ACTIVATION OF THE TRKB NEUROTROPHIN RECEPTOR BY ANTIDEPRESSANT DRUGS

HANNA ANTILA

Neuroscience Center
&
Division of Pharmacology and Pharmacotherapy
Faculty of Pharmacy
University of Helsinki
&
Doctoral Programme Brain & Mind

ACADEMIC DISSERTATION

To be presented for public examination with the permission of the Faculty of Pharmacy of the University of Helsinki at University of Helsinki Main Building, Auditorium XII, on 14th of September 2016 at 12 o'clock noon

Supervisors

Docent Tomi Rantamäki, PhD
Department of Biosciences
University of Helsinki
Finland

Professor Eero Castrén, MD, PhD
Neuroscience Center
University of Helsinki
Finland

Reviewers

Associate professor Annakaisa Haapasalo, PhD
Department of Neurobiology
University of Eastern Finland
Finland

Docent Mikko Airavaara, PhD (pharm.)
Institute of Biotechnology
University of Helsinki
Finland

Opponent

Professor Moses Chao, PhD
Skirball Institute of Biomolecular Medicine
New York University Langone Medical Center
New York, United States of America

Custos

Professor Raimo Tuominen, MD, PhD
Division of Pharmacology and Pharmacotherapy
Faculty of Pharmacy
University of Helsinki
Finland

ISBN 978-951-51-2424-1 (paperback)
ISBN 978-951-51-2425-8 (PDF)
ISNN 2342-3161 (paperback)
ISNN 2342-317X (PDF)

Hansaprint
Helsinki, Finland 2016

In memory of
Mumma and Matti

CONTENTS

ABSTRACT

Major depressive disorder is one of the most significant causes of disability worldwide. Currently, the main treatment options for depression are psychotherapy and antidepressant drugs that pharmacologically target the monoamine systems – such as serotonin transporter (SERT) blocker fluoxetine. It has been hypothesized that impairments in synaptic function and plasticity, caused for example by stress, could underlie the manifestation of depression. Moreover, in rodent models chronic treatment with antidepressant drugs has been shown to enhance plasticity of the adult brain via brain-derived neurotrophic factor (BDNF). The effects of BDNF mediated via its receptor tropomyosin receptor kinase B (TrkB) promote synapse function and thus could facilitate recovery from depression. Interestingly, antidepressant drugs with different main pharmacological targets seem to share the ability to activate the TrkB receptor, however, the mechanisms how antidepressant drugs activate TrkB are not known.

The delayed onset of action and limited therapeutic efficacy of antidepressant drugs has promoted interest toward finding more rapid-acting and effective treatment options for depression. Electroconvulsive therapy has been the treatment of choice for treatment resistant depressed patients, however, side effects and the disrepute among general public has limited its use. Recently, subanesthetic doses of dissociative anesthetic ketamine have been shown to rapidly alleviate depression symptoms in depressed patients who do not respond to conventional antidepressant drugs. The effects of ketamine on mood appear already couple of hours after single intravenous infusion and last for about one week. Ketamine has been shown to induce mammalian target of rapamycin (mTOR) via BDNF-TrkB signaling, rapidly promote synaptogenesis and alter neural network function. Furthermore, in small human studies another anesthetic isoflurane has rapidly alleviated symptoms of depressed patients. Yet, the potential of isoflurane in the treatment of depression has not been studied in large clinical trials.

Since TrkB receptor is involved in regulation of synaptic plasticity, drugs that act as agonists or positive allosteric modulators of TrkB could be potentially beneficial in the treatment of CNS disorders characterized by impaired plasticity. The first aim of our studies was to develop a platform suitable for high-throughput screening of compounds regulating TrkB activity. We developed an *in situ* ELISA (enzyme-linked immunosorbent assay) method that detects phosphorylated TrkB receptors from cultured cells. The main advantage of the *in situ* ELISA compared to conventional ELISA is that the cells are cultivated directly on the ELISA plate making the additional transfer step of the cellular material from the cell culture plate to the ELISA plate unnecessary. To further validate the *in situ* ELISA method, we conducted a proof-of-concept screening of a small chemical library and found several compounds that dose-dependently activated TrkB receptor or inhibited BDNF-induced TrkB activation.

The second aim was to examine the mechanism how the antidepressant drugs activate TrkB. Interestingly, we found that antidepressant drugs activate TrkB independently of BDNF. Moreover, SERT, the main pharmacological target of fluoxetine, was not required for the fluoxetine-induced TrkB activation. Furthermore, the antidepressant-induced TrkB activation was developmentally regulated. The ability of antidepressants to activate TrkB appeared around postnatal day 12. Interestingly, at this same developmental timepoint (P12) the ability of BDNF to activate TrkB decreased dramatically.

Finally, we aimed to characterize the neurobiological basis for the possible antidepressant effects of isoflurane. We found that brief isoflurane anesthesia rapidly and transiently activated the TrkB-mTOR signaling and produced antidepressant-like behavioral response in the forced swim test in a TrkB-dependent manner. Single isoflurane treatment also produced an antidepressant-like phenotype in behavioral paradigms that normally require chronic treatment with conventional antidepressant drugs, suggesting that isoflurane may have rapid antidepressant effects similar to ketamine. Moreover, isoflurane facilitated hippocampal long-term potentiation when measured 24 hours after the treatment and affected the general neural network function by increasing activity of the parvalbumin-positive inhibitory interneurons in the hippocampus.

In conclusion, our results improve the understanding of the mechanism of action of conventional antidepressant drugs and provide plausible neurobiological basis for the antidepressant effects of isoflurane. Our findings also support examining further the potential of anesthetics in the treatment of depressed patients who do not respond to the current treatment options.

TIIVISTELMÄ

Masennus on merkittävä kansanterveydellinen ongelma, jonka kehittymiseen on ehdotettu liittyvän esimerkiksi pitkäkestoisen stressin aiheuttamia häiriöitä aivojen muovautuvuudessa ja hermosoluyhteyksien toiminnassa. Masennuksen hoito perustuu pääasiassa psykoterapiaan ja masennuslääkkeisiin. Kaikki kliinisessä käytössä olevat masennuslääkkeet vaikuttavat aivojen monoaminergisiin järjestelmiin, ja masennuslääkkeiden vaikutusten onkin pitkään ajateltu välittyvän yksinomaan näiden järjestelmien kautta. Eläinkokeissa masennuslääkkeiden on havaittu voimistavan aivojen muovautuvuutta aivoperäisen hermokasvutekijän (BDNF) välityksellä. BDNF osallistuu hermoyhteyksien toiminnan säätelyyn TrkB (tropomyosin receptor kinase B) –reseptorin välityksellä ja masennuslääkkeiden vaikutukset BDNF-TrkB –signalointiin saattavatkin osaltaan edesauttaa masennuksesta toipumista. Kyky aktivoida TrkB-reseptoria vaikuttaisikin olevan yhteinen ominaisuus muutoin eri kohdemolekyyleihin vaikuttavilla masennuslääkkeillä. Tarkempaa mekanismia masennuslääkkeiden aikaansaaman TrkB-reseptorin aktivaation taustalla ei kuitenkaan vielä tunneta.

