III)

Mice were placed individually in glass cylinders (19 cm in diameter, 24 cm high) filled with water at 21 ± 1 °C to a height of 14 cm. If used, the test compounds and vehicle were administered 45 minutes prior to testing. The time spent immobile (passive floating, during which the animal was motionless or moving the tail or one hind limb only slightly) was measured during the 6 min test. The latency to the first bout of immobility was also recorded. The results were normalized to the respective controls and expressed as a percentage of the control.

4.3.5 Novel object recognition (I)

A pair of identical objects was left overnight in the home cage and removed the next morning. After 4 hours, a new pair of objects, one identical to the previous pair and a novel object, was placed side by side at the back of the cage (the side of the novel object was randomized between days). The behavior of the animal was monitored for 5 minutes, and the approaches to the objects were counted. Four pairs of objects were used [three of them were Duplo^{R} toy building blocks (Lego, Denmark)] that were similar in size to the mouse; the last pair of objects was a drinking glass and a metal tea jar (height ~ 9 cm). The total number of approaches to the objects and novelty preference was counted over the four days. The formula for novelty preference was as follows:

[(visits to novel – visits to familial)/total visits] * 100%

4.3.6 Marble Burying (I)

The test was conducted in the home cage (27 cm x 45 x cm 14.5 cm). In the afternoon, 1 l of extra bedding was added to the cage bottom, and 9 glass marbles (1 cm in diameter) were left on the top of the new bedding in a 3 x 3 array. The next morning, the number of uncovered marbles was counted.

4.3.7 Fear conditioning, extinction, renewal and reinstatement (IV)

Freezing behavior was measured with an automatic infrared beam detection system, which was placed on the sides of the chamber of the fear conditioning apparatus (TSE Systems GmbH, Bad Homburg, Germany). The mouse was considered to be frozen only if it was not moving for at least 3 s, and the measurement was expressed as the percentage of the time spent freezing. Fear conditioning and extinction occurred in two different contexts unless otherwise stated. Fear conditioning context (A) was a transparent Plexiglas chamber with metal grids on the floor, whereas extinction context (B) was a black nontransparent Plexiglas chamber with a planar floor. Both context A and context B were cleaned before each session with 70% ethanol or 70% 2-propanol, respectively.

Fear conditioning. During the fear conditioning, the mice were conditioned with 5 pairings of the CS (total CS duration 30 s, 1 Hz, white noise, 80 dB) with the US (1 s foot shock of 0.6 mA, inter-trial interval: 20-120 s). The US co-terminated with the CS. The freezing level during the first CS, preceding the first US, was taken as the baseline freezing during CS.

Fear extinction. After fear conditioning (Days 2 and 3), mice were submitted to extinction training in context B. During this training, the mice received 12 presentations of the CS on each day (inter-trial interval: 20-60 s). Spontaneous recovery and context-dependent fear renewal were tested 7 days later in context B and context A, respectively, using 4 presentations of the CS (inter-trial interval: 20-60 s).

Fear renewal. After fear conditioning, the mice were assigned to 2 groups with equal levels of freezing: one received fluoxetine in the drinking water until the end of the experiment, while the other received tap water. On days 14 and 15, the conditioned mice were submitted to extinction training in context B, during which they received 12 daily presentations of the CS (inter-trial interval: 20-60 s). Spontaneous recovery and context-dependent fear renewal were tested 7 days later in context B and context A, respectively, using 4 presentations of the CS (inter-trial interval: 20-60 s).

Fear reinstatement. All experimental procedures were conducted in context A. After fear conditioning, mice were assigned to 2 groups with equal levels of freezing: one received fluoxetine in the drinking water until the end of the experiment, while the other received tap water. On days 14 and 15, the conditioned mice were submitted to extinction training, during which they received 12

daily presentations of the CS (inter-trial interval: 20-60 s). 7 days later, the mice received 5 unsignaled US; 24 hours later, fear reinstatement was tested using 4 presentations of the CS (inter-trial interval: 20-60 s). To control for the context specificity of the fear reinstatement test, mice were additionally tested in the new context B 2 hours later using 4 presentations of the CS (inter-trial interval: 20-60 s), during which the mice did not exhibit elevated freezing behavior.

4.4 Biochemical analysis (II)

4.4.1 Preparation of biological samples

Animals were stunned with CO_2 and killed by rapid decapitation, and hippocampi were rapidly dissected on a cooled dish. Samples were stored at - 80 °C until use. Frozen tissue was homogenized in cooled NP^{++} lysis buffer [137 mM NaCl; 20 mM Tris, pH 8.0; 1% NP-40; 10% glycerol, 50 mM NaF, 2× Complete Mini (Roche Diagnostics, Hertforshire, UK) and 2 mM Na_3VO_4], proteins were extracted, and Trk receptor phosphorylation was measured as previously described (Aloyz et al., 1999; Rantamäki et al., 2007; Saarelainen et al., 2003).

4.4.2 Western blotting

All glycosylated proteins, including Trk receptors, were precipitated using lectin beads (wheat germ agglutinin; EY Laboratories, San Mateo, CA, USA). Next, the beads were washed extensively with NP++ buffer, and specifically bound proteins eluted upon heating in Laemmli sample buffer. Samples were separated by SDS-PAGE and blotted on a PVDF membrane for pTrk immunoblot detection (anti-pY705/6; 1:1000; Cell Signaling Technology, Danvers, MA, USA). The efficiency of lectin precipitation was confirmed by Ponceau staining. Trk receptor phosphorylation levels were normalized to total Trk-receptor levels with the SC-11 (1:2000, Santa Cruz Biotechnology, Dallas, Texas, USA) antibody as previously described (Aloyz et al., 1999; Rantamäki et al., 2007; Saarelainen et al., 2003).

4.4.3 ELISA

BDNF protein levels were analyzed using a BDNF ELISA (enzyme-linked immunosorbent assay) according to Karpova *et al.* (2010). The results are shown as the percentage of the respective controls.

4.5 Statistical analysis

Data obtained from behavioral tests were analyzed with STATVIEW software (SAS, Cary, NC, USA) or with SPSS for Windows 14.0 software. For comparisons between 2 groups, an unpaired, two-

tailed Student's t-test was performed for parametric data, and the Mann-Whitney U-test was used for non-parametric data. Two-way ANOVA was used to reveal the main effect and interaction between the factors, followed by Fisher's PLSD test. For the fear conditioning test, statistical analyses were performed using repeated-measures ANOVA followed by Student's paired or unpaired two-tailed *t*-test. The criterion for significance was $P<0.05$. All values reported in the text, table and figures represent the mean ± SEM.

5 Results

5.1 Behavioral phenotyping of TrkB-signaling deficient mice (*I*)

To examine the influence of decreased TrkB signaling on depression- and anxiety-like behavior, we tested mice overexpressing a dominant negative form of the TrkB receptor (TrkB.T1). Both male and female mice were tested in two cohorts of mice: young adult and middle aged. In addition, to compare BDNF-ligand-dependent and –independent transgenic models, heterozygous BDNF KO mice were tested to identify differences in comparison with TrkB.T1 mice.

5.1.1 TrkB.T1 mice show lack of motivation and indifference to surrounding environment

The spontaneous activity and exploration behavior of TrkB-signaling-deficient mice (TrkB.T1) were examined in a novel empty cage. Young adult TrkB.T1 mice displayed a non-significant toward a decrease in total distance traveled (two-way ANOVA, P=0.091) (*I*, Figure 1.A). However, this trend became significant in aged mice (two-way ANOVA, P=0.004) and was particularly apparent in female mice (*I*, Figure 1.B). In rearing behavior, young adult male TrkB.T1 mice exhibited a decreased number of rearings (P=0.014) compared to female mice, who were indistinguishable from their littermate controls (*I*, Figure 1.C).

The anxiety–related behavior of TrkB.T1 mice was tested in the EPM test. Both young adult and middle-aged TrkB.T1 mice were indistinguishable from their littermate controls when total arm entries were measured, indicating that the TrkB.T1 mice explored the maze similarly to WT mice (*I*, Figure 2.C-D). Both young adult (P=0.032) and middle-aged male (two-way ANOVA, genotype: P=0.017) TrkB.T1 mice displayed a significant increase in the time spent in open arms, more likely indicating anxiolytic-like than anxiogenic-like behavior (*I*, Figure 2.A-B).

To measure object xenophobia, middle-aged TrkB.T1 mice were subjected to the MB and NOR tests. In the MB test, TrkB.T1 mice buried an equal amount of marbles compared to the controls (*I*, Figure 3.A). However, in the NOR test, TrkB.T1 mice showed less interest (approaches) to the novel objects (two-way ANOVA, P<0.001). Because both male and female mice made fewer approaches to the familiar objects, their preference for the novel objects was indistinguishable from their controls (P=0.25) (*I*, Figure 3.B-C).

The depression-like behavior of young adult TrkB.T1 mice was characterized in the FST. Two-way ANOVA revealed significant differences between genotypes (P=0.004), sexes (P=0.027) and a genotype x sex interaction (P=0.009) in total immobility time (*I*, Figure 4.A). Similarly, there were significant differences in the latency to the first immobility between genotype x sex interaction (P=0.012) but not between genotype or sex alone (*I*, Figure 4.B). Thus, male TrkB.T1 mice exhibited

increased immobility time (P<0.001) and decreased latency to immobility (P=0.004) compared to WT littermate controls, while female mice displayed no changes in their behavior.

5.1.2 Behavioral phenotype of BDNF$^{(+/-)}$ animals

The spontaneous activity and exploration behavior of the heterozygous BDNF KO mice (*BDNF*$^{(+/-)}$) were examined in a novel empty cage. Both young adult and middle-aged *BDNF*$^{(+/-)}$ mice were indistinguishable from their littermate controls in exploratory behavior (*I*, Figure 5.A-D). However, middle-aged female mice traveled longer during test time compared to the middle-aged male mice (P=0.028).

The anxiety–related behavior of the middle-aged *BDNF*$^{(+/-)}$ mice was tested in the EPM test. When compared to their control groups, both male and female *BDNF*$^{(+/-)}$ mice displayed a similar level of arm entries (*I*, Figure 6.B). However, both sexes spent more time in the open arms of the EPM (two-way ANOVA, P=0.001) (*I*, Figure 6.A).

To measure object xenophobia, middle-aged *BDNF*$^{(+/-)}$ mice were subjected to the MB and NOR tests. In the MB test, *BDNF*$^{(+/-)}$ animals displayed the genotype X sex interaction (P=0.003); male TrkB.T1 mice buried more marbles than their controls, while female mice and their controls buried equal numbers of marbles (*I*, Figure 7.A). In the NOR test, no genotype effect was evident, but a significant difference between sexes was observed, as female mice made a greater number of approaches to the novel objects compared to male mice (P<0.001) (*I*, Figure 7.B-C). However, a significant difference between genotypes in novelty preference was observed in both sexes (P=0.034).

The depression-like behavior of young adult male *BDNF*$^{(+/-)}$ mice was measured in the FST. No significant differences were observed in the immobility time or the latency to immobility of these mice (*I*, Figure 8.A-B).

5.2 Behavioral phenotyping of the TrkB^{F616A} mice

The TrkB^{F616A} mice were tested in the behavioral test battery. 4 groups were formed with two genotypes (WT or TrkB^{F616A}) and two treatments (water or 1NMPP1) (Table 7). Contrary to our expectations, in the absence of an inhibitor, the TrkB^{F616A} mice exhibited significant differences in the LD and EPM tests and trends in the FST (Table 7). The mice also exhibited differences in baseline freezing and altered contextual memory in the FC test (data not shown). Furthermore, the 1NMPP1 inhibitor appeared to influence the behavior of the WT mice but did not alter the behavior of the TrkB^{F616A} mice.

Table 7. The behavior of the TrkB^{F616A} mice. All data are presented as group the mean±SEM. Data were analyzed by two-way ANOVA followed by Fischer's post hoc –test. * P<0.05, ** P<0.01, *** P<0.001 vs. WT/Control group; # P<0.05, ## P<0.01 vs. TrkB^{F616A}/Control group.

	WT		TrkB^{F616A}		
	Control	1NMPP1	Control	1NMPP1	
Number of animals:	6	8	8	6	P-value
L/D:					
Zone entries to light (no)	32.2±4.9	24.6±1.6**	16.8±2.9***	22.6±0.9**	GT 0.010; GT vs. TREAT 0.039
Time in light (s)	197.2±41.5	128.9±15.2$^{\#\#}$	323.3±65.1	174.7±39.2$^{\#}$	TREAT 0.037
Total resting time (s)	383.9±7.6	400.6±12.0	433.1±22.8	404.1±10.7	n.s.
OF:					
Distance traveled (cm)	2082±107	2059±272	1741±264	2114±279	n.s.
EPM:					
Time in open arms (%)	14.6±6.1	1.5±0.9*	1,6±0.9*	8.7±5.8	GT vs. TREAT 0.018
Total arm entries (no)	15.2±1.4	9.6±0.7*	11.4±1.0*	10.0±1.6*	TREAT 0.008
FST:					
Immobility time (s)	155.8±14.0	83.5±28.2	101.62±32.0	46.4.±18.1*	TREAT 0.025
Latency to immobility (s)	72.5±9.7	160.0±38.5	171.9±32.8	171.9±31.4	n.s.