Osa masennuspotilaista ei riittävästi hyödy nykyisistä masennuslääkkeistä ja masennuslääkkeiden terapeuttiset vaikutukset ilmenevät viiveellä. Tämän vuoksi masennuksen hoitoon yritetään jatkuvasti kehittää uusia, tehokkaampia ja nopeammin toimivia lääkkeitä. Sähköhoito (ECT) on tällä hetkellä käytössä olevista hoitomuodosta tehokkain, mutta se aiheuttaa muistihäiriöitä ja sen käyttöä rajoittavat lisäksi voimakkaat ennakkoluulot. Viime aikoina nukutusaine ketamiinin on havaittu nopeasti (muutamassa tunnissa) lievittävän masennusoireita muihin hoitoihin reagoimattomilla potilailla. Ketamiinin masennusta lievittävien vaikutusten taustalla on esitetty olevan sen kyky aktivoida BDNF-TrkB-mTOR (mammalian target of rapamycin) –signalointia, nopeasti lisätä uusien hermosoluyhteyksien määrää ja muuttaa hermoverkkojen toimintaa. Ihmisillä tehdyissä tutkimuksissa on lisäksi havaittu toisen nukutusaineen, isofluraanin, lievittävän masennusoireita nopeasti. Isofluraanin tehoa masennuksen hoidossa ei ole kuitenkaan vielä tutkittu laajemmissa kliinisissä tutkimuksissa.

TrkB-reseptorin välittämiä plastisuusvaikutuksia voitaisiin mahdollisesti hyödyntää myös muiden keskushermostosairauksien kuin masennuksen hoidossa. Tarkoituksenamme olikin kehittää menetelmä, jonka avulla voitaisiin seuloa TrkB-reseptoriin vaikuttavia uusia molekyylejä. Kehitimme *in situ* ELISA (enzyme-linked immunosorbent assay) –menetelmän, joka tunnistaa TrkB-reseptorin fosforyloituneen eli aktivoituneen muodon solunäytteistä. *In situ* ELISA eroaa tavallisesta ELISAsta siten, että solut kasvatetaan suoraan ELISA-levyllä. *In situ* ELISA soveltuu myös suurten kirjastojen seulomiseen, koska siinä vältytään työläältä näytteiden siirrolta soluviljelylevyltä ELISA-levylle. Osoittaaksemme menetelmän soveltuvuuden seulomistarkoitukseen, seuloimme 2000 yhdisteen kirjaston ja identifioimme useita Trk-reseptoria aktivoivia sekä BDNF:n vaikutuksia estäviä yhdisteitä.

Tämän jälkeen tutkimme, miten masennuslääkkeet saavat aikaan TrkB-reseptorin aktivoitumisen hiiressä. Yllättäen havaitsimme, että masennuslääkkeet aktivoivat TrkB-reseptorin ilman BDNF:ä, transaktivaation välityksellä. Lisäksi fluoksetiinin aikaansaama TrkB-reseptorin aktivoituminen tapahtui ilman, että sen täytyi sitoutua pääasialliseen kohdemolekyylinsä serotoniinitransportteriin (SERT). Masennuslääkkeiden aikaansaama TrkB-reseptorin aktivoituminen oli myös

kehityksellisesti säädeltyä ilmeten vasta 12 päivän ikäisillä hiirillä. Tässä samassa kehitysvaiheessa BDNF:n aikaansaama TrkB-reseptorin aktivoituminen taas väheni merkittävästi.

Lopuksi selvitimme, minkälaisten neurobiologisten prosessien kautta nukutusaine isofluraanin mahdolliset masennusta lievittävät vaikutukset voisivat välittyä. Tutkimuksemme osoittivat isofluraanin aktivoivan hiiressä TrkB-mTOR –signalointia ja aiheuttavan pakotetussa uintitestissä (forced swim test) masennuslääkkeen kaltaisen käyttäytymisvasteen, joka välittyi TrkB-reseptorin kautta. Lisäksi yksi isofluraani-nukutus sai aikaan masennuslääkkeen kaltaisen käyttäytymisvasteen testeissä, jotka normaalisti vaativat pitkäaikaisen käsittelyn masennuslääkkeillä. Tämä osoittaakin, että isofluraani saattaisi toimia ketamiinin tapaan nopeavaikutteisena masennuslääkkeenä. Kestotehostuminen (LTP, long-term potentiation) voimistui ja parvalbumiinia ilmentävien estävien välineuronien aktiivisuus lisääntyi hippokampuksessa 24 tuntia isofluraani-käsittelyn jälkeen, osoittaen että yhdellä nukutuksella on pitkäkestoisia vaikutuksia myös hermoverkkojen toimintaan.

Tutkimustuloksemme tuovat lisää tietoa masennuslääkkeiden vaikutusmekanismeista ja voivat selittää, minkä vuoksi isofluraanilla saattaa olla masennusoireita lievittäviä vaikutuksia. Lisäksi tulostemme perusteella nukutusaineiden käyttökelpoisuutta muihin hoitoihin reagoimattomien masennuspotilaiden hoidossa kannattaisi tutkia lisää.

ABBREVIATIONS

5-HT	5-hydroxytryptamine, serotonin
AD	Antidepressant drug
AKT	Protein kinase B
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	Brain-derived neurotrophic factor
BrdU	Bromodeoxyuridine
CA	Cornu ammonis (in hippocampus)
CaMKII	Calcium/calmodulin-dependent protein kinase II
CNS	Central nervous system
CREB	Cyclic AMP response element-binding protein
DAG	Diacylglycerol
DNA	Deoxyribonucleic acid
ECT	Electroconvulsive therapy
eEF2	Eucaryotic elongation factor 2
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinase
FST	Forced swim test
GABA	Gamma-aminobutyric acid
GPCR	G-protein coupled receptor
GSK3β	Glycogen synthase kinase 3 beta
HFS	High frequency stimulation
HNK	Hydroxynorketamine
IgG	Immunoglobulin G
IP_3	Inositol trisphosphate
LRR	Leucine-rich repeat
LTD	Long-term depression
LTP	Long-term potentiation
MAO	Monoamine oxidase
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin
NBQX	2,3- Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NT-3	Neurotrophin 3
NT-4	Neurotrophin 4
PACAP	Pituitary adenylate cyclase-activating polypeptide
$p75^{NTR}$	P75 neurotrophin receptor
PC	Pro-convertase
PFC	Prefrontal cortex
PI3k	Phosphoinositide 3-kinase
PKC	Protein kinase C
PLCγ	Phospholipase C-gamma
PNS	Peripheral nervous system
PP1	Protein phosphatase 1
proBDNF	Pro-form of Brain-derived neurotrophic factor
qPCR	Quantitavive real-time polymerase chain reaction
Shc	Src-homology 2 domain-containing
SERT	Serotonin transporter
SGZ	Subgranular zone of hippocampus
SSRI	Selective serotonin reuptake inhibitor
SVZ	Subventricular zone
tPA	Tissue plasminogen activator
Trk	Tropomyocin receptor kinase
VEP	Visually evoked potential
VTA	Ventral tegmental area
Y	Tyrosine