5.3 The behavioral effects of glutamatergic drugs in WT and *BDNF$^{(+/-)}$* mice (*II*)

To investigate the acute and long-lasting antidepressant-like effects of glutamatergic drugs, mice were treated with the NMDA receptor antagonist ketamine and the AMPA receptor potentiator LY 451646 45 min or 7 days prior to testing. BDNF$^{(+/-)}$ mice were used to examine the importance of BDNF signaling in the mechanism of action of these drugs.

5.3.1 Ketamine induced an antidepressant-like effect in WT and BDNF$^{(+/-)}$ mice without changes in phosphorylated-Trk or BDNF protein levels

Acute (45 min) ketamine administration (50 mg/kg) induced antidepressant-like effects in WT mice in the FST. Ketamine decreased total immobility time (P=0.011) and increased latency to the first appearance of immobility (P=0.016) (Table 8). This antidepressant-like effect disappeared at 7 days after ketamine administration. However, ketamine did not influence the total distance traveled or time spent in the open area in the OF test.

Table 8. Behavioral effects of ketamine (50 mg/kg) in WT mice in the FST and OF. FST data are presented as the percentage of the control as the group mean±SEM and OF data as the group mean±SEM. Data were analyzed from the original values by an unpaired t-test. * P<0.05.

		Control	N	Ketamine	N	P-value
FST: After 45 min	Latency (%)	100±10.3	8	208.5±38.3*	8	0.016
	Immobility (%)	100±12.3	8	32±19.1*	8	0.011
FST: After 7 days	Latency (%)	100±24.0	9	94±20.8	10	n.s.
	Immobility (%)	100±16.5	9	93±16.4	10	n.s.
OF: After 35 min	Time spent in center (s)	272.8±44.4	8	280.4±83.0	6	n.s.
	Distance traveled (cm)	3672±395	8	4492±751	7	n.s.

Furthermore, ketamine did not induce any changes in phosphorylated-Trk or BDNF protein levels, either acutely or 7 days after the ketamine injections (Table 9).

Table 9. Influence of ketamine (50 mg/kg) on BDNF protein and pTrk levels in the HC of WT mice. All data are presented as the percentage of the control as the group mean±SEM. Data were analyzed by an unpaired t-test. * P<0.05.

		Control	N	Ketamine	N	P-value
Acute: after 60 min	pTrk/Trk	100±10.1	9	132.7±18.3	10	n.s.
	BDNF prot.	100±4.1	4	98.4±1.6	5	n.s.
After 7 days	pTrk/Trk	100±10.7	11	143.1±23.6	11	n.s.
	BDNF prot.	100±3.2	9	96.1±4.9	10	n.s.

In *BDNF*$^{(+/-)}$ mice, acute ketamine administration caused antidepressant-like effects in the FST by decreasing the total immobility time (two-way ANOVA, genotype x treatment P=0.0069; WT/control vs. WT/ketamine P=0.0169; WT/control vs. *BDNF*$^{(+/-)}$/ketamine P=0.0022; *BDNF*$^{(+/-)}$/ control vs. *BDNF*$^{(+/-)}$/ketamine P=0.0022) (Figure 3). Meanwhile, the effect of the tricyclic AD imipramine was lost in these mice.

Furthermore, ketamine did not induce any changes in BDNF protein levels acutely or at 7 days after injections (Figure 4). However, as described in the literature (Rantamäki et al., 2013), total BDNF protein levels were decreased by approximately 40% in BDNF$^{(+/-)}$ mice when compared to their WT littermates (P<0.0001).

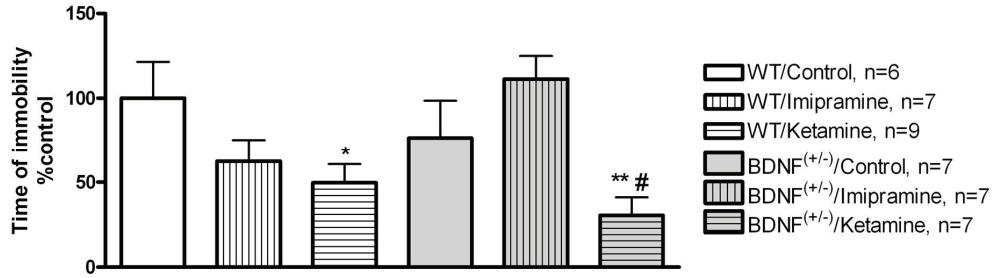

Figure 3. Acute (after 45 min) behavioral effects of ketamine (50 mg/kg) and imipramine (30 mg/kg) in *BDNF*$^{(+/-)}$mice in the FST. All data are presented as a percentage of the control as the group mean±SEM. Data were analyzed from original values by two-way ANOVA followed by Fischer's *post hoc*–test. * P<0.05, ** P<0.01 vs. WT/Control group; # P<0.05 vs. BDNF$^{(+/-)}$/Control.

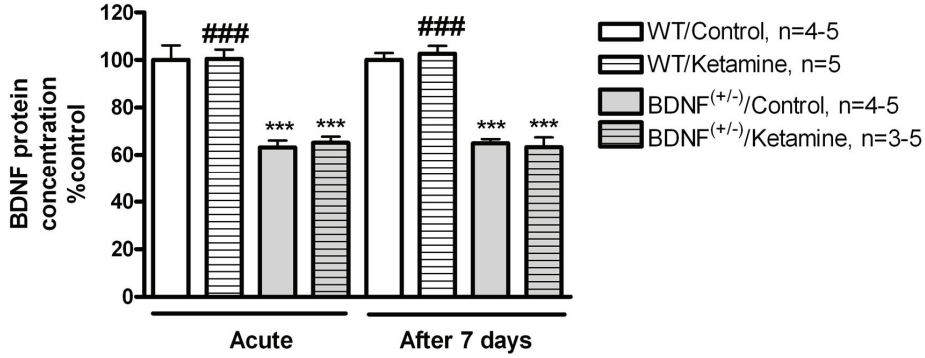

Figure 4. BDNF protein levels acutely (after 60 min) and 7 days after a single ketamine injection (50 mg/kg) in WT and *BDNF*$^{(+/-)}$ mice. All data are presented as the percentage of the control as the group mean±SEM. Data were analyzed by two-way ANOVA followed by Fischer's *post hoc*–test. *** P<0.001 vs. WT/Control group; ### P<0.001 vs. BDNF$^{(+/-)}$/Control.

5.3.2 An AMPA potentiator induced antidepressant-like effects in WT and BDNF$^{(+/-)}$ mice without changes in phosphorylated-Trk or BDNF protein levels

Acute (45 min) LY 451646 administration (5 mg/kg) induced antidepressant-like effects in WT mice in the FST. LY 451646 decreased total immobility time (P=0.0001) and increased latency to the first appearance of immobility (P=0.043) (Table 10). However, LY 451646 did not influence the total distance traveled or the time spent in the center area in the OF test. Furthermore, no changes in phosphorylated-Trk or BDNF protein levels were observed acutely or at 7 days after LY 451646 injection (Table 11).

Table 10. Behavioral effects of LY 451646 (5 mg/kg) as observed in the FST and OF with WT mice. FST data are presented as the percentage of the control as the group mean±SEM and OF data as the group mean±SEM. Data were analyzed from original values with an unpaired t-test. * P<0.05, *** P<0.001.

		Control	N	LY 451646	N	P-value
FST: after 45 min	Latency (%)	100±30.7	8	211.8±39.9*	8	0.043
	Immobility (%)	100±15.9	8	8.1±5.9***	8	0.0001
OF: after 35 min	Time spent in center (s)	272.8±44.4	8	277.4±58.3	8	n.s.
	Distance traveled (cm)	3672±395	8	3496±360	8	n.s.

Table 11. Influence of LY 451646 (5 mg/kg) on BDNF protein and pTrk levels in the HC of WT mice. All data are presented as the percentage of the control as the group mean±SEM. Data were analyzed with an unpaired t-test.

		Control	N	LY 451646	N	P-value
Acute: after 60 min	pTrk/Trk	100±7.2	4	95.4±1.1	4	n.s.
	BDNF prot.	100±10.0	4	87.8±2.1	4	n.s.
After 7 days	pTrk/Trk	100±7.2	4	103.2±6.9	4	n.s.
	BDNF prot.	100±10.0	4	88.7±3.1	4	n.s.

In BDNF$^{(+/-)}$ mice, acute LY 451646 administration caused an antidepressant-like effect in the FST by decreasing the total immobility time (two-way ANOVA: treatment, P=0.0001; WT/control vs. WT/LY 451646, P<0.001; WT/control vs. *BDNF*$^{(+/-)}$/LY 451646, P<0.001; *BDNF*$^{(+/-)}$/control vs. *BDNF*$^{(+/-)}$ /LY 451646, P=0.003) (Figure 5).

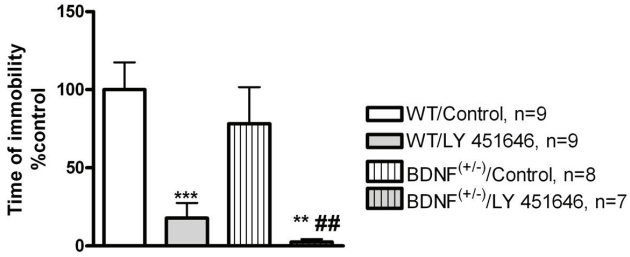

Figure 5. Acute (after 45 min) behavioral effects of LY 451646 (5 mg/kg) in *BDNF*$^{(+/-)}$mice in the FST. All data are presented as the percentage of the control as the group mean±SEM. Data were analyzed from original values by two-way ANOVA followed by Fischer's post hoc test. ** P<0.01, *** P<0.001 vs. WT/Control group; ## P<0.01 vs. BDNF$^{(+/-)}$/Control.

5.4 Potential behavioral alterations induced by early postnatal fluoxetine treatment (*III*)

To examine the long-term behavioral effects of early exposure of mice to ADs, male C57Bl/6J mice were treated during postnatal days P4-P21 with daily injections of saline or fluoxetine (10 mg/kg). Furthermore, to measure the potential rescuing effect of adult AD treatment, starting from day P90, these two groups were randomly divided further into two groups: mice drinking regular water and mice receiving fluoxetine in the drinking water (0.08 mg/ml). Treatments started 21 days prior to behavioral testing and lasted until sacrifice. The experimental groups are shown in Figure 6. The depression- and anxiety-like phenotypes of these mice were tested using a behavioral test battery.

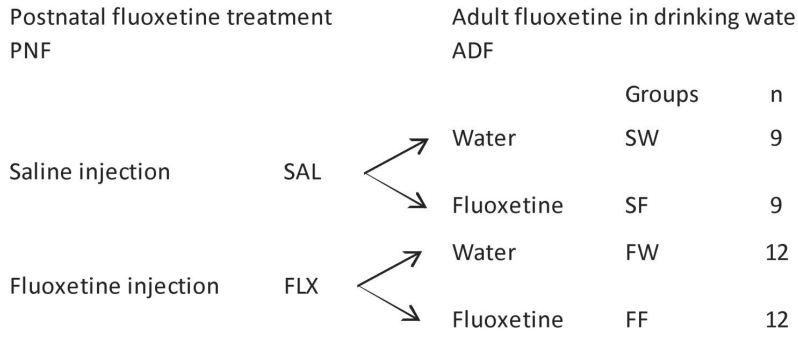

Figure 6. Experimental groups in the early fluoxetine exposure study.

5.4.1 Postnatal fluoxetine treatment decreases body weight and explorative activity

Postnatal fluoxetine treatment significantly decreased the body weight of mice ($t=3.714$, $DF=40$, $P<0.001$), and this decrease was observed throughout the entire lifespan of the mice (P90, $P=0.006$) (Figure 7). Later in life, fluoxetine treatment rescued this weight loss to the control level. However, adult fluoxetine treatment did not have any effect on the weight of the mice who received saline injections in early life (two-way ANOVA: ADF, $P<0.0001$; PNF x ADF interaction, $P<0.05$).

In the OF test, PNF treatment slightly decreased the distance mice traveled during 30 minutes (two-way ANOVA: $P<0.05$) (III, Table 1). However, no differences in the ADF or PNF x ADF interaction were identified. According to Fisher's (PLSD) post hoc test, no statistically significant difference was identified. Moreover, PNF treatment significantly decreased (two-way ANOVA, $P<0.0001$) and ADF increased (two-way ANOVA, $P<0.001$) the total rearing time. Post hoc analysis revealed that the total rearing time was decreased in the FW group compared to the controls.

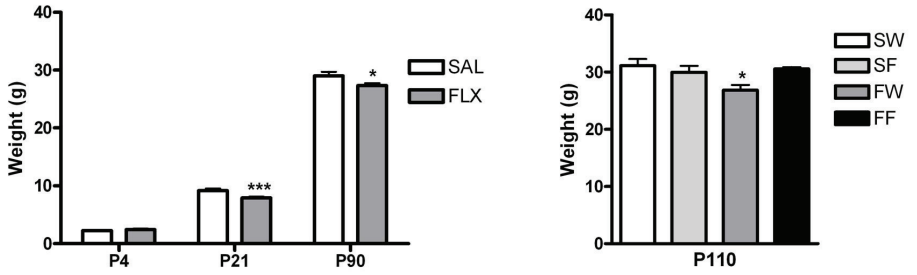

Figure 7. Effect of postnatal (10 mg/kg, daily P4-P21) and adult (0.08 mg/ml *via* drinking water, starting from P90) fluoxetine treatments on the weight of mice. All data are presented as the group mean±SEM. Data were analyzed by unpaired t-test or two-way ANOVA followed by Fischer's *post hoc* test. * P<0.05, *** P<0.001.

5.4.2 Postnatal fluoxetine treatment-induced anxiety-like behavior was rescued by adult fluoxetine treatment

The effects of PNF and ADF treatments on anxiety-like behavior were measured using the OF and LD tests. In the OF test, PNF-treated mice displayed increased total immobility time (two-way ANOVA, P<0.01), with a trend toward moving later to walls (two-way ANOVA, P=0.09) (*III*, Table 1). In the *post hoc* analysis (Fisher's PLSD), the FW group showed a statistically significant increase in the total and central area immobility times (P<0.05); this phenotype was rescued by ADF treatment. However, the time in the central area, the distance traveled in the central area, the center-wall transition and the rearing time in the central area were not affected by the treatments.

In the LD test, PNF treatment decreased the total distance traveled (two-way ANOVA, P<0.001) and total rearing time (two-way ANOVA, P<0.0001), while ADF increased the same parameters (two-way ANOVA, P<0.05 and P<0.0001, respectively) (*III*, Table 1). In addition, PNF increased (two-way ANOVA, P<0.001) and ADF decreased (two-way ANOVA, P<0.05) total immobility time. Moreover, PNF-treated mice showed a tendency toward increased latency to enter the dark compartment (two-way ANOVA, P=0.012). However, the distance traveled in, the time spent in or the entries to the light compartment were not affected by the treatments.

5.4.3 Effects on depression-like behavior

The effects of the PNS and ADF treatments on depression-like behavior were measured in the FST. PNF-treated mice were less immobile compared to the ADF-treated mice (two-way ANOVA, P<0.001), while the latencies to immobility were longer and shorter for PNF and ADF, respectively (two-way ANOVA, P<0.05) (Figure 8). However, the *post hoc* analysis (Fischer's PLSD) revealed no

statistically significant differences between groups in latencies to immobility, and differences in immobility time were observed only between the SW and FW groups (P<0.05).

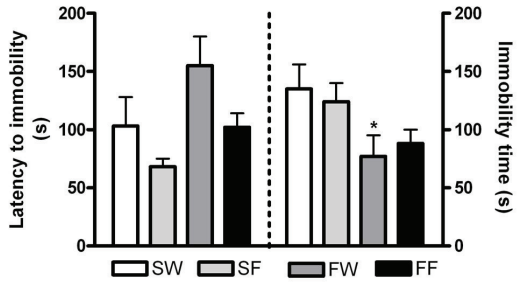

Figure 8. Effect of postnatal (10 mg/kg, daily P4-P21) and adult (0.08 mg/ml *via* drinking water, starting from P90) fluoxetine treatment on the depression-like behavior of mice. All data are presented as the group mean±SEM. Data were analyzed with two-way ANOVA followed by Fischer's *post-hoc* test. * P<0.05.

5.5 Fluoxetine-induced re-opening of developmental-type plasticity in fear circuitry: the role of TrkB (*IV*)

In this study, we investigated whether fluoxetine can open developmental-type plasticity and enable, in conjunction with an environmental stimulus, recovery of permanent fear extinction memory similar to what has been observed in the rat visual cortex. Four different study setups were performed. All mice were conditioned to the CS (a 30 s white noise), followed by the US (1 s foot shock) in context A. After fear conditioning (on Days 2 and 3), mice were submitted to extinction training in context B.

Setup 1. Mice received water or fluoxetine (0.08 mg/ml *via* drinking water) for 3 weeks prior to and during the testing. Spontaneous recovery and context-dependent fear renewal were tested at 7 days after extinction in context B and context A, respectively, using 4 presentations of the CS. After extinction control, mice displayed spontaneous recovery (P<0.05) and fear renewal (P<0.05), while the fear extinction in the fluoxetine-treated mice was permanent (*IV*, Figure 1). However, both control and fluoxetine-treated mice without extinction displayed a similar fear response in the fear renewal test. Moreover, fluoxetine-treated mice with extinction showed significantly reduced freezing when compared with fluoxetine-treated mice without extinction (P<0.001), indicating the effectiveness of the extinction training.

Setup 2. After fear conditioning, mice were assigned to two groups with equal levels of freezing, one of which received fluoxetine in the drinking water until the end of the experiment, while the other received tap water. On days 14 and 15, the conditioned mice were submitted to

extinction training in context B, during which they received 12 presentations of the CS each day. Spontaneous recovery and context-dependent fear renewal were tested 7 days later in context B and context A, respectively, using 4 presentations of the CS. Similar to Setup 1, control mice exhibited spontaneous recovery and fear renewal ($P<0.05$), while the fear extinction of fluoxetine-treated mice was permanent (*IV*, Figure 2.C). However, both control and fluoxetine-treated mice without extinction exhibited a similar fear response in the fear renewal test. Moreover, fluoxetine-treated mice with extinction displayed significantly reduced freezing when compared with fluoxetine-treated mice without extinction ($P<0.05$).

Setup 3. After fear conditioning, mice were assigned to two groups with equal levels of freezing, one of which received fluoxetine in the drinking water until the end of the experiment, while the other received tap water. On days 14 and 15, the conditioned mice were submitted to extinction training, during which they received 12 presentations of the CS each day. Seven days later, the mice received 5 unsignaled US; 24 hours later, fear reinstatement was tested using 4 presentations of the CS. Although both groups displayed increased freezing (control, $P<0.01$; fluoxetine, $P<0.01$) compared to the results of the extinction training, the fluoxetine-treated group displayed significantly reduced freezing compared to the control mice ($P<0.05$) (*IV*, Figure 2.C).

Setup 4. Control and fluoxetine-treated $BNDF^{(+/-)}$ mice and their littermate WT control mice were exposed to similar conditions as in Setup 1. While fluoxetine-treated WT mice exhibited permanent fear extinction, $BNDF^{(+/-)}$ mice exhibited a similar level of freezing when compared to the control animals (*IV*, Figure 4.B).

6 Discussion

6.1 Challenges of measuring depression-like behavior in mice

The discovery of antidepressants almost 60 years ago revolutionized the treatment of MDD. ADs were shown to increase serotonin and norepinephrine levels in the brain, which led to the formation of the monoamine theory of depression (Schildkraut, 1965). However, clinical findings and biochemical studies suggest that the pathology of depression is more complex and that monoamine deficiency alone cannot explain depression (Hindmarch, 2002). Furthermore, the unsatisfying interpretive ability of the monoamine theory and the identification of a connection between the neurotrophin BDNF and depression, particularly antidepressant effects, resulted in the formation of the neurotrophin hypothesis of depression. Increased support of this theory has led to countless studies evaluating the connection between BDNF and depression and the importance of BDNF in the mechanism of action of ADs (Adachi et al., 2008; Castrén et al., 2007; Duman & Monteggia, 2006).

Broad variety and individuality in MDD patients and their symptoms makes modeling this complex disorder challenging. After the discovery of ADs a half century ago, several "depression models" were introduced. These models focused on a monoamine-based mechanism of action for ADs. Many potential new ADs may give false-negative results in these models. Behavioral despair models of depression (LH, FST and TST) give positive results for all clinically active AD treatments, including ECT, but do they really model depression? The major drawback of despair models is that, in practice, they do not have construct validity as a depression model because ADs work acutely in these tests, whereas in clinical care, long-term treatments are needed (Willner & Mitchell, 2006). Several other behavioral depression models have been established. The most auspicious tests are related to chronic stress; CMS and OB both enhance biochemical changes that are similar to those observed in depressed patients (Papp et al., 1996; Shepherd, 2006). In addition, these models also mimic, at least in part, symptoms of depression. Moreover, chronic treatment with ADs yields positive results in these models (Roche et al., 2007; Strekalova et al., 2006). However, how can depression be diagnosed in a mouse or rat models, and do these animals actually experience depression? Thus, more precise tests are needed to model depressive symptoms.

What constitutes a good mouse model of depression or depressive symptoms? A single test measuring the behavioral despair of a mouse is insufficient to model this complex disorder. However, combining several tests that measure different symptoms may be a better solution because evaluating different aspects of the symptomology of depression increases the face validity of a model. Furthermore, a good model of MDD should also have some predictive validity; several classes of ADs should give positive results in chronic use. Dzirasa and Covington (2012) have introduced criteria for mouse affective syndrome (MAS). This model includes several tests in three domains. The first

domain includes reward-related symptoms of MDD: anhedonia and decreased concentration. The first can be measured by an intracranial self-stimulation or sucrose preference test, and the second can be measured with a 5-hole nose poke test. The second domain (homeostatic factors) contains three symptomatic groups: psychomotor retardation or agitation, insomnia or hypersomnia and changes in appetite or weight, which can easily be measured by observing changes in dark-cycle locomotion, sleep patterns, food consumption and weight, respectively. As the third domain, Dzirasa and Covington (2012) listed changes in biochemical, molecular and neurophysiological markers, such as alterations in cortical gene expression or enhanced cortico-limbic network synchrony.

6.2 Effects of the genetic manipulation of TrkB on the depression-like behavior of mice

As the number of genetically modified mouse lines increases, adequate behavioral measurements of depression-like behavior become more important; MAS is a good basis for this. MAS mimics several symptoms of depression and requires alterations in biochemical and neurophysiological markers similar to those that occur in depressed patients (Dzirasa & Covington, 2012). However, the authors do not specify these markers or the etiological basis of depression and leave interpretation to the adapters of the model. As Willner and Mitchell (2006) described, a good depression model should also exhibit responsiveness to current AD treatments, preferably upon chronic administration. Thus, when evaluating the equivalence of a transgenic mouse model to depression, pharmacological responses should also be considered. Furthermore, although the authors omitted behavioral despair tests from their model, it may be beneficial to add them to the test battery because they are currently the only tests that attempt to measure the “mood” of mice. Other tests that could be included are those that measure the emotional exploratory behavior of mice, as the majority of depressed patients also suffer from anxiety disorders.

Regardless of the large number of genetically modified mouse lines, genetic studies have not clarified the connection between BDNF signaling and depression. BDNF ligand-dependent transgenic mice models have not facilitated the characterization of the depression-like phenotype. Moreover, full-TrkB receptor KO mice are not viable, so other approaches are needed. TrkB.T1 knock-in mice overexpress a natural form of the truncated TrkB receptor in neurons, leading to decreased BDNF-TrkB signaling. Similar to $BNDF^{(+/-)}$ mice, these mice have impairments in memory, and the antidepressant-like effects of ADs are blocked in TrkB.T1 knock-in mice (Saarelainen et al. 2000; 2003). These mice have been subsequently backcrossed to other genetic backgrounds, increasing interest in their use. In contrast to previous findings, we found that in the new background, these mice appear to exhibit depression-like behavior in the FST. The dissimilarity between these two phenotypes is primarily due to their different genetic backgrounds, which has been shown to

strongly influence the behavior of genetically modified mice (Crawley et al., 1997; Crawley, 2008; Holmes et al., 2003a; Metz et al., 2006). Furthermore, in our experiments, the TrkB.T1 mice displayed alterations in anxiety-like behavior, decreased interest in novel objects, psychomotor retardation during the dark (active) phase and no changes in activity during the light (testing) phase (data not shown). When stressed, these animals displayed social withdrawal behavior and decreased appetite, and in general, they gained less weight and had significantly decreased BDNF protein levels compared to the WT mice (Razzoli et al., 2011). However, stress did not appear to influence the metabolic hormone or cytokine levels or weight of these mice (Razzoli et al., 2011). When these findings are compared to the symptomatic modeling of depression in Table 3, the TrkB.T1 mouse appears to have at least mediocre success as a model of depression-like behavior; these mice exhibit social withdrawal when stressed, impairments in learning and memory, decreased locomotor/explorative activity and changes in appetite and body weight. However, the results are less encouraging when these mice are compared to the MAS criteria, mostly because the selected tests for MAS are not included in the test battery.

However, because our studies only encompass basic behavioral phenotyping, additional studies are needed before a final conclusion can be made about the suitability of TrkB.T1 mice as a depression model. Future studies should include at least test measurements of anhedonic behavior (sucrose preference) and changes in corticosterone levels when animals are stressed and/or treated with ADs. The responsiveness of these mice to ADs has been examined previously (Saarelainen et al., 2003). Because these studies were performed with mice of different genetic background and the results of the FST differed from previous studies, AD effects should be evaluated further.

Chen et al. (2005) produced promising new genetic mice with a point mutation in the TrkB receptor. This mutation enabled the activity of the TrkB receptor to be turned off at the selected time point with a specific kinase inhibitor. This mouse appears to be an ideal model to study the effects of the time-dependent inactivation of the TrkB receptor on mouse behavior. However, our findings suggest that the “silent” mutation in $TrkB^{F616A}$ mice is not that silent, at least from a behavioral point of view. Even a single mutation in the receptor changes its conformation enough to change the function of the receptor. Thus, this “ideal” mouse model was not as appropriate as anticipated.