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I **Antila H**, Autio H, Turunen L, Harju K, Tammela P, Wennerberg K, Yli-Kauhaluoma J, Huttunen H, Castrén E, Rantamäki T: Utilization of in situ ELISA method for examining TrkB receptor phosphorylation in cultured cells. J Neurosci Methods, 2013 Nov 12;222C,142-146

II Rantamäki T, Vesa L*, **Antila H***, di Lieto A, Tammela P, Schmitt A, Lesch KP, Rios M, Castrén E: Antidepressant drugs transactivate TrkB neurotrophin receptors in the adult rodent brain independently of BDNF and monoamine transporter blockade. PloSOne 2011;6(6):e20567 *Equal contribution

III Di Lieto A, Rantamäki T, Vesa L, Yanpallewar S, **Antila H**, Lindholm J, Rios M, Tessarollo L, Castrén E: The responsiveness of TrkB to BDNF and antidepressant drugs is differentially regulated during mouse development. PLoS One 2012;7(3):e32869

IV **Antila H**, Casarotto P, Popova D, Sipilä P Guirado R, Kohtala S, Ryazantseva M, Vesa L, Lindholm J, Yalcin I, Sato V, Nurkkala H, Lemprière S, Cordeira J, Autio H, Kislin M, Rios M, Joca S, Khiroug L, Lauri S, Varjosalo M, Grant SGN, Taira T, Castrén E, Rantamäki T : TrkB signaling underlies the rapid antidepressant effects of isoflurane. Submitted manuscript.

1. INTRODUCTION

Major depression is the largest contributor to the worldwide disease burden when measured as years lost to disability. This occurs primarily because depression can persist for many years and a large number of individuals (~350 million) suffer from it (Smith, 2014). Antidepressant drugs (AD) and psychotherapy are the main treatment options for depression, however, significant amount of depressed patients do not respond to the treatment.

It has been suggested that stress-induced changes in neuronal connectivity and resulting disturbances in network function are important factors in the pathophysiology of depression (Castrén & Hen, 2013). Brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin receptor kinase B (TrkB) are known to regulate neuronal plasticity, pathology of depression and the mechanism of action of ADs. ADs promote the expression of BDNF and activate its receptor TrkB (Nibuya et al., 1995a; Rantamäki et al., 2007; Saarelainen et al., 2003). Since BDNF and TrkB are involved in the regulation of neuronal excitability, cell survival and plasticity, the ability of ADs to increase their expression has been suggested to underlie the therapeutic effects of ADs.

However, delayed onset of action and poor efficacy of ADs limits their therapeutic use. Significant attempts to find novel drugs for treatment of depression have been conducted. Electroconvulsive therapy (ECT) remains the treatment of choice for patients unresponsive to multiple trials with different ADs and psychotherapy. Recently, however, a subanesthetic dose of ketamine has been shown to rapidly alleviate depression symptoms and to reduce suicidal ideation in treatment resistant depressed (TRD) patients and BDNF-TrkB signaling has been shown to be involved in these therapeutic effects of ketamine (Autry et al., 2011; Berman et al., 2000; Zarate CA et al., 2006). Ketamine, however, may produce hallucinogenic effects and has significant abuse potential, thus it is not an optimal drug to replace the conventional antidepressant treatments. Intriguingly, volatile anesthetic isoflurane has been shown to relieve depression symptoms of TRD patients as effectively as ECT but without the cognitive side effects characterized with ECT (Langer et al., 1985, 1995; Weeks et al., 2013). Altogether these preliminary findings encourage investigating further the antidepressant potential of anesthetics in animal models and human patients.

In this thesis the role of BDNF and TrkB in the central nervous system and in the effects of antidepressant drugs will be discussed. In the experimental section we have developed tools to screen for novel TrkB activators, investigated the mechanisms of antidepressant-induced TrkB activation, and dissected the neurobiological basis for the antidepressant effects of isoflurane.

2. REVIEW OF THE LITERATURE

2.1 BDNF AND TRKB

2.1.1 Discovery of neurotrophins

The discovery of the first neurotrophin, the nerve growth factor (NGF), and the characterization of its effects on the survival and target innervation of subpopulation of neurons in the peripheral nervous system (PNS) were done by Rita Levi-Montalcini, Victor Hamburger and Stanley Cohen (Levi-Montalcini, 1987). These findings were seminal to the idea that the non-neural target tissue secretes factors that affect the survival of neurons innervating it; a concept nowadays known as the neurotrophic hypothesis (Bothwell, 2014). According to the hypothesis, now supported by massive amounts of experimental data, neurotrophins are released from the target tissue in very limited amounts allowing the survival of only a small number of neurons during early development (Bothwell, 2014).

NGF alone was not sufficient to understand all of the neurotrophic effects detectable during early development, thus brain-derived neurotrophic factor (BDNF) was discovered. As the name implies, BDNF was first extracted from pig brain tissue (Barde et al., 1982), indicating that neurotrophins also act at the level of central nervous system (CNS). The characterization of the other members of the neurotrophin family - Neurotrophin-3 (NT-3) and Neurotrophin 4 (NT-4) - was facilitated by the technical development in molecular biology, especially the discovery of polymerase chain reaction (PCR), since the gene structures of already known neurotrophins could be exploited to find similar proteins (Hallböök et al., 1991; Maisonpierre et al., 1990a). Currently the mammalian neurotrophin family consists of four structurally and functionally similar members: NGF, BDNF, NT-3 and NT-4.

The signaling effects of neurotrophins are mediated via the p75 neurotrophin receptor ($p75^{NTR}$) and the Trk receptor tyrosine kinases. All the neurotrophins can activate the signaling via $p75^{NTR}$, but their binding affinities to the Trk receptors are more specific: NGF binds to TrkA, BDNF and NT-4 to TrkB and NT-3 to TrkC (Klein et al., 1991a, 1991b, 1992; Lamballe et al., 1991).

Although all neurotrophins act as target-derived survival factors in the PNS during the development, their functions in the CNS appear much more diverse and complex. The literature review focuses on BDNF since it is the most abundant neurotrophin in the brain and it has been linked with the pathophysiology of depression, mechanism of action of antidepressant drugs and brain plasticity. These issues and the basic neurobiology of BDNF and TrkB will be introduced in the subsequent sections.

2.1.2 Bdnf gene structure and regulation

The *bdnf* gene consists of eight 5' non-coding, regulatory exons and one 3' coding exon (exon IX) (Aid et al., 2007) (Fig 1A). The complexity of the *bdnf* gene allows precise temporal and spatial regulation of BDNF expression. The exons are controlled by distinct promoters that can be differentially regulated. Importantly however, all the transcripts eventually encode the same BDNF protein. Various stimuli can activate different transcription factors, which can then bind to different promoter regions of the *bdnf* exons

resulting in transcription of specific *bdnf* transcripts (West et al., 2014). Furthermore, the transcripts have distinct localization, stability and translational regulation inside the cell. Deletion of promoter regions IV and VI from the *bdnf* gene results in a robust reduction of *bdnf* expression in the hippocampus and prefrontal cortex (PFC), whereas deletion of promoter regions I and II reduces BDNF expression in the hypothalamus (Maynard et al., 2015). These studies support the area specific roles of the *bdnf* promoters.

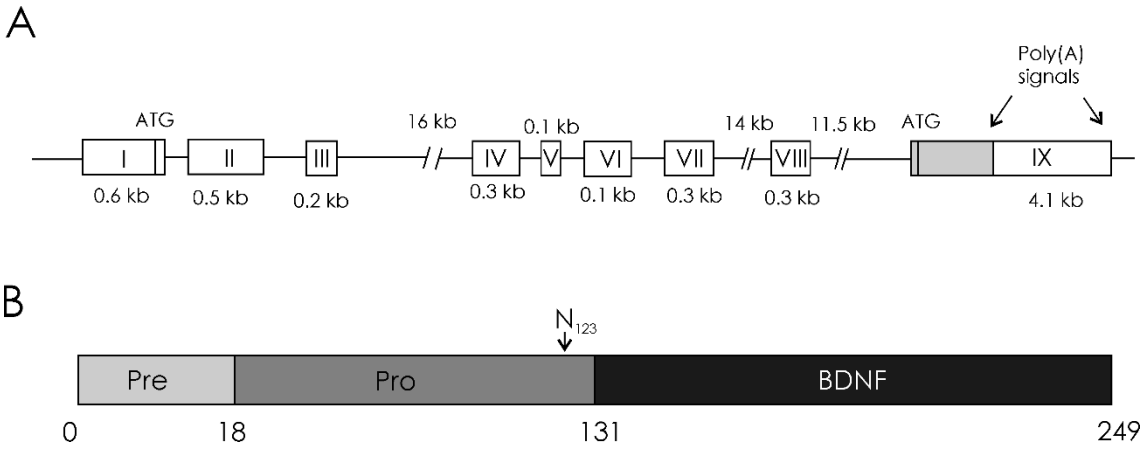

Fig 1. A Structure of the *bdnf* gene (modified from Aid et al. 2007). The grey are represents the protein coding region. **B** Structure of the BDNF protein showing the different domains and the N-glycosylation site.

The distinct effects of the individual *bdnf* transcripts is further supported by the altered behavior and serotonergic functions of mice in which BDNF production is disrupted from the promoters I, II, IV or VI (Maynard et al., 2015). For example, exon I and II specific knock out mice show increased aggressive behavior whereas mice lacking exon IV or VI do not. It has been previously shown however, that *bdnf* deletion from the ventromedial and dorsomedial hypothalamus does not cause aggressive behavior (Unger et al. 2007), suggesting that the effects of BDNF on hypothalamic circuits regulating aggression are derived from developmental abnormalities in the network formation. Exon I knockouts show increased expression of the serotonin (5-HT) transporter (SERT), the $5HT_{2A}$ receptor and parvalbumin in the prefrontal cortex (Maynard et al., 2015). Exon IV and VI knockouts have reduced gene expression of markers for GABAergic interneurons, e.g. parvalbumin (only in exon IV knockout mice), cortistatin and somatostatin in the PFC.

Neuronal activity strongly regulates *bdnf* transcription. Specifically the expressions of *bdnf* exons I, II and IV are regulated in an activity-dependent manner (West et al., 2014). *Bdnf* exon IV expression is strongly induced by elevations in intracellular calcium concentration (Hong et al., 2008; Tao et al., 1998). Transcription factor cAMP response element binding protein (CREB) is an important mediator of the activity- and calcium-dependent transcription of *bdnf* (Chen and Russo-Neustadt, 2009; Tao et al., 1998). Indeed, mutation in the CREB-binding site in the *bdnf* promoter IV impairs the activity-dependent *bdnf* transcription (Hong et al., 2008). In addition, calcium-responsive

transcription factor (CaRF) regulates *bdnf* exon IV transcription in neurons in an activity-dependent manner (Tao et al., 2002).

Potassium chloride and kainic acid are widely used agents to increase excitatory neurotransmission and neuronal activity. Stimulation with potassium chloride or kainic acid increased *bdnf* expression AMPAR- (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) and calcium-dependently in cultured neurons (Zafra et al., 1990). Even though NMDA (N-methyl-D-aspartate) receptors were not required for these effects, NMDA receptor -mediated activity is important in more physiological conditions since NMDA antagonists can prevent increases in *bdnf* messenger ribonucleic acid (mRNA) normally observed during maturation in neuronal cultures (Zafra et al. 1991). Most importantly, sensory stimuli, or lack thereof, strongly regulate *bdnf* transcription *in vivo*. For example, dark rearing decreases *bdnf* expression in the rat visual cortex but re-exposure to light quickly restores *bdnf* mRNA levels (Castrén et al., 1992). Moreover, increased neuronal activity following physical exercise, drug treatment, or seizures increase *bdnf* transcription (Chen and Russo-Neustadt, 2009; Nibuya et al., 1995a; Russo-Neustadt et al., 1999; Zafra et al., 1991). In contrast, pharmacologically-induced neuronal inhibition or reduction of neuronal excitability decreases *bdnf* mRNA levels *in vivo* (Zafra et al. 1991). *Bdnf* transcription also appears to be stress-responsive since acute and chronic stress reduce hippocampal *bdnf* mRNA levels, while increasing *bdnf* expression in the hypothalamus and pituitary (Smith et al., 1995a, 1995b).

2.1.3 Expression and localization of BDNF

BDNF protein is widely expressed in the brain, with highest levels detected in the cerebral cortex and the hippocampus (Conner et al., 1997; Ernfors et al., 1992; Hofer et al., 1990). The expression of BDNF was initially thought to be limited to neurons (Zafra et al., 1990) but it is now widely accepted that brain microglia – particularly activated microglia – can take up and release BDNF as well (Parkhurst et al., 2013). BDNF seems to be expressed mainly in principal glutamatergic neurons but not in inhibitory interneurons (Gorba and Wahle, 1999; Kuczewski et al., 2009). Although certain areas of the brain essentially lack *bdnf* mRNA expression, transported BDNF protein can be detected in these areas (Altar et al., 1997).