6.3 Role of BDNF in the antidepressant-like effect of glutamatergic drugs

Heterozygous BDNF KO mice have, at best, a mild depression- and anxiety-like behavioral phenotype (Chen et al., 2006; Duman et al., 2007; Li et al., 2010b). As our results confirm, these mice are indistinguishable from control animals in the behavioral despair model and yield contradictory

findings in tests of anxiety-related behaviors. However, as we and others have reported, the effects of the classical ADs appear to be inhibited in the $BNDF^{(+/-)}$ mice, suggesting the importance of BDNF for the mechanism of action of the classical ADs (Ibaarguen-Vargas et al., 2009; Saarelainen et al., 2003). Based on these findings, we sought to determine if glutamate-based drugs, which have been shown to have fast and robust antidepressant effects in humans and animal models (Autry et al., 2011; Berman et al., 2000; Koike et al., 2011; Li et al., 2010a; Machado-Vieira et al., 2009; Maeng et al., 2008; Popik et al., 2008; Reus et al., 2011; Zarate et al., 2006), have a similar effect in the behavioral despair test as observed in $BNDF^{(+/-)}$ mice. Surprisingly, we observed that the dissociative anesthetic ketamine functioned similarly in both WT and $BNDF^{(+/-)}$ mice to induce an antidepressant-like effect in the FST.

AMPA receptor potentiators (e.g., LY 392098 and LY 451646) have been shown to have antidepressant-like effects in rodent models of depression-like behavior (Bai et al., 2001; Farley et al., 2010). We observed that exposure to LY 451646 yielded an antidepressant-like effect in both $BNDF^{(+/-)}$ mice and WT mice, similar to ketamine. Furthermore, neither ketamine nor LY 451646 appeared to have an effect on BDNF protein or Trk-phosphorylation levels in the HC of WT or $BDNF^{(+/-)}$ mice. These results indicate that the antidepressant-like effect of glutamate-based drugs may be independent from BDNF-TrkB signaling.

However, our findings are inconsistent with some studies suggesting a connection between BDNF signaling and the antidepressant-like effect of ketamine (Autry et al., 2011). What could explain the differences in the results of these studies? First, Autry et al. (2011) used a more specific deletion of BDNF at a selected time point, while our mice lacked half of the BDNF protein during their development and adulthood. Thus, only 50% reductions in BDNF protein levels may not be sufficient to block the AD effects of ketamine, whereas behavioral responsiveness to classical ADs is more sensitive to alterations in BDNF levels. The discrepancies in the results of these studies could also be due to differences in ketamine dosage [our dosage of ketamine was more than ten-fold greater than that used in Autry et al. (2011)] and different genetic backgrounds.

Furthermore, in our studies, we observed behavioral changes soon after a single ketamine administration but not after longer periods of time. This result is in contrast to previous reports of an antidepressant-like effect even two weeks after a single ketamine injection (Koike et al., 2011; Maeng et al., 2008; Popik et al., 2008). These differences may also be due to the selected dose and genetic background of the mice, as Autry et al. (2011) and Maeng et al. (2008) both observed long-lasting behavioral effects with smaller doses of ketamine (2.5-5 mg/kg). In retrospect, smaller doses of ketamine should also have been examined.

Another AMPA potentiator, LY 392098, has been shown to regulate BDNF expression in primary cultured neurons (Legutko et al., 2001). Furthermore, Mackowiak et al. (2002) observed that

both acute and chronic injection of LY 451646 increased BDNF mRNA expression in the rat HC. In contrast to these findings, we did not observe a change in BDNF protein levels in response to LY 451646. However, our study set-up differs significantly from that of Mackowiak et al. (2002); in our experiment, we used a fivefold higher dose of this potentiator, and we used mice instead of rats. Most importantly, in our study, animals were sacrificed 60 minutes after the drug injection, whereas Mackowiak et al. (2002) sacrificed their animals six hours after drug administration. Potential alterations in BDNF levels in whole tissue may only be observed after longer time periods.

However, in practice, the use of large doses of ketamine is not appropriate because of its notable side effects, increased risk of abuse and impracticality for extensive clinical use. Thus, an orally active and fast-acting AD would be highly beneficial. A recent study revealed that ketamine activates the mammalian target of rapamycin (mTOR) pathway (Li et al., 2010a), which is involved in protein synthesis and synaptic plasticity (Hoeffer & Klann, 2010). Direct inhibition of this pathway in the medial prefrontal cortex (with rapamycin) blocks ketamine-induced pathway activation and the antidepressant-like effect of ketamine. Furthermore, imipramine, fluoxetine and ECT did not activate this pathway. Because classical ADs enhance neuronal plasticity *via* BDNF-TrkB signaling, the effects of glutamate-based drugs may be channeled through the mTOR pathway. In conclusion, as Cryan and O'Leary suggested (2010), this pathway could be the target for new, fast-acting ADs.

6.4 Long-term behavioral effects of fluoxetine exposure in postnatal and adult mice

ADs have controversial effects on adult depression-like behavior in rodents when given during early life; postnatal treatment with clomipramine produces lifelong depression- and anxiety-like behavior in rodents. Similarly, SERT-KO mice have been shown to have a depression- and anxiety-like phenotype. These behavioral phenotypes have been suggested to be the result of serotonin-induced overactivation of presynaptic 5-HT1A-autoreceptors. The findings of Gross et al. (2002) support this hypothesis by demonstrating that the 5-HT1A receptor is required during postnatal exposure to drugs to induce adult anxiety-like behaviors. In this study, we observed that postnatal treatment with fluoxetine (PNF) induced a decrease in weight and activity-related parameters. Furthermore, no changes in anxiety-related behaviors were observed, and in contrast to the previous findings, PNF decreased immobility time in the FST. However, the dosage and time period of fluoxetine treatment may be responsible for the differences between the present and previous findings.

Encouraged by the finding that adult fluoxetine treatment can induce developmental-like plasticity and recover impairments induced by early fluoxetine exposure (Maya Vetencourt et al., 2008), the PNF-treated mice were treated with fluoxetine as adults. We observed that these ADF mice appeared to recover from some PNF-induced changes; the weight of the animals was

normalized, as were some but not all of the behavioral alterations. Early fluoxetine treatment might interfere with the formation and function of the neuronal network, leading to permanent behavioral changes. However, the rescuing effect of fluoxetine in adulthood may result from the reactivation of developmental-like plasticity and reorganization of the neuronal network. Furthermore, it is unclear if SSRIs have permanent effects in humans if exposed during neonatal or early postnatal life (Nulman et al., 1997; 2002; Wisner et al., 2009). In particular, long-term influences should be studied. However, the possible long-term influence of the SSRIs should be taken into account when the pharmacotherapy of children and pregnant women are evaluated. In conclusion, these findings suggest that even if there is a risk of permanent behavioral and neurobiological changes with early SSRI treatment, these changes may be reversible.

Stressful and fearful life events can produce long-lasting pathological fear responses in both mice and humans, and these reactions can be erased by exposure therapy (Bisson & Andrew, 2007). This fear erasure effect is usually not permanent, and fear reactions return after a period of time. However, when performed during the critical period of development, extinction training has been shown to be permanent (Gogolla et al., 2009; Kim & Richardson, 2010). Because fluoxetine opens critical period-like plasticity in the adult rat visual cortex, we investigated whether this compound could also open a similar type of plasticity in fear circuits. We determined that treatment with fluoxetine in conjunction with an environmental stimulus (extinction training) enabled permanent fear extinction when employed before and, more importantly and relevant from a clinical perspective, after a fearful experience. Moreover, extinction training or fluoxetine treatment alone did not induce permanent fear erasure. These results also indicate that BDNF plays a role in this fluoxetine-induced recovery because the effect of fluoxetine was blocked in $BDNF^{(+/-)}$ mice. In conclusion, our results suggest that, as in the visual cortex, fluoxetine can also open developmental-like plasticity in fear circuits, leading to permanent fear extinction. However, for long-lasting effects, both drug treatment and exposure therapy are needed. These findings emphasize the clinical necessity of both drug treatment and psychotherapy for the treatment of depression.

6.5 New ideas and future studies

In this thesis, we examined the importance of BDNF-TrkB signaling in the effect of antidepressant action and depression-like behavior. We observed that mice with blunted TrkB signaling (TrkB.T1, dominant negative) displayed indifference to the surrounding environment, indicating potential depression-like behavior. However, as mentioned previously, the behavioral test battery lacked some important tests, including anhedonia models. Responsiveness to stress and classical ADs remain to be examined in future studies. We also observed that the NMDA receptor

antagonist ketamine and the AMPA receptor potentiator LY 451646 displayed antidepressant-like effects in heterozygous BDNF KO mice, indicating a BDNF-independent mechanism of action. However, as discussed above, the results of another study indicated the opposite effect (Autry et al., 2011). Thus, it would be interesting to study these drugs in TrkB.T1 mice to examine their relationship with TrkB signaling.

As we showed, effects of fluoxetine on the reformation of the neuronal network in mice fear circuits are dependent on BDNF signaling. Furthermore, in this study, this phenomenon was demonstrated only with the SSRI fluoxetine, and the potential effects of other ADs remain uncertain. However, as all classes of ADs induce TrkB signaling and therefore increase plasticity (Rantamäki et al., 2007), it is likely that these results can be generalized to a wider spectrum of ADs. However, it is unclear if this paradigm will also be applicable to ketamine and LY 451646 and other fast-acting ADs. Furthermore, as the AD effect of these drugs appears to occur independently of BDNF, it would be of interest to determine if ketamine could, *via* some other mechanism of action, induce plastic changes similar to those obtained with fluoxetine. The findings of Sawtell et al. (2003) support this possibility by demonstrating that adult ocular dominance plasticity requires the presence of the NMDA receptor. Future studies will reveal whether this phenomenon can also be extended to ketamine and other non-classical fast-acting ADs.

7 Conclusions

The main aims of this thesis were to characterize the anxiety- and depression-like phenotypes of TrkB signaling-deficient mice (TrkB.T1), to examine the role of BDNF-TrkB signaling in the antidepressant-like effects of ketamine and other glutamatergic drugs, to study the network theory of ADs in a mouse fear extinction paradigm and to investigate the behavioral effects of adult fluoxetine treatment in mice exposed to fluoxetine early in life. Based on the results obtained, the main conclusions are as follows:

I. The phenotype of TrkB.T1 mice is characterized by decreased spontaneous activity, indifference to the surrounding environment or novel objects, increased behavioral despair and a lack of motivation, with some similarities with *BDNF*$^{(+/-)}$ mice. Therefore, TrkB.T1 mice may serve as a rodent model of depression.

II. Both ketamine and the AMPA potentiator LY451646 induced antidepressant-like effects in WT and *BDNF*$^{(+/-)}$ mice. Furthermore, neither ketamine nor LY 451646 affected the levels of phosphorylated-Trk or BDNF protein. These results indicate that the antidepressant effect of glutamatergic drugs may be independent of BDNF-TrkB signaling.

III. Early postnatal fluoxetine exposure resulted in long-lasting behavioral alterations in adult mice. When given again in adulthood, chronic fluoxetine treatment rescued some of these behavioral changes. These results demonstrate that ADs may have long-lasting effects on infants and children that should be considered when formulating treatment recommendations. More importantly, we determined that these changes may be recovered with adult AD treatment.

IV. The initiation of fluoxetine treatment before or after fear conditioning facilitated long-lasting fear extinction in adult mice. Furthermore, neither fluoxetine nor extinction training alone produced permanent fear memory erasures; both were needed for a long-lasting effect. These findings suggest that fluoxetine can also open developmental-like plasticity in the fear circuitry of adult mice, leading to the permanent reformation of fear responses.

Taken as a whole, these findings strengthen the previous hypothesis that BDNF and TrkB signaling are involved in phenomena associated with depression and, more specifically, to the mechanism of action of ADs. Furthermore, the AD fluoxetine, when used in combination with an environmental stimulus, can facilitate long-lasting changes in the neuronal network. Finally, our results suggest that there might be other fast-acting ADs with a mechanism of action other than BDNF-TrkB signaling.

References

aan het Rot, M., Collins, K. A., Murrough, J. W., Perez, A. M., Reich, D. L., Charney, D. S., & Mathew, S. J. 2010. Safety and efficacy of repeated-dose intravenous ketamine for treatment-resistant depression. Biological Psychiatry 67, 139-145.

Adachi, M., Barrot, M., Autry, A. E., Theobald, D., & Monteggia, L. M. 2008. Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. Biological Psychiatry 63, 642-649.

Adriaan Bouwknecht, J., Olivier, B., & Paylor, R. E. 2007. The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: A review of pharmacological and genetic studies in the mouse. Neuroscience and Biobehavioral Reviews 31, 41-59.

Alexandre, C., Popa, D., Fabre, V., Bouali, S., Venault, P., Lesch, K., Hamon, M., & Adrien, J. 2006. Early life blockade of 5-hydroxytryptamine 1A receptors normalizes sleep and depression-like behavior in adult knock-out mice lacking the serotonin transporter. Journal of Neuroscience 26, 5554-5564.

Aloyz, R., Fawcett, J. P., Kaplan, D. R., Murphy, R. A., & Miller, F. D., 1999. Activitydependent activation of TrkB neurotrophin receptors in the adult CNS. Learning & Memory 6, 216-231.

Alpermann, H., Schacht, U., Usinger, P., & Hock, F. 1992. Pharmacological effects of hoe-249 - a new potential antidepressant. Drug Development Research 25, 267-282.

Alt, A., Nisenbaum, E., Bleakman, D., & Witkin, J. 2006. A role for AMPA receptors in mood disorders. Biochemical Pharmacology 71, 1273-1288.

American Psychiatric Association (APA). 1952. Diagnostic and Statistical Manual: Mental Disorders, First edition (DSM-I).

American Psychiatric Association (APA). 1994. Diagnostic and Statistical Manual: Mental Disorders, 4th edition (DSM-IV).