BDNF expression in the brain is strongly regulated during development. The expression of *bdnf* mRNA gradually increases during early postnatal life, plateauing in rodents around 3 weeks of age (Maisonpierre et al., 1990b; Rauskolb et al., 2010). In the human dorsolateral prefrontal cortex (dlPFC*) bdnf* expression increases about one-third from postnatal levels to adulthood peaking during early adulthood (around 22 years of age) (Webster et al., 2002). Importantly, the peak of *bdnf* expression in the dlPFC is seen at the age when the structural and functional maturation of the PFC occurs. In the hippocampus *bdnf* mRNA levels seem to stay relatively constant during human life span, including the aging brain (Webster et al., 2006).

In cultured neurons BDNF protein is found in the soma, as well as also in axons and in dendrites (Adachi et al., 2005; Conner et al., 1997; Kohara et al., 2001). *In vivo* the dendritic expression of *bdnf* mRNA has been demonstrated in apical dendrites of hippocampal CA1 neurons (An et al., 2008; Tongiorgi et al., 2004). BDNF protein has been primarily located in dense-core vesicles of the presynaptic terminals of excitatory neurons (Dieni et al., 2012). These partially controversial findings of *in vitro* versus *in*

vivo BDNF localization may be explained by the differential and complex regulatory processes of synapse formation and intracellular trafficking of neurons in the adult brain compared to neuronal cultures.

The retrograde transport of neurotrophins in the PNS is the basis for the neurotrophic hypothesis, where survival of the innervating neurons is regulated by the neurotrophin released from the target tissue in a constitutive manner. Although the situation is more complex in the brain, retrograde transport of BDNF has been demonstrated for example in the eye, from where BDNF could be transported into the isthmo-optic nucleus (von Bartheld et al., 1996). Striatal infusion of BDNF resulted in retrograde BDNF transport to brain regions known to project to striatum e.g. thalamic areas and substantia nigra pars compacta (Mufson et al., 1994). Application of BDNF to dendrites but not to axons induced immediate early gene expression (c-fos, Arc) in the soma; indicating that BDNF-induced signals from dendrites are conveyed to the soma (Cohen et al., 2011).

One of the first studies showing anterograde transport of BDNF was done by Zhou et al. in primary sensory neurons (Zhou and Rush, 1996). BDNF was shown to be transported both retrogradely and anterogradely, functioning as a modulator of synaptic transmission or as a trophic factor for the organs that the neurons were innervating. Kohara et al. (2001) demonstrated the anterograde axonal transport of BDNF in cultured cortical neurons. Follow-up studies from the same group showed that the transport of BDNF in axons is mainly anterograde, whereas in dendrites BDNF seemed to be not moving at all or moving back and forth in a slower fashion (no clear retrograde transport) (Adachi et al., 2005). In the striatum BDNF protein, but not mRNA is present; indicating that BDNF is transported to the striatum anterogradely from the cortex by projection neurons (Kolbeck et al., 1999). In the nucleus accumbens *bdnf* mRNA levels are low and BDNF protein is transported there mainly from the ventral tegmental area (Altar et al., 1997; Conner et al., 1997; Horger et al., 1999). In BDNF knockout animals the number of parvalbumin-expressing neurons in the striatum was reduced at two weeks of age suggesting that the BDNF transported to the area is regulating the maturation or survival of this neuronal population (Altar et al., 1997). Noradrenergic neurons projecting to the cortex were shown to express BDNF and transport it anterogradely to the cortex to regulate the survival of the target neurons (Fawcett et al., 1998).

2.1.4 Processing and secretion of BDNF protein

BDNF is synthesized as pre-proBDNF, a precursor protein that is further processed in the endoplasmic reticulum to proBDNF (Greenberg et al., 2009) (Fig 1B). The proBDNF isoform is then N-glycosylated and glycosulfated (Mowla et al., 2001). The glycosylation increases the stability of proBDNF during processing and subcellular trafficking. The pro-domain participates in the proper folding and intracellular sorting of BDNF (Brigadski et al., 2005; Lee et al., 2001b). The translated protein is further processed in the Golgi and *trans*-Golgi network and directed to synaptic vesicles for release. The proBDNF isoform can be cleaved to produce the mature form of BDNF (mBDNF) inside the cell in *trans*-Golgi network or *post*-Golgi compartments by pro-convertases and furin, or outside the cell by matrix metalloproteinases (MMP-7) or plasmin (Lee et al., 2001b; Pang et al., 2004; Seidah et al., 1996). The efficacy of proBDNF cleavage to mBDNF seems to vary during development. Postnatally and during adolescence both proBDNF and mBDNF are expressed at similar levels, but in the adult brain the mature form dominates. Furin is the main cleavage enzyme of the constitutive pathway guiding

the neurotrophins to the constitutive pathway (for example in fibroblasts) (Mowla et al., 1999). The processing enzymes in the regulated and constitutive secretion pathways are different and the cleavage of BDNF occurs in different subcellular compartments. In CNS neurons BDNF is primarily directed to the regulated secretion pathway where furin is not the essential enzyme for the processing of BDNF in the CNS neurons (Mowla et al., 1999). In neurons the pro-convertases (PC) cleave the neurotrophin precursors inside immature secretory vesicles in the *trans*-Golgi and are involved in the processing of neurotrophins in the regulatory pathway. In the hippocampus and amygdala, PC7, an enzyme of the proprotein convertase family, is involved in the intracellular processing of proBDNF to BDNF (Wetsel et al., 2013).

In non-neuronal tissue BDNF appears to be constitutively released but in the brain the secretion is mainly regulated through activity-dependent mechanisms (Mowla et al., 1999). BDNF is stored in dense-core vesicles in the membrane fraction of synaptic terminals (Fawcett et al., 1997). *In vitro* BDNF can be released both pre- and postsynaptically and in cultured hippocampal neurons BDNF has been detected in dendrites and axons (Adachi et al., 2005; Jakawich et al., 2010; Matsuda et al., 2009). Interestingly pre- and postsynaptic release of BDNF seems to require different patterns of stimulation. For example, the dendritic release of BDNF is dependent on calcium influxes *via* NMDA receptors and L-type voltage-gated calcium channels. Furthermore, BDNF can induce its own release *via* a positive feedback loop including stimulation of TrkB receptors, activation of phospholipase C-gamma (PLCγ) and mobilization of intracellular calcium stores (Canossa et al., 1997, 2001). Stimulation of metabotropic glutamate receptors can also activate PLCγ and induce calcium release from intracellular storages via inositol trisphosphate ($IP_{3)}$ resulting in BDNF release (Canossa et al., 2001). Interestingly, the effect of glutamate on BDNF release can be blocked with AMPA receptor antagonists but not NMDA receptor antagonists, and AMPA can increase BDNF release from hippocampal slices and cultured neurons.

In vivo BDNF is co-expressed with the cleaved pro-peptide in dense core vesicles presynaptically in the hippocampus, indicating that BDNF is cleaved inside the vesicles and possibly released together with the cleaved pro-domain (Dieni et al., 2012). Since there are enzymes that are able to cleave proBDNF to mBDNF also extracellularly, it has been debated whether proBDNF can be released from the neurons or if it is processed to mature BDNF in secretory vesicles before release. Lee et al. (2001b) suggested that proBDNF is released from endothelial cells and can then be processed by tissue plasminogen activator (tPA) and MMPs, and Chen et al. (2004) showed that proBDNF is primarily released from the PC12 cells but is then quickly cleaved outside the cell. In another study where proBDNF and mBDNF levels were measured in hippocampal neuron cultures from the cell lysate and medium (secreted), the authors found that in the presence of plasmin inhibitor α_2-antiplasmin and in the culture conditions where glial cell amount is reduced, proBDNF but not mBDNF is found in the medium, supporting the importance of extracellular cleavage of secreted proBDNF (Yang et al., 2009). When hippocampal neurons were infected with proBDNF expressing virus, proBDNF was secreted into the medium and the amount of proBDNF in the medium increased over time (Mowla et al., 1999). Also cultured cortical neurons were shown to release proBDNF into the medium (Teng et al., 2005). In a study by Matsumoto et al. (2008) the authors suggest that in hippocampal neurons endogenous proBDNF is processed into BDNF already intracellularly (Matsumoto et al., 2008). Altogether the

release of BDNF/proBDNF has been mainly studied in overexpression systems or non-neuronal cell lines using transfection of BDNF complementary DNA (cDNA), and Matsumoto et al. suggest that in these situations the cells may lack the machinery for proper processing of proBDNF or the capacity to process proBDNF to mature BDNF is saturated, which could lead to biased results. Thus, it is difficult to reliably measure BDNF secretion *in vivo*.

2.1.5 TrkB gene and mRNA

The BDNF receptor TrkB was first cloned from mouse brain tissue by virtue of its high sequence homology to the NGF receptor TrkA (Klein et al., 1989). The *TrkB* gene (NTRK2) is capable of producing multiple transcripts including the full-length catalytic tyrosine kinase receptor but also TrkB receptor variants that lack the catalytic kinase domain (Klein et al., 1990; Middlemas et al., 1991; Stoilov et al., 2002). The full-length TrkB receptor consists of an extracellular ligand-binding domain, a transmembrane anchoring domain and an intracellular domain that includes the highly conserved catalytic kinase domain (Klein et al., 1990). The truncated TrkB receptors (TrkB.T1 and TrkB.T2) share similar extracellular and transmembrane domains to the full-length receptor but have only a short intracellular part consisting of a unique sequence of amino acid residues. In the human brain there is no expression of TrkB.T2, however, an additional isoform, TrkB.Shc, is present and lacks the tyrosine kinase domain but contains the intracellular Shc site (Stoilov et al., 2002). In mouse a TrkB receptor isoform lacking the extracellular leucine rich repeats completely or in part has been identified (Ninkina et al., 1997).

The human TrkB gene is large, consisting of 24 exons that produce multiple mRNAs which can produce up to 10 different protein isoforms (Stoilov et al., 2002) (Fig 2A). Three isoforms, TrkB.FL, TrkB.T1 and TrkB.Shc are predominantly expressed at the protein level. The exons 6-15 of the *TrkB* gene encode the extracellular domain, the transmembrane domain, and a part of the juxtramembrane domain of the receptor. Exon 16 includes a stop codon and is part of the TrkB.T1. Exons 17-18 encode the intracellular juxtramembrane part and exon 19 is an alternative terminating exon involved in the TrkB.Shc. Exons 20-24 encode the tyrosine kinase domain and the PLCγ site of the receptor.

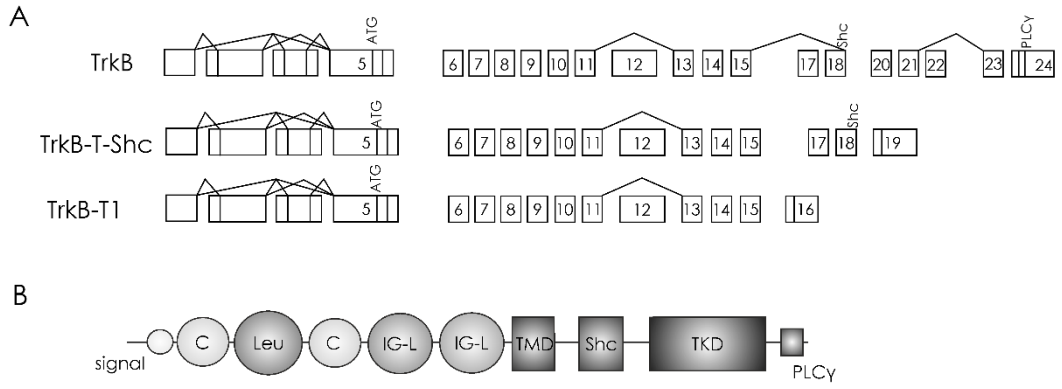

Fig 2. **A** Exon structures of the three predominant TrkB isoforms: Full-length TrkB, TrkB.Shc lacking the tyrosine kinase domain but containing the Shc binding site and TrkB.T1 lacking the intracellular domains. **B** TrkB protein domains; C, cysteine rich region; Leu, leucine rich region; IG-L, immunoglobulin like-domain; TMD, transmembrane domain; Shc, Shc binding site; TKD, tyrosine kinase domain; PLCγ, PLCγ binding site. Modified from. Luberg et al. 2010.

In humans, additional N-terminal truncated TrkB receptors have been identified (Luberg et al., 2010). These receptors lack the signal peptide that targets the receptors to the membrane and also the leucine-rich repeats (LRR) and one cysteine-rich domain from the extracellular domain of the receptor. The N-terminal truncated TrkB receptors can be phosphorylated even though they are not targeted to the membrane and most are unable to be activated by BDNF (Luberg et al., 2010).

2.1.6 Functional domains and post-transcriptional processing of TrkB

The extracellular domain of Trk receptors consists of a membrane-targeting signal peptide, two cysteine clusters that are located around LRRs followed by two immunoglobulin G (IgG)-like structures adjacent to the transmembrane domain (Schneider and Schweiger, 1991) (Fig 2B). The intracellular domains of TrkB include a tyrosine kinase domain and tyrosine motifs. Tyrosine phosphorylation controls the kinase activity of TrkB receptors and can regulate the binding of adaptor molecules to the receptor (Segal et al., 1996). The catalytic domain inside the tyrosine kinase domain is highly conserved among all the receptor tyrosine kinases (Klein et al., 1989; Lee et al., 2001a; Segal et al., 1996). The IgG-like domain adjacent to the transmembrane domain acts as a binding site for neurotrophins and determines the ligand specificity of the receptor (Urfer et al., 1995). Neurotrophin binding to this domain can induce activation of the catalytic tyrosine kinase domain of the receptor. The other IgG domain and the leucine- and cysteine-rich repeats also seem to participate in ligand binding either directly or by inducing conformational changes (Huang and Reichardt, 2003; Ninkina et al., 1997). In general, the LRRs are thought to participate in protein-protein interactions. Deletion of the LRRs prevents the ligand from binding to TrkB and blocks the survival enhancing effects of BDNF in serum-depleted NIH3T3 cells (Ninkina et al., 1997). Specifically the second LRR of TrkB seems to bind BDNF (Windisch et al., 1995). Co-expression of $p75^{NTR}$ can alter the extracellular sites of TrkB required for BDNF binding (Zaccaro et al., 2001). In the absence of $p75^{NTR}$ BDNF binds to the IgG-C2 domain of TrkB but when $p75^{NTR}$ and TrkB are expressed together, BDNF binding requires the LRR