Andlin-Sobocki, P., Jonsson, B., Wittchen, H. U., & Olesen, J. 2005. Cost of disorders of the brain in europe. European Journal of Neurology 12 Suppl 1, 1-27.

Ansorge, M., Zhou, M., Lira, A., Hen, R., & Gingrich, J. 2004. Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. Science 306, 879-881.

Ashcroft, G. W., Blackburn.I.M., Eccleston, D., Murray, L. G., Glen, A. I. M., Cramford, T. B. B., Pullar, I. A., Shields, P. J., Walter, D. S., Connechan, J., & Lonergan, M. 1972. Modified amine hypothesis for etiology of affective illness. Lancet 2, 573-577.

Autry, A. E., Adachi, M., Cheng, P., & Monteggia, L. M. 2009. Gender-specific impact of brain-derived neurotrophic factor signaling on stress-induced depression-like behavior. Biological Psychiatry 66, 84-90.

Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. F., Cheng, P. F., Kavalali, E. T., & Monteggia, L. M. 2011. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 475, 91-95.

Bai, F., Li, X., Clay, M., Lindström, T., & Skolnick, P. 2001. Intra- and interstrain differences in models of "behavioral despair". Pharmacology Biochemistry and Behavior 70, 187-192.

Barrientos, R., Sprunger, D., Campeau, S., Higgins, E., Watkins, L., Rudy, J., & Maier, S. 2003. Brain-derived neurotrophic factor mRNA downregulation produced by social isolation is blocked by intrahippocampal interleukin-1 receptor antagonist. Neuroscience 121, 847-853.

Bath, K. G., Mandairon, N., Jing, D., Rajagopal, R., Kapoor, R., Chen, Z. Y., Khan, T., Proenca, C. C., Kraemer, R., Cleland, T. A., Hempstead, B. L., Chao, M. V., & Lee, F. S. 2008. Variant brain-derived neurotrophic factor (Val66Met) alters adult olfactory bulb neurogenesis and spontaneous olfactory discrimination. The Journal of Neuroscience 28, 2383-2393.

Baumeister, A. A., Hawkins, M. F., & Uzelac, S. M. 2003. The myth of reserpine-induced depression: Role in the historical development of the monoamine hypothesis. Journal of the History of the Neurosciences 12, 207-220.

Belzung, C., & Griebel, G. 2001. Measuring normal and pathological anxiety-like behaviour in mice: A review. Behavioural Brain Research 125, 141-149.

Benbow, S. M. 2005. The ECT Handbook: Second Edition, Chapter 18: Adverse effects of ECT (edit. Scott, A. I. F.). The Royal College of Psychiatrists, London. 170-174.

Bergami, M., Rimondini, R., Santi, S., Blum, R., Gotz, M., & Canossa, M. 2008. Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. Proceedings of the National Academy of Sciences of the United States of America 105, 15570-15575.

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S., & Krystal, J. H. 2000. Antidepressant effects of ketamine in depressed patients. Biological Psychiatry 47, 351-354.

Bernal, M., Haro, J. M., Bernert, S., Brugha, T., de Graaf, R., Bruffaerts, R., Lepine, J. P., de Girolamo, G., Vilagut, G., Gasquet, I., Torres, J. V., Kovess, V., Heider, D., Neeleman, J., Kessler, R., Alonso, J., & ESEMED/MHEDEA Investigators. 2007. Risk factors for suicidality in europe: Results from the ESEMED study. Journal of Affective Disorders 101, 27-34.

Berton, O., McClung, C., DiLeone, R., Krishnan, V., Renthal, W., Russo, S., Graham, D., Tsankova, N., Bolanos, C., Rios, M., Monteggia, L., Self, D., & Nestler, E. 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311, 864-868.

Bishop, A., Kung, C., Shah, K., Witucki, L., Shokat, K., & Liu, Y. 1999. Generation of monospecific nanomolar tyrosine kinase inhibitors via a chemical genetic approach. Journal of the American Chemical Society 121, 627-631.

Bisson, J., & Andrew, M. 2007. Psychological treatment of post-traumatic stress disorder (PTSD). The Cochrane Database of Systematic Reviews (3), CD003388.

Borsini, F., Podhorna, J., & Marazziti, D. 2002. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? Psychopharmacology 163, 121-141.

Bostwick, J. M., & Pankratz, V. S. 2000. Affective disorders and suicide risk: A reexamination. The American Journal of Psychiatry 157, 1925-1932.

Bourin, M., & Hascoet, M. 2003. The mouse light/dark box test. European Journal of Pharmacology 463, 55-65.

Bremner, J., Narayan, M., Anderson, E., Staib, L., Miller, H., & Charney, D. 2000. Hippocampal volume reduction in major depression. American Journal of Psychiatry 157, 115-117.

Brunoni, A. R., Teng, C. T., Correa, C., Imamura, M., Brasil-Neto, J. P., Boechat, R., Rosa, M., Caramelli, P., Cohen, R., Del Porto, J. A., Boggie, P.S., & Fregni, F. 2010. Neuromodulation approaches for the treatment of major depression challenges and recommendations from a working group meeting. Arquivos De Neuro-Psiquiatria 68, 433-451.

Burke, H., Davis, M., Otte, C., & Mohr, D. 2005. Depression and cortisol responses to psychological stress: A meta-analysis. Psychoneuroendocrinology 30, 846-856.

Burghardt, N. S., & Bauer, E. P. 2013. Acute and chronic effects of selective serotonin reuptake inhibitor treatment on fear conditioning: Implications for underlying fear circuits. Neuroscience 247, 253-272.

Buwalda, B., Kole, M., Veenema, A., Huininga, M., de Boer, S., Korte, S., & Koolhaas, J. 2005. Long-term effects of social stress on brain and behavior: A focus on hippocampal functioning. Neuroscience and Biobehavioral Reviews 29, 83-97.

Carola, V., & Gross, C. 2010. BDNF moderates early environmental risk factors for anxiety in mouse. Genes, Brain, and Behavior 9, 379-389.

Castrèn, E. 2005. Opinion - is mood chemistry? Nature Reviews Neuroscience 6, 241-246.

Castrén, E. 2004. Neurotrophic effects of antidepressant drugs. Current Opinion in Pharmacology 4, 58-64.

Castrén, E., & Rantamäki, T. 2010. The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. Developmental Neurobiology 70, 289-297.

Castrén, E., Voikar, V., & Rantamäki, T. 2007. Role of neurotrophic factors in depression. Current Opinion in Pharmacology 7, 18-21.

Cavanagh, J., Carson, A., Sharpe, M., & Lawrie, S. 2003. Psychological autopsy studies of suicide: A systematic review. Psychological medicine 33, 395-405.

Checkley, S. 1996. The neuroendocrinology of depression and chronic stress. British Medical Bulletin 52, 597-617.

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

Chen, X., Ye, H., Kuruvilla, R., Ramanan, N., Scangos, K., Zhang, C., Johnson, N., England, P., Shokat, K., & Ginty, D. 2005. A chemical-genetic approach to studying neurotrophin signaling. Neuron 46, 13-21.

Chen, F., Madsen, T. M., Wegener, G., & Nyengaard, J. R. 2008. Changes in rat hippocampal CA1 synapses following imipramine treatment. Hippocampus 18, 631-639.

Chen, Z., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C., Herrera, D. G., Toth, M., Yang, C., McEwen, B. S., Hempstead, B. L., & Lee, F. S.. 2006. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science 314, 140-143.

Chourbaji, S., Brandwein, C., & Gass, P. 2011. Altering BDNF expression by genetics and/or environment: Impact for emotional and depression-like behaviour in laboratory mice. Neuroscience and Biobehavioral Reviews 35, 599-611.

Chourbaji, S., Hellweg, R., Brandis, D., Zorner, B., Zacher, C., Lang, U. E., Henn, F. A., Hortnagl, H., & Gass, P. 2004. Mice with reduced brain-derived neurotrophic factor expression show decreased choline

acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. Molecular Brain Research 121, 28-36.

Coppell, A. L., Pei, Q., & Zetterstrom, T. S. C. 2003. Bi-phasic change in BDNF gene expression following antidepressant drug treatment. Neuropharmacology 44, 903-910.

Coppen, A. 1967. The biochemistry of affective disorders. The British Journal of Psychiatry: The Journal of Mental Science 113, 1237-1264.

Crawley, J. N. 2008. Behavioral phenotyping strategies for mutant mice. Neuron 57, 809-818.

Crawley, J. N., Belknap, J. K., Collins, A. et al. 1997. Behavioral phenotypes of inbred mouse strains: Implications and recommendations for molecular studies. Psychopharmacology 132, 107-124.

Crowley, J. J., Jones, M. D., O'Leary, O. F., & Lucki, I. 2004. Automated tests for measuring the effects of antidepressants in mice. Pharmacology, Biochemistry, and Behavior 78, 269-274.

Cryan, J. F., & Holmes, A. 2005. The ascent of mouse: Advances in modelling human depression and anxiety. Nature Reviews: Drug Discovery 4, 775-790.

Cryan, J. F., Markou, A., & Lucki, I. 2002. Assessing antidepressant activity in rodents: Recent developments and future needs. Trends in Pharmacological Sciences 23, 238-245.

Cryan, J. F., & Mombereau, C. 2004. In search of a depressed mouse: Utility of models for studying depression-related behavior in genetically modified mice. Molecular Psychiatry 9, 326-357.

Cryan, J. F., Mombereau, C., & Vassout, A. 2005. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. Neuroscience and Biobehavioral Reviews 29, 571-625.

Cryan, J. F., & O'Leary, O. F. 2010. Neuroscience. A glutamate pathway to faster-acting antidepressants? Science 329, 913-914.

Dalla, C., Pitychoutis, P. M., Kokras, N., & Papadopoulou-Daifoti, Z. 2010. Sex differences in animal models of depression and antidepressant response. Basic & Clinical Pharmacology & Toxicology 106, 226-233.

Davison, K. 2006. Historical aspects of mood disorders. Psychiatry 5, 115-118.

Daws, L. C. 2009. Unfaithful neurotransmitter transporters: Focus on serotonin uptake and implications for antidepressant efficacy. Pharmacology & Therapeutics 121, 89-99.

De Boer, S. F., & Koolhaas, J. M. 2003. Defensive burying in rodents: Ethology, neurobiology and psychopharmacology. European Journal of Pharmacology 463, 145-161.

Deltheil, T., Guiard, B. P, Cerdan, J., David, D. J., Tanaka, K. F., Repérant, C., Guilloux, J. P., Coudoré, F., Hen, R., & Gardier, A. M. 2008. Behavioral and serotonergic consequences of decreasing or increasing hippocampus brain-derived neurotrophic factor protein levels in mice. Neuropharmacology 55, 1006-14.

Depont, F., Rambelomanana, S., Le Puil, S., Begaud, B., Verdoux, H., & Moore, N. 2003. Antidepressants: Psychiatrists' opinions and clinical practice. Acta Psychiatrica Scandinavica 108, 24-31.

Diazgranados, N., Ibrahim, L., Brutsche, N. E., Newberg, A., Kronstein, P., Khalife, S., Kammerer, W. A., Quezado, Z., Luckenbaugh, D. A., Salvadore, G., Machado-Vieira, R., Manji, H. K., & Zarate, C. A. ,Jr. 2010.

A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. Archives of General Psychiatry 67, 793-802.

Doris, A., Ebmeier, K., & Shajahan, P. 1999. Depressive illness. Lancet 354, 1369-1375.

Dranovsky, A., & Hen, R. 2006. Hippocampal neurogenesis: Regulation by stress and antidepressants. Biological Psychiatry 59, 1136-1143.

Dulawa, S. C., & Hen, R. 2005. Recent advances in animal models of chronic antidepressant effects: The novelty-induced hypophagia test. Neuroscience and Biobehavioral Reviews 29, 771-783.

Duman, C. H., Schlesinger, L., Russell, D. S., & Duman, R. S. 2008. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. Brain Research 1199, 148-158.

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

Duman, R. S., Heninger, G. R., & Nestler, E. J. 1997. A molecular and cellular theory of depression. Archives of General Psychiatry 54, 597-606.

Duman, R. S., & Monteggia, L. M. 2006. A neurotrophic model for stress-related mood disorders. Biological Psychiatry 59, 1116-1127.

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

Dzirasa, K., & Covington, H. E.,3rd. 2012. Increasing the validity of experimental models for depression. Annals of the New York Academy of Sciences 1265, 36-45.

Eisch, A., Bolanos, C., de Wit, J., Simonak, R., Pudiak, C., Barrot, M., Verhaagen, J., & Nestler, E. 2003. Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: A role in depression. Biological Psychiatry 54, 994-1005.

Ellis, P., & Royal Australian and New Zealand College of Psychiatrists Clinical Practice Guidelines Team for Depression. 2004. Australian and new zealand clinical practice guidelines for the treatment of depression. The Australian and New Zealand Journal of Psychiatry 38, 389-407.

Enkel, T., Spanagel, R., Vollmayr, B., & Schneider, M. 2010. Stress triggers anhedonia in rats bred for learned helplessness. Behavioural Brain Research 209, 183-186.

Ernfors, P., Lee, K. F., & Jaenisch, R. 1994. Mice lacking brain-derived neurotrophic factor develop with sensory deficits. Nature 368, 147-150.

Farley, S., Apazoglou, K., Witkin, J. M., Giros, B., & Tzavara, E. T. 2010. Antidepressant-like effects of an AMPA receptor potentiator under a chronic mild stress paradigm. The International Journal of Neuropsychopharmacology 13, 1207-1218.