and the cysteine 2 domains. Juxtamembrane domain (short sequence of about 80 amino acids that is located between the transmembrane and tyrosine kinase domain) of TrkB regulates the internalization of the receptor after ligand binding (Sommerfeld et al., 2000). The extracellular domain of TrkB can be N-glycosylated and the glycosylation of the receptor appears to increase during early development (Fryer et al., 1996; Haniu et al., 1995). Glycosylation is required for the localization of the Trk receptors to the cell membrane and can inhibit ligand-independent activation of the receptor (Watson et al., 1999a)

2.1.7 Expression and subcellular localization of TrkB

TrkB receptor is widely expressed in both the peripheral and central nervous systems. *In situ* hybridization analysis has shown that *trkB* transcripts are expressed in the cerebral cortex, hippocampus, thalamus, choroid plexus, granular layer of the cerebellum, brain stem and spinal cord (Klein et al., 1993). In the PNS *trkB* transcripts have been detected in the cranial ganglia, retina, ophthalmic nerve, vestibular system, multiple facial structures, submaxillary glands and dorsal root ganglia. TrkB protein is found in olfactory bulb, hippocampus, thalamus, hypothalamus, septum, basal ganglia, midbrain nuclei and cerebellum (Yan et al., 1997).

The expression patterns of the full-length and truncated TrkB receptors differ in the CNS. During early development TrkB.FL is the main receptor in the brain and is highly expressed in the dendrites; however, around postnatal day 10-15 (P10-P15) the expression of the TrkB.T1 receptor increases and exceeds the TrkB.FL especially in cortical areas (Fryer et al., 1996). In human prefrontal cortex the expression of TrkB.FL mRNA and protein peaks in toddlers and decreases slightly with aging, whereas the expression of the TrkB.T1 mRNA is regulated the opposite way with lowest expression levels in toddlers (Luberg et al., 2010). The TrkB.T1 protein expression seems to increase until teenage years after which it declines slightly. TrkB.Shc expression is low compared to the other two isoforms and the expression is reduced during aging compared to the expression levels in infants (Luberg et al., 2010).

Functional full-length TrkB receptors can be found in postsynaptic densities (Wu et al., 1996). TrkB receptors are found in axons and dendrites intracellularly and on the cell surface throughout development (Gomes et al., 2006). During maturation TrkB receptors localize to excitatory synapses in cortical neurons. The expression pattern of TrkB in interneurons and their responsiveness to neurotrophins varies during development (Gorba and Wahle, 1999). The TrkB receptor is not expressed by all types of interneurons but especially interneurons expressing parvalbumin coexpress TrkB and BDNF-induced TrkB activation can promote parvalbumin expression *via* phosphoinositide 3-kinase (PI3K) signaling in early developmental timepoints (Patz et al., 2004).

In cultured cells the truncated form of TrkB was heavily expressed on the cell surface in normal conditions but high levels of the full-length receptor were found in granular structures near the cytoplasm, suggesting that the majority of TrkB receptors are located inside the cell (Haapasalo et al., 2002). Similarly to BDNF, the synthesis, expression and intracellular transport of TrkB is regulated by neuronal activity (Merlio et al., 1993). Du et al. (2000) found that in cultured neurons TrkB receptors are mainly located in the cytoplasm and after electrical stimulation are recruited heavily to the membrane.

Apparently the receptors can be quickly translocated to the membrane from intracellular pools e.g. by BDNF stimulation or as a result of increased neuronal activity. In hippocampal neurons high frequency electrical stimulation induced surface expression of both the full-length and truncated TrkB receptors in a calcium- and calcium/calmodulin-dependent protein kinase II (CaMKII)-dependent manner (Du et al., 2000). Increase in calcium concentration leads to a cyclin-dependent kinase 5 (Cdk5) -dependent phosphorylation of the receptor at serine 478 which induces TrkB insertion into the cell membrane (Zhao et al., 2009). In cultured retinal ganglion cells and spinal motor neurons full-length TrkB receptors were inserted into the cell membrane after an increase in the amount of intracellular cyclic adenosine monophosphate (cAMP) (Meyer-Franke et al., 1998). TrkB can also be inserted to the cell membrane after intracellular transactivation (Puehringer et al., 2013). It has been suggested that TrkB could act as a "synaptic tag" for plasticity promoting proteins (e.g. BDNF) to promote late-phase long-term potentiation (L-LTP) since its expression on the plasma membrane increases after neuronal activity thereby marking active synapses (Lu et al., 2011).

In addition to the insertion of TrkB into the cell membrane, neuronal activity and increase in intracellular calcium concentration regulate the internalization of TrkB receptors (Du et al., 2003). The kinetics of the receptor insertion to the membrane and its subsequent internalization can define the signaling pathways and other downstream actions of the receptor. A short treatment (15 s) with potassium chloride (KCl) did not increase TrkB surface expression in hippocampal primary neurons or TrkB.TK+ transfected N2a cells but BDNF did (Haapasalo et al., 2002). However, a 5 minute treatment with BDNF already reduced the surface expression of TrkB and the levels remained low for at least 24h, possibly due to endocytosis following receptor activation. Pretreatment of neuronal cell cultures with KCl prevented the decrease in TrkB surface expression that normally occurs after BDNF stimulation elongating the effect of BDNF on TrkB signaling, including phosphorylation of the receptor and its downstream targets extracellular signal-regulated kinase (ERK), protein kinase B (Akt) and PLCγ (Guo et al., 2014). Inserting more TrkB receptors to the cell membrane compensates for the endocytosis occurring after ligand binding, and the TrkB signaling shifts from a transient event to sustained state.

After endocytosis receptors are brought back to the membrane, degraded or transported towards the cell soma depending on whether the receptors are targeted to recycling endosomes, early endosomes or late-endosomes/lysosomes (IJzendoorn, 2006). TrkB.T1 and TrkB.FL receptors seem to be differentially recycled after BDNF-induced endocytosis with TrkB.FL receptor degraded (targeted to the lysosomes) more quickly than TrkB.T1 (Huang et al., 2009). The Rab11-positive endosomes regulate dendritic trafficking of the TrkB receptors after ligand binding and have an important role in the dendritic branching promoting effects of BDNF (Lazo et al., 2013). The Rab11-positive endosomes carrying TrkB receptor enrich to dendrites and increase TrkB expression in the plasma membrane (Watson et al., 1999b).