Fava, M. 2003. Diagnosis and definition of treatment-resistant depression. Biological Psychiatry 53, 649-659.

Fava, M., Uebelacker, L. A., Alpert, J. E., Nierenberg, A. A., Pava, J. A., & Rosenbaum, J. F. 1997. Major depressive subtypes and treatment response. Biological Psychiatry 42, 568-576.

Fernandez, S. P., & Gaspar, P. 2012. Investigating anxiety and depressive-like phenotypes in genetic mouse models of serotonin depletion. Neuropharmacology 62, 144-154.

Fibiger, H. C., & Phillips, A. G. 1981. Increased intracranial self-stimulation in rats after long-term administration of desipramine. Science 214, 683-685.

Freitas, A. E., Machado, D. G., Budni, J., Neis, V. B., Balen, G. O., Lopes, M. W., de Souza, L. F., Dafre, A. L., Leal, R. B., & Rodrigues, A. L. 2013. Fluoxetine modulates hippocampal cell signaling pathways implicated in neuroplasticity in olfactory bulbectomized mice. Behavioural Brain Research 237, 176-184.

Forbes, N. F., Stewart, C. A., Matthews, K., & Reid, I. C. 1996. Chronic mild stress and sucrose consumption: Validity as a model of depression. Physiology & Behavior 60, 1481-1484.

Fuchs, E., & Gould, E. 2000. In vivo neurogenesis in the adult brain: Regulation and functional implications. European Journal of Neuroscience 12, 2211-2214.

Garcia, L. S. B., Comim, C. M., Valvassori, S. S. Reus, G. Z., Barbosa, L. M., Andreazza, A. C., Stertz, L., Fries, G. R., Gavioli, E. C., Kapczinski, F., & Quevedo, J. 2008. Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. Progress in Neuro-Psychopharmacology & Biological Psychiatry 32, 140-144.

Garcia-Gutierrez, M. S., Perez-Ortiz, J. M., Gutierrez-Adan, A., & Manzanares, J. 2010. Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. British Journal of Pharmacology 160, 1773-1784.

Garcia-Toro, M., Rubio, J. M., Gili, M., Roca, M., Jin, C. J., Liu, S. M., Bastianoni, C., & Blanco, C. 2013. Persistence of chronic major depression: A national prospective study. Journal of Affective Disorders 151, 306-312.

Gardier, A. M. 2009. Mutant mouse models and antidepressant drug research: Focus on serotonin and brain-derived neurotrophic factor. Behavioural Pharmacology 20, 18-32.

Gardier, A. M., Guiard, B. P., Guilloux, J. P., Reperant, C., Coudore, F., & David, D. J. 2009. Interest of using genetically manipulated mice as models of depression to evaluate antidepressant drugs activity: A review. Fundamental & Clinical Pharmacology 23, 23-42.

Gogolla, N., Caroni, P., Luthi, A., & Herry, C. 2009. Perineuronal nets protect fear memories from erasure. Science 325, 1258-1261.

Gould, E., McEwen, B. S., Tanapat, P., Galea, L. A., & Fuchs, E. 1997. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. The Journal of Neuroscience 17, 2492-2498.

Gould, E., Tanapat, P., McEwen, B., Flugge, G., & Fuchs, E. 1998. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proceedings of the National Academy of Sciences of the United States of America 95, 3168-3171.

Govindarajan, A., Rao, B. S., Nair, D., Trinh, M., Mawjee, N., Tonegawa, S., & Chattarji, S. 2006. Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. Proceedings of the National Academy of Sciences of the United States of America 103, 13208-13213.

Gratacos, M., Gonzalez, J. R., Mercader, J. M., de Cid, R., Urretavizcaya, M., & Estivill, X. 2007. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: Meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. Biological Psychiatry 61, 911-922.

Gross, C., Zhuang, X., Stark, K., Ramboz, S., Oosting, R., Kirby, L., Santarelli, L., Beck, S., & Hen, R. 2002. Serotonin(1A) receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 416, 396-400.

Gruenberg, A. M., Goldstein, R. D., & Pincus, H. A. 2005. Classification of Depression: Research and Diagnostic Criteria: DSM-IV and ICD-10, in Biology of Depression: From Novel Insights to Therapeutic Strategies, edit. Licinio J and Wong M-L, Wiley-VCH Verlag GmbH & Co, Published online.

Gustavsson, A., Svensson, M., Jacobi, F. *et al.* 2011. Cost of disorders of the brain in Europe 2010. European Neuropsychopharmacology 21, 718-779.

Guze, S. B., & Robins, E. 1970. Suicide and primary affective disorders. British Journal of Psychiatry 117, 437-438.

Hajszan, T., MacLusky, N., & Leranth, C. 2005. Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. European Journal of Neuroscience 21, 1299-1303.

Hammen, C. 2005. Stress and depression. Annual Review of Clinical Psychology 1, 293-319.

Harkin, A., Kelly, J., & Leonard, B. 2003. A review of the relevance and validity of olfactory bulbectomy as a model of depression. Clinical Neuroscience Research 3, 253-262.

Hawton, K., Casanas I Comabella, C., Haw, C., & Saunders, K. 2013. Risk factors for suicide in individuals with depression: A systematic review. Journal of Affective Disorders 147, 17-28.

Hayashi, E., Shimamura, M., Kuratani, K., Kinoshita, M., & Hara, H. 2011. Automated experimental system capturing three behavioral components during murine forced swim test. Life Sciences 88, 411-417.

Hebert, M., Evenson, A., Lumley, L., & Meyerhoff, J. 1998. Effects of acute social defeat on activity in the forced swim test: Parametric studies in DBA/2 mice using a novel measurement device. Aggressive Behavior 24, 257-269.

Heisler, L., Chu, H., Brennan, T., Danao, J., Bajwa, P., Parsons, L., & Tecott, L. 1998. Elevated anxiety and antidepressant-like responses in serotonin 5-HT1A receptor mutant mice. Proceedings of the National Academy of Sciences of the United States of America 95, 15049-15054.

Henriksson, M. M., Aro, H. M., Marttunen, M. J., Heikkinen, M. E., Isometsä, E. T., Kuoppasalmi, K. I., & Lönnqvist, J. K. 1993. Mental disorders and comorbidity in suicide. The American Journal of Psychiatry 150, 935-940.

Hindmarch, I. 2002. Beyond the monoamine hypothesis: Mechanisms, molecules and methods. European Psychiatry 17 Suppl 3, 294-299.

Hirschfeld, R. M. 2000. History and evolution of the monoamine hypothesis of depression. The Journal of Clinical Psychiatry 61 Suppl 6, 4-6.

Hodes, G. E., Hill-Smith, T. E., & Lucki, I. 2010. Fluoxetine treatment induces dose dependent alterations in depression associated behavior and neural plasticity in female mice. Neuroscience Letters 484, 12-16.

Hoeffer, C. A., & Klann, E. 2010. mTOR signaling: At the crossroads of plasticity, memory and disease. Trends in Neurosciences 33, 67-75.

Hogg, S. 1996. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacology, Biochemistry, and Behavior 54, 21-30.

Holmes, A., Li, Q., Murphy, D., Gold, E., & Crawley, J. 2003a. Abnormal anxiety-related behaviour in serotonin transporter null mutant mice: The influence of genetic background. Genes Brain and Behavior 2, 365-380.

Holmes, A., Murphy, D., & Crawley, J. 2003b. Abnormal behavioral phenotypes of serotonin transporter knockout mice: Parallels with human anxiety and depression. Biological Psychiatry 54, 953-959.

Holmes, A., Yang, R., Lesch, K., Crawley, J., & Murphy, D. 2003c. Mice lacking the serotonin transporter exhibit 5-HT1A receptor-mediated abnormalities in tests for anxiety-like behavior. Neuropsychopharmacology 28, 2077-2088.

Holmes, A., Yang, R., Murphy, D., & Crawley, J. 2002. Evaluation of antidepressant-related behavioral responses in mice lacking the serotonin transporter. Neuropsychopharmacology 27, 914-923.

Huang, E. J., & Reichardt, L. F. 2001. Neurotrophins: Roles in neuronal development and function. Annual Review of Neuroscience 24, 677-736.

Huang, Y. Z., Pan, E., Xiong, Z. Q., & McNamara, J. O. 2008. Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. Neuron 57, 546-558.

Huang, Z. J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M. F., Maffei, L., & Tonegawa, S. 1999. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98, 739-755.

Ibarguen-Vargas, Y., Surget, A., Touma, C., Palme, R., & Belzung, C. 2008. Multifaceted strain-specific effects in a mouse model of depression and of antidepressant reversal. Psychoneuroendocrinology 33, 1357-1368.

Ibarguen-Vargas, Y., Surget, A., Vourc'h, P., Leman, S., Andres, C. R., Gardier, A. M., & Belzung, C. 2009. Deficit in BDNF does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress. Behavioural Brain Research 202, 245-251.

Jacobson, L. H., & Cryan, J. F. 2007. Feeling strained? influence of genetic background on depression-related behavior in mice: A review. Behavior Genetics 37, 171-213.

Juszczak, G. R., Lisowski, P., Sliwa, A. T., & Swiergiel, A. H. 2008. Computer assisted video analysis of swimming performance in a forced swim test: Simultaneous assessment of duration of immobility and swimming style in mice selected for high and low swim-stress induced analgesia. Physiology & Behavior 95, 400-407.

Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J. M., & Bertschy, G. 2005. Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biological Psychiatry 57, 1068-1072.

Karpova, N.N., Rantamäki, T., Di Lieto, A., Lindemann, L., Höener, M.C., & Castrén, E., 2010. Darkness reduces BDNF expression in the visual cortex and induces repressive chromatin remodeling at the BDNF gene in both hippocampus and visual cortex. Cellular and Molecular Neurobiology 30, 1117-1123.

Kernie, S. G., Liebl, D. J., & Parada, L. F. 2000. BDNF regulates eating behavior and locomotor activity in mice. The EMBO Journal 19, 1290-1300.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. Archives of General Psychiatry 62, 593-602.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K. R., Rush, A. J., Walters, E. E., & Wang, P. S. 2003. The epidemiology of major depressive disorder - results from the national comorbidity survey replication (NCS-R). Jama-Journal of the American Medical Association 289, 3095-3105.

Kessler, R. C., & Bromet, E. J. 2013. The epidemiology of depression across cultures. Annual Review of Public Health 34, 119-138.

Kikusui, T., Ichikawa, S., & Mori, Y. 2009. Maternal deprivation by early weaning increases corticosterone and decreases hippocampal BDNF and neurogenesis in mice. Psychoneuroendocrinology 34, 762-772.

Kim, Y. K., Lee, H. P., Won, S. D. Park, E. Y., Lee, H. Y., Lee, B. H., Lee, S. W., Yoon, D., Han, C., Kim, D. J., & Choi, S. H.. 2007. Low plasma BDNF is associated with suicidal behavior in major depression. Progress in Neuro-Psychopharmacology & Biological Psychiatry 31, 78-85.

Kim, J. H., & Richardson, R. 2010. New findings on extinction of conditioned fear early in development: Theoretical and clinical implications. Biological Psychiatry 67, 297-303.

Klein, R., Smeyne, R. J., Wurst, W., Long, L. K., Auerbach, B. A., Joyner, A. L., & Barbacid, M. 1993. Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. Cell 75, 113-122.

Koike, H., Iijima, M., & Chaki, S. 2011. Involvement of AMPA receptor in both the rapid and sustained antidepressant-like effects of ketamine in animal models of depression. Behavioural Brain Research 224, 107-111.

Koponen, E., Rantamäki, T., Voikar, V., Saarelainen, T., MacDonald, E., & Castrén, E. 2005. Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. Cellular and Molecular Neurobiology 25, 973-980.

Koponen, E., Voikar, V., Riekki, R., Saarelainen, T., Rauramaa, T., Rauvala, H., Taira, T., & Castrén, E. 2004. Transgenic mice overexpressing the full-length neurotrophin receptor trkB exhibit increased activation of the trkB-PLC gamma pathway, reduced anxiety, and facilitated learning. Molecular and Cellular Neuroscience 26, 166-181.

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., & Bonhoeffer, T. 1995. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proceedings of the National Academy of Sciences of the United States of America 92, 8856-8860.

Kozisek, M. E., Middlemas, D., & Bylund, D. B. 2008. Brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B in the mechanism of action of antidepressant therapies. Pharmacology & Therapeutics 117, 30-51.

Krishnan, V., & Nestler, E. J. 2010. Linking molecules to mood: New insight into the biology of depression. The American Journal of Psychiatry 167, 1305-1320.

Krystal, J. H., Tolin, D. F., Sanacora, G., Castner, S. A., Williams, G. V., Aikins, D. E., Hoffman, R. E., & D'Souza, D. C. 2009. Neuroplasticity as a target for the pharmacotherapy of anxiety disorders, mood disorders, and schizophrenia. Drug Discovery Today 14, 690-697.

Kudryavtseva, N., Bakshtanovskaya, I., & Koryakina, L. 1991. Social model of depression in mice of C57bl/6j strain. Pharmacology Biochemistry and Behavior 38, 315-320.

Kuhn, R. 1958. The treatment of depressive states with G 22355 (imipramine hydrochloride). The American Journal of Psychiatry 115, 459-464.

Kupfer, D., & Frank, E. 2003. Comorbidity in depression. Acta Psychiatrica Scandinavica 108, 57-60.

Lam, R. W., Kennedy, S. H., Grigoriadis, S., McIntyre, R. S., Milev, R., Ramasubbu, R., Parikh, S. V., Patten, S. B., & Ravindran, A. V. 2009. Canadian network for mood and anxiety treatments (CANMAT) clinical guidelines for the management of major depressive disorder in adults. ill. pharmacotherapy. Journal of Affective Disorders 117, S26-S43.

LeDoux, J. E. 2000. Emotion circuits in the brain. Annual Review of Neuroscience 23, 155-184.

Lee, F. S., & Chao, M. V. 2001. Activation of trk neurotrophin receptors in the absence of neurotrophins. Proceedings of the National Academy of Sciences of the United States of America 98, 3555-3560.

Legutko, B., Li, X., & Skolnick, P. 2001. Regulation of BDNF expression in primary neuron culture by LY392098, a novel AMPA receptor potentiator. Neuropharmacology 40, 1019-1027.

Li, N., Lee, B., Liu, R., Banasr, M., Dwyer, J. M., Iwata, M., Li, X., Aghajanian, G., & Duman, R. S. 2010a. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329, 959-964.

Li, W. J., Yu, H., Yang, J. M., Gao, J., Jiang, H., Feng, M., Zhao, Y. X., & Chen, Z. Y. 2010b. Anxiolytic effect of music exposure on BDNFMet/Met transgenic mice. Brain Research 1347, 71-79.

Li, X., Tizzano, J. P., Griffey, K., Clay, M., Lindström, T., & Skolnick, P. 2001. Antidepressant-like actions of an AMPA receptor potentiator (LY392098). Neuropharmacology 40, 1028-1033.

Li, X., Witkin, J. M., Need, A. B., & Skolnick, P. 2003. Enhancement of antidepressant potency by a potentiator of AMPA receptors. Cellular and Molecular Neurobiology 23, 419-430.

Liebenauer, L. L., & Slotnick, B. M. 1996. Social organization and aggression in a group of olfactory bulbectomized male mice. Physiology & Behavior 60, 403-409.

Liebrenz, M., Borgeat, A., Leisinger, R., & Stohler, R. 2007. Intravenous ketamine therapy in a patient with a treatment-resistant major depression. Swiss Medical Weekly 137, 234-236.

Linnarsson, S., Bjorklund, A., & Ernfors, P. 1997. Learning deficit in BDNF mutant mice. The European Journal of Neuroscience 9, 2581-2587.

Liu, I. Y., Lyons, W. E., Mamounas, L. A., & Thompson, R. F. 2004. Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning. The Journal of Neuroscience 24, 7958-7963.

Lopez, A. D., Mathers, C. D., Ezzati, M., Jamison, D. T., & Murray, C. J. L. (edit.). 2003. Global Burden of Disease and Risk Factors.

Lu, B., Pang, P., & Woo, N. 2005. The yin and yang of neurotrophin action. Nature Reviews Neuroscience 6, 603-614.

Lucki, I., Dalvi, A., & Mayorga, A. J. 2001. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. Psychopharmacology 155, 315-322.

Lyons, W. E., Mamounas, L. A., Ricaurte, G. A., Coppola, V., Reid, S. W., Bora, S. H., Wihler, C., Koliatsos, V. E., & Tessarollo, L. 1999. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proceedings of the National Academy of Sciences of the United States of America 96, 15239-15244.

Machado, D. G., Cunha, M. P., Neis, V. B., Balen, G. O., Colla, A., Grando, J., Brocardo, P. S., Bettio, L. E., Capra, J. C., & Rodrigues, A. L. 2012. Fluoxetine reverses depressive-like behaviors and increases hippocampal acetylcholinesterase activity induced by olfactory bulbectomy. Pharmacology, Biochemistry, and Behavior 103, 220-229.

Machado-Vieira, R., Yuan, P., Brutsche, N., DiazGranados, N., Luckenbaugh, D., Manji, H. K., & Zarate, C. A., Jr. 2009. Brain derived neurotrophic factor and initial antidepressant response to an NMDA antagonist. Bipolar Disorders 11, 58-59.

Mackowiak, M., O'Neill, M. J., Hicks, C. A., Bleakman, D., & Skolnick, P. 2002. An AMPA receptor potentiator modulates hippocampal expression of BDNF: An in vivo study. Neuropharmacology 43, 1-10.

MacQueen, G. M., Ramakrishnan, K., Croll, S. D., Siuciak, J. A., Yu, G. H., Young, L. T., & Fahnestock, M. 2001. Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behavioral Neuroscience 115, 1145-1153.

Madsen, T., Treschow, A., Bengzon, J., Bolwig, T., Lindvall, O., & Tingstrom, A. 2000. Increased neurogenesis in a model of electroconvulsive therapy. Biological Psychiatry 47, 1043-1049.

Maeng, S., Zarate, C. A., Jr., Du, J., Schloesser, R. J., McCammon, J., Chen, G., & Manji, H. K. 2008. Cellular mechanisms underlying the antidepressant effects of ketamine: Role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biological Psychiatry 63, 349-352.

Malberg, J., Eisch, A., Nestler, E., & Duman, R. 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. Journal of Neuroscience 20, 9104-9110.

Marais, L., Stein, D. J., & Daniels, W. M. U. 2009. Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. Metabolic Brain Disease 24, 587-597.

Maya Vetencourt, J. F., Sale, A., Viegi, A., Baroncelli, L., De Pasquale, R., O'Leary, O. F., Castrén, E., & Maffei, L. 2008. The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science 320, 385-388.

Mazure, C. 1998. Life stressors as risk factors in depression. Clinical Psychology-Science and Practice 5, 291-313.

McEwen, B. 2000. Effects of adverse experiences for brain structure and function. Biological Psychiatry 48, 721-731.

McKittrick, C., Magarinos, A., Blanchard, D., Blanchard, R., McEwen, B., & Sakai, R. 2000. Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. Synapse 36, 85-94.

Merali, Z., Du, L., Hrdina, P., Palkovits, M., Faludi, G., Poulter, M., & Anisman, H. 2004. Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptorsubunits in frontal cortical brain region. Journal of Neuroscience 24, 1478-1485.

Metz, A. V., Chynoweth, J., & Allan, A. M. 2006. Influence of genetic background on alcohol drinking and behavioral phenotypes of 5-HT3 receptor over-expressing mice. Pharmacology, Biochemistry, and Behavior 84, 120-127.

Milad, M. R., Rauch, S. L., Pitman, R. K., & Quirk, G. J. 2006. Fear extinction in rats: Implications for human brain imaging and anxiety disorders. Biological Psychology 73, 61-71.

Millstein, R. A., & Holmes, A. 2007. Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. Neuroscience and Biobehavioral Reviews 31, 3-17.

Mineur, Y., Prasol, D., Belzung, C., & Crusio, W. 2003. Agonistic behavior and unpredictable chronic mild stress in mice. Behavior Genetics 33, 513-519.

Minichiello, L., Korte, M., Wolfer, D., Kuehn, R., Unsicker, K., Cestari, V., Rossi-Arnaud, C., Lipp, H. P., Bonhoeffer, T., & Klein, R. 1999. Essential role for TrkB receptors in hippocampus-mediated learning. Neuron 24, 401-414.

Minichiello, L. 2009. TrkB signalling pathways in LTP and learning. Nature Reviews Neuroscience 10, 850-860.

Monteggia, L. M., Barrot, M., Powell, C. M., Berton, O., Galanis, V., Gemelli, T., Meuth, S., Nagy, A., Greene, R. W., & Nestler, E. J. 2004. Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proceedings of the National Academy of Sciences of the United States of America 101, 10827-10832.

Monteggia, L. M., Luikart, B., Barrot, M., Theoloold, D., Malkovska, I., Nef, S., Parada, L. F., & Nestler, E. J. 2007. Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biological Psychiatry 61, 187-197.

Monteleone, P., Serritella, C., Martiadis, V., & Maj, M. 2008. Decreased levels of serum brain-derived neurotrophic factor in both depressed and euthymic patients with unipolar depression and in euthymic patients with bipolar I and II disorders. Bipolar Disorders 10, 95-100.

Montgomery, K. C. 1955. The relation between fear induced by novel stimulation and exploratory behavior. Journal of Comparative and Physiological Psychology 48, 254-260.

Muscat, R., Papp, M., & Willner, P. 1992. Reversal of stress-induced anhedonia by the atypical antidepressants, fluoxetine and maprotiline. Psychopharmacology 109, 433-438.

Myers, K. M., & Davis, M. 2007. Mechanisms of fear extinction. Molecular Psychiatry 12, 120-150.

Nagappan, G., Woo, N. H., & Lu, B. 2008. Ama "zinc" link between TrkB transactivation and synaptic plasticity. Neuron 57, 477-479.

Nakajo, Y., Miyamoto, S., Nakano, Y., Xue, J. H., Hori, T., & Yanamoto, H. 2008. Genetic increase in brain-derived neurotrophic factor levels enhances learning and memory. Brain Research 1241, 103-109.

Nemeroff, C. B. 2007. Prevalence and management of treatment-resistant depression. The Journal of Clinical Psychiatry 68 Suppl 8, 17-25.

Newport, D., Stowe, Z., & Nemeroff, C. 2002. Parental depression: Animal models of an adverse life event. American Journal of Psychiatry 159, 1265-1283.

Nibuya, M., Morinobu, S., & Duman, R. S. 1995. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. The Journal of Neuroscience 15, 7539-7547.

Nicolas, L. B., Kolb, Y., & Prinssen, E. P. M. 2006. A combined marble burying-locomotor activity test in mice: A practical screening test with sensitivity to different classes of anxiolytics and antidepressants. European Journal of Pharmacology 547, 106-115.

Nulman, I., Rovet, J., Stewart, D., Wolpin, J., Gardner, H., Theis, J., Kulin, N., & Koren, G. 1997. Neurodevelopment of children exposed in utero to antidepressant drugs. New England Journal of Medicine 336, 258-262.

Nulman, I., Rovet, J., Stewart, D., Wolpin, J., Pace-Asciak, P., Shuhaiber, S., & Koren, G. 2002. Child development following exposure to tricyclic antidepressants or fluoxetine throughout fetal life: A prospective, controlled study. American Journal of Psychiatry 159, 1889-1895.

Olchanski, N., McInnis Myers, M., Halseth, M., Cyr, P. L., Bockstedt, L., Goss, T. F., & Howland, R. H. 2013. The economic burden of treatment-resistant depression. Clinical Therapeutics 35, 512-522.

O'Leary, O. F., Wu, X., & Castrén, E. 2009. Chronic fluoxetine treatment increases expression of synaptic proteins in the hippocampus of the ovariectomized rat: Role of BDNF signalling. Psychoneuroendocrinology 34, 367-381.

Olesen, J., Gustavsson, A., Svensson, M., Wittchen, H. U., Jonsson, B., CDBE2010 study group, & European Brain Council. 2012. The economic cost of brain disorders in europe. European Journal of Neurology 19, 155-162.

Olesen, J., & Leonardi, M. 2003. The burden of brain diseases in europe. European Journal of Neurology 10, 471-477.

O'Neil, M., & Moore, N. 2003. Animal models of depression: Are there any? Human Psychopharmacology-Clinical and Experimental 18, 239-254.

Oestergaard, S., & Moldrup, C. 2011. Improving outcomes for patients with depression by enhancing antidepressant therapy with non-pharmacological interventions: A systematic review of reviews. Public Health 125, 357-367.

Pampallona, S., Bollini, P., Tibaldi, G., Kupelnick, B., & Munizza, C. 2004. Combined pharmacotherapy and psychological treatment for depression - A systematic review. Archives of General Psychiatry 61, 714-719.

Papaleo, F., Silverman, J. L., Aney, J., Tian, Q., Barkan, C. L., Chadman, K. K., & Crawley, J. N. 2011. Working memory deficits, increased anxiety-like traits, and seizure susceptibility in BDNF overexpressing mice. Learning & Memory 18, 534-544.

Papp, M., Moryl, E., & Willner, P. 1996. Pharmacological validation of the chronic mild stress model of depression. European Journal of Pharmacology 296, 129-136.

Park, H., & Poo, M. M. 2013. Neurotrophin regulation of neural circuit development and function. Nature Reviews: Neuroscience 14, 7-23.

Parks, C., Robinson, P., Sibille, E., Shenk, T., & Toth, M. 1998. Increased anxiety of mice lacking the serotonin(1A) receptor. Proceedings of the National Academy of Sciences of the United States of America 95, 10734-10739.

Pawlak, J., Dmitrzak-Weglarz, M., Skibinska, M., Szczepankiewicz, A., Leszczynska-Rodziewicz, A., Rajewska-Rager, A., Zaremba, D., Czerski, P., & Hauser, J. 2013. Suicide attempts and clinical risk factors in patients with bipolar and unipolar affective disorders. General Hospital Psychiatry 35, 427-432.

Paykel, E. 2003. Life events and affective disorders. Acta Psychiatrica Scandinavica 108, 61-66.

Pedersen, S. H., Stage, K. B., Bertelsen, A., Grinsted, P., Kragh-Sorensen, P., & Sorensen, T. 2001. ICD-10 criteria for depression in general practice. Journal of Affective Disorders 65, 191-194.

Petit-Demouliere, B., Chenu, F., & Bourin, M. 2005. Forced swimming test in mice: A review of antidepressant activity. Psychopharmacology 177, 245-255.

Poo, M. M. 2001. Neurotrophins as synaptic modulators. Nature Reviews Neuroscience 2, 24-32.

Popik, P., Kos, T., Sowa-Kucma, M., & Nowak, G. 2008. Lack of persistent effects of ketamine in rodent models of depression. Psychopharmacology 198, 421-430.

Porsolt, R., Bertin, A., & Jalfre, M. 1978. Behavioral despair in rats and mice - strain differences and effects of imipramine. European Journal of Pharmacology 51, 291-294.

Porsolt, R., Lepichon, M., & Jalfre, M. 1977. Depression - new animal-model sensitive to antidepressant treatments. Nature 266, 730-732.

Pothion, S., Bizot, J., Trovero, F., & Belzung, C. 2004. Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behavioural Brain Research 155, 135-146.

Preskorn, S. H., Baker, B., Kolluri, S., Menniti, F. S., Krams, M., & Landen, J. W. 2008. An innovative design to establish proof of concept of the antidepressant effects of the NR2B subunit selective N-methyl-D-aspartate antagonist, CP-101,606, in patients with treatment-refractory major depressive disorder. Journal of Clinical Psychopharmacology 28, 631-637.

Psotta, L., Lessmann, V., & Endres, T. 2013. Impaired fear extinction learning in adult heterozygous BDNF knock-out mice. Neurobiology of Learning and Memory 103C, 34-38.

Rantamäki, T., Hendolin, P., Kankaanpää, A., Mijatovic, J., Piepponen, P., Domenici, E., Chao, M. V., Männistö, P. T., & Castrén, E. 2007. Pharmacologically diverse antidepressants rapidly activate brain-derived neurotrophic factor receptor TrkB and induce phospholipase-cgamma signaling pathways in mouse brain. Neuropsychopharmacology 32, 2152-2162.

Rantamäki, T., Kemppainen, S., Autio, H., Stavén, S., Koivisto, H., Kojima, M., Antila, H., Miettinen, P. O., Kärkkäinen, E., Karpova, N., Vesa, L., Lindemann, L., Höener, M. C., Tanila, H., Castrén, E. 2013. The impact of bdnf gene deficiency to the memory impairment and brain pathology of APPswe/PS1dE9 mouse model of alzheimer's disease. PloS One 8, e68722.

Rasmusson, A. M., Shi, L., & Duman, R. 2002. Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with footshock. Neuropsychopharmacology 27, 133-142.

Razzoli, M., Domenici, E., Carboni, L., Rantamäki, T., Lindholm, J., Castrén, E., & Arban, R. 2011. A role for BDNF/TrkB signaling in behavioral and physiological consequences of social defeat stress. Genes Brain and Behavior 10, 424-433.

Remick, R. A. 2002. Diagnosis and management of depression in primary care: A clinical update and review. CMAJ : Canadian Medical Association Journal = Journal De l'Association Medicale Canadienne 167, 1253-1260.

Reus, G. Z., Stringari, R. B., Ribeiro, K. F., Ferraro, A. K., Vitto, M. F., Cesconetto, P., Souza, C. T., & Quevedo, J. 2011. Ketamine plus imipramine treatment induces antidepressant-like behavior and increases CREB and BDNF protein levels and PKA and PKC phosphorylation in rat brain. Behavioural Brain Research 221, 166-171.

Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R. M., & Jaenisch, R. 2001. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Molecular Endocrinology 15, 1748-1757.

Roceri, M., Cirulli, F., Pessina, C., Peretto, P., Racagni, G., & Riva, M. 2004. Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biological Psychiatry 55, 708-714.

Roceri, M., Hendriks, W., Racagni, G., Ellenbroek, B. A., & Riva, M. A. 2002. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Molecular Psychiatry 7, 609-616.

Roche, M., Harkin, A., & Kelly, J. P. 2007. Chronic fluoxetine treatment attenuates stressor-induced changes in temperature, heart rate, and neuronal activation in the olfactory bulbectomized rat. Neuropsychopharmacology 32, 1312-1320.

Rogoz, Z., Skuza, G., Maj, J., & Danysz, W. 2002. Synergistic effect of uncompetitive NMDA receptor antagonists and antidepressant drugs in the forced swimming test in rats. Neuropharmacology 42, 1024-1030.

Russo-Neustadt, A., Beard, R., & Cotman, C. 1999. Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. Neuropsychopharmacology 21, 679-682.

Saarelainen, T., Pussinen, R., Koponen, E., Alhonen, L., Wong, G., Sirviö, J., & Castrén, E. 2000. Transgenic mice overexpressing truncated trkB neurotrophin receptors in neurons have impaired long-term spatial memory but normal hippocampal LTP. Synapse 38, 102-104.

Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., Agerman, K., Haapasalo, A., Nawa, H., Aloyz, R., Ernfors, P., & Castrén, E. 2003. Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. Journal of Neuroscience 23, 349-357.

Sairanen, M., Lucas, G., Ernfors, P., Castrén, M., & Castrén, E. 2005. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. The Journal of Neuroscience 25, 1089-1094.

Sale, A., Maya-Vetencourt, J. F., Medinin, P., Cenni, M. C., Baroncelli, L., De Pasquale, R., & Maffei, L. 2007. Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. Nature Neuroscience 10, 679-681.

Sallert, M., Rantamäki, T., Vesikansa, A., Anthoni, H., Harju, K., Yli-Kauhaluoma, J., Taira, T., Castrén, E., & Lauri, S. E. 2009. Brain-derived neurotrophic factor controls activity-dependent maturation of CA1 synapses by downregulating tonic activation of presynaptic kainate receptors. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 29, 11294-11303.

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

Sawtell, N. B., Frenkel, M. Y., Philpot, B. D., Nakazawa, K., Tonegawa, S., & Bear, M. F. 2003. NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. Neuron 38, 977-985.

Schildkraut, J. J. 1965. The catecholamine hypothesis of affective disorders: A review of supporting evidence. The American Journal of Psychiatry 122, 509-522.

Schmidt, H. D., & Duman, R. S. 2010. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology 35, 2378-2391.

Sen, S., Duman, R., & Sanacora, G. 2008. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: Meta-analyses and implications. Biological Psychiatry 64, 527-532.

Sheline, Y., Wang, P., Gado, M., Csernansky, J., & Vannier, M. 1996. Hippocampal atrophy in recurrent major depression. Proceedings of the National Academy of Sciences of the United States of America 93, 3908-3913.

Shepherd, G. M. 2006. Behaviour: Smells, brains and hormones. Nature 439, 149-151.

Shirayama, Y., Chen, A. C., Nakagawa, S., Russell, D. S., & Duman, R. S. 2002. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. Journal of Neuroscience 22, 3251-3261.

Sik, A., van Nieuwehuyzen, P., Prickaerts, J., & Blokland, A. 2003. Performance of different mouse strains in an object recognition task. Behavioural Brain Research 147, 49-54.

Simon, G. E. 2003. Social and economic burden of mood disorders. Biological Psychiatry 54, 208-215.

Siuciak, J. A., Lewis, D. R., Wiegand, S. J., & Lindsay, R. M. 1997. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacology Biochemistry and Behavior 56, 131-137.

Skolnick, P., Popik, P., & Trullas, R. 2009. Glutamate-based antidepressants: 20 years on. Trends in Pharmacological Sciences 30, 563-569.

Slattery, D. A., Markou, A., & Cryan, J. F. 2007. Evaluation of reward processes in an animal model of depression. Psychopharmacology 190, 555-568.

Smirnov, I., Walsh, J. T., & Kipnis, J. 2013. Chronic mild stress eliminates the neuroprotective effect of copaxone after CNS injury. Brain, Behavior, and Immunity 31, 177-182.

Song, C., & Leonard, B. E. 2005. The olfactory bulbectomised rat as a model of depression. Neuroscience and Biobehavioral Reviews 29, 627-647.

Steru, L., Chermat, R., Thierry, B., & Simon, P. 1985. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology 85, 367-370.

Strekalova, T., Couch, Y., Kholod, N., Boyks, M., Malin, D., Leprince, P., & Steinbusch, H. M. W. 2011. Update in the methodology of the chronic stress paradigm: Internal control matters. Behavioral and Brain Functions 7:9.

Strekalova, T., Gorenkova, N., Schunk, E., Dolgov, O., & Bartsch, D. 2006. Selective effects of citalopram in a mouse model of stress-induced anhedonia with a control for chronic stress. Behavioural Pharmacology 17, 271-287.

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F. A., & Gass, P. 2004. Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. Neuropsychopharmacology 29, 2007-2017.

Tennant, C. 2002. Life events, stress and depression: A review of recent findings. Australian and New Zealand Journal of Psychiatry 36, 173-182.

Thase, M., & Rush, A. 1997. When at first you don't succeed: Sequential strategies for antidepressant nonresponders. Journal of Clinical Psychiatry 58, 23-29.

Thoenen, H. 1995. Neurotrophins and neuronal plasticity. Science 270, 593-598.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. 2009. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology 204, 361-373.

Tsankova, N., Berton, O., Renthal, W., Kumar, A., Neve, R., & Nestler, E. 2006. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nature Neuroscience 9, 519-525.

Valverde, O., & Torrens, M. 2012. CB1 receptor-deficient mice as a model for depression. Neuroscience 204, 193-206.

Verhagen, M., van der Meij, A., van Deurzen, P. A., Janzing, J. G., Arias-Vasquez, A., Buitelaar, J. K., & Franke, B. 2008. Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: Effects of gender and ethnicity. Molecular Psychiatry 15, 260-271.

Vogel, G., Neill, D., Hagler, M., & Kors, D. 1990. A new animal-model of endogenous-depression - a summary of present findings. Neuroscience and Biobehavioral Reviews 14, 85-91.

Vollenweider, F. X., & Kometer, M. 2010. OPINION the neurobiology of psychedelic drugs: Implications for the treatment of mood disorders. Nature Reviews Neuroscience 11, 642-651.

Vollmayr, B., & Henn, F. 2003. Stress models of depression. Clinical Neuroscience Research 3, 245-251.

Wahlsten, D., Metten, P., Phillips, T., Boehm, S., Burkhart-Kasch, S., Dorow, J., Doerksen, S., Downing, C., Fogarty, J., Rodd-Henricks, K., Hen, R., McKinnon, C., Merrill, C., Nolte, C., Schalomon, M., Schlumbohm, J., Sibert, J., Wenger, C., Dudek, B., & Crabbe, J. 2003. Different data from different labs: Lessons from studies of gene-environment interaction. Journal of Neurobiology 54, 283-311.

Walsh, R., & Cummins, R. 1976. Open-field test - critical-review. Psychological Bulletin 83, 482-504.

Willner, P. 2005. Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52, 90-110.

Willner, P., & Mitchell, P. 2002. The validity of animal models of predisposition to depression. Behavioural Pharmacology 13, 169-188.

Willner, P., & Mitchell, P.J. 2006. Animal Models of Depression, in Animal Models of Neuropsychiatric Diseases, edit. Koch, M., Imperial College Press, London.

Wisner, K. L., Sit, D. K. Y., Hanusa, B. H., Moses-Kolko, E. L., Bogen, D. L., Hunker, D. F., Perel, J. M., Jones-Ivy, S., Bodnar, L. M., & Singer, L. T. 2009. Major depression and antidepressant treatment: Impact on pregnancy and neonatal outcomes. American Journal of Psychiatry 166, 557-566.

World Health Organization (WHO). 2001. The World Health Report 2001, Mental Health: New understanding, New Hope.

World Health Organization (WHO). 2010. International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) Version for 2010.

World Health Organization (WHO). 1994. The Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death 10th edition (ICD-10).

World Health Organization (WHO). 1948. The Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death 6th edition (ICD-6).

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jonsson, B., Olesen, J., Allgulander, C., Alonso, J., Faravelli, C., Fratiglioni, L., Jennum, P., Lieb, R., Maercker, A., van Os, J., Preisig, M., Salvador-Carulla, L., Simon, R., & Steinhausen, H. C.. 2011. The size and burden of mental disorders and other disorders of the brain in Europe 2010. European Neuropsychopharmacology 21, 655-679.

Wu, X., & Castrén, E. 2009. Co-treatment with diazepam prevents the effects of fluoxetine on the proliferation and survival of hippocampal dentate granule cells. Biological Psychiatry 66, 5-8.

Yu, H., Wang, D. D., Wang, Y., Liu, T., Lee, F. S., & Chen, Z. Y. 2012. Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. The Journal of Neuroscience 32, 4092-4101.

Zarate, C. A., Jr., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., Charney, D. S., & Manji, H. K. 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Archives of General Psychiatry 63, 856-864.

Zetterstrom, T., Pei, Q., & Grahame-Smith, D. 1998. Repeated electroconvulsive shock extends the duration of enhanced gene expression for BDNF in rat brain compared with a single administration. Molecular Brain Research 57, 106-110.

Zörner, B., Wolfer, D. P., Brandis, D., Kretz, O., Zacher, C., Madani, R., Grunwald, I., Lipp, H. P., Klein, R., Henn, F. A., Gass, P. 2003. Forebrain-specific trkB-receptor knockout mice: Behaviorally more hyperactive than "depressive". Biological Psychiatry 54, 972-982.

Zueger, M., Urani, A., Chourbaji, S., Zacher, C., Roche, M., Harkin, A., & Gass, P. 2005. Olfactory bulbectomy in mice induces alterations in exploratory behavior. Neuroscience Letters 374, 142-146.