Recently, cell-surface protein SLIT- and NTRK-like protein 5 (Slitrk5) has been implicated in the regulation of BDNF-induced TrkB signaling (Song et al., 2015). The extracellular LRR domain 1 of Slitrk5 specifically interacts with the LRR-domain of TrkB receptors after BDNF stimulation. In the absence of Slitrk5 BDNF stimulation induced normal TrkB phosphorylation, however, prolonged BDNF treatment did not produce

increase in primary dendrite formation as was seen in wild type neuronal cultures. The authors found that TrkB degradation was increased in the absence of Slitrk5 due to reduced targeting of TrkB to recycling endosomes, which are responsible for the recycling of the endocytosed receptors back to the membrane. These findings implicate Slitrk5 in targeting of TrkB to recycling endosomes and regulation of TrkB signaling.

Receptor internalization and the following dynein-dependent transport of TrkB receptor from axon to soma (retrograde transport) are necessary for its survival-promoting effects in sensory neurons (Heerssen et al., 2004). The first observation of retrograde transport of activated Trk receptors was done using sciatic nerve injury, after which the phosphorylated Trk receptors were accumulating in the distal side of the injury indicating that the receptors were transported in clathrin-coated vesicles from the axon terminal towards the soma (Bhattacharyya et al., 1997). In addition, anterograde transport of TrkB following sciatic nerve injury has been reported (Yano et al., 2001).

2.1.8 TrkB activation by BDNF and downstream signaling

TrkB is a receptor tyrosine kinase and thus catalyzes upon activation transfer of a phosphate group to a tyrosine of another protein. TrkB receptors form homodimers upon ligand binding and this allows the receptors to phosphorylate tyrosines 706 and 707 in each other's catalytic domain resulting in increased kinase activity of the receptor (Reichardt, 2006). In addition to the catalytic domain, other tyrosines of the receptor can be phosphorylated with the most extensively studied phosphorylation sites being Y515 and Y816 (Middlemas et al., 1994; Segal et al., 1996). Phosphorylated Y515 and Y816 (pY515 and pY816, respectively) can serve as docking site for Src homology 2 (SH2) adaptor proteins and phosphotyrosine binding domain containing proteins. Shc, Frs2 (fibroblast growth factor receptor substrate 2) and PLCγ are the major interactor proteins directly binding to TrkB receptors and activating Trk-associated signaling pathways Ras, PI3k and PLCγ1 (Obermeier et al., 1993) (Fig 3).

Shc binding to pY515 can activate signaling via PI3K and Ras by inducing a cascade of protein-protein interactions that further recruit serine/threonine kinases Akt and ERK (Hallberg et al., 1998; Obermeier et al., 1994). ERK can also be activated by signaling initiated by PLCγ binding to pY816 in TrkB (Stephens et al., 1994). Shc binding site signaling is linked to survival and axon outgrowth (Atwal et al., 2000). The Shc-PI3K-Akt pathway and the Shc-Ras-Raf-ERK pathway both promote survival and differentiation (Yao and Cooper, 1995). In addition to Shc also Frs2 can bind to pY515. Frs2 interacts with Shp2 and Grb2 to induce ERK activation via Ras (Easton et al., 2006). It has been suggested that competition of Shc and Frs2 for the binding site could regulate the induction of proliferation vs. differentiation by the neurotrophins (Meakin et al., 1999). The survival promoting effects of ERK are mediated via inhibition of pro-apoptotic factors and increases in transcription of pro-survival factors (Bonni et al., 1999). BDNF-TrkB-ERK signaling promotes dendritic growth and increases the number of spines in a subgroup of hippocampal neurons (Alonso et al., 2004). The PI3K-Akt pathway leads to activation of mTOR which regulates P70S6k and 4eBP1 to promote translation of proteins that affect cell survival, proliferation, differentiation and dendritic growth (Kumar et al., 2005; Takei et al., 2004).

In addition to activating ERK, BDNF-TrkB signaling also induces the translocation of ERK to the nucleus and thus affects the transcription factors regulated by ERK, (e.g.

cyclic AMP response element-binding protein, CREB) (Patterson et al., 2001; Ying et al., 2002). ERK cannot, however, directly phosphorylate CREB but requires Rsk to phosphorylate the serine 133 in CREB (Watson et al., 2001). CREB is one of the transcription factors linked to increased transcription of genes required for late-phase LTP and cell survival (Minichiello et al., 2002; Watson et al., 2001).

Minichiello *et al.* generated a genetically modified mouse with a point mutation at the Shc binding site of TrkB receptor (Y515 → phenylalanine (F)) (Minichiello et al., 1998). Cultured nodose and trigeminal ganglion neurons from these mice responded poorly to NT4 stimulation, suggesting that the Shc site activation is important for NT4 induced TrkB activation. *In vivo*, neurons known to depend on NT4 signaling were missing from $TrkB^{Y515\rightarrow F}$ mice, however, most of the neurons that are lost in BDNF knockout animals were not affected in the mice with the Shc site mutation. ERK signaling is significantly reduced in $TrkB^{Y515\rightarrow F}$ mice but CREB activation by BDNF stimulation appears to be normal (Minichiello et al., 1998, 2002). In contrast to the findings in cultured neurons the $TrkB^{Y515\rightarrow F}$ mice did not show any deficits in the differentiation of CNS neurons, however, a mutation in the Shc site impaired axonal regeneration *in vivo* (Hollis et al., 2009; Minichiello et al., 1998).

The PLCγ1 binding site (Y816) of TrkB is in close proximity to the C-terminal region of the receptor and can be phosphorylated by ligand binding (Middlemas et al., 1994). Upon binding to pY816 the membrane-bound enzyme PLCγ is activated and can then hydrolyze phosphatidyl(4,5)inositolbisphosphate (PIP2) to second messengers diacylglycerol (DAG) and IP_3 (Carpenter and Ji, 1999). DAG is a lipid that cannot diffuse into the cytoplasm but stays in the plasma membrane and activates protein kinase C (PKC) signaling. In contrast, IP_3 can enter the cytoplasm and activate the release of calcium from the intracellular storages. PKC activation and calcium release can lead to the activation of ERK, CaMKIV, and CREB and to release of neurotrophins (Canossa et al., 2001; Finkbeiner et al., 1997; West et al., 2001). BDNF can potentiate excitatory synaptic transmission by increasing intracellular calcium concentration via TrkB-PLCγ-IP_3-PKC signaling (Carmignoto et al., 1997; Levine et al., 1995; Li et al., 1998). In contrast to Y515, Y816-mediated signaling seems to be required for synaptic plasticity, especially for hippocampal long-term potentiation (Korte et al., 2000; Minichiello et al., 2002).

TrkB-mediated PLCγ activation is also required for epileptogenesis (Gu et al., 2015; He et al., 2010). TrkB Y816 phosphorylation is increased during status epilepticus and preventing the signaling of the Y816-residue reduced PLCγ activation and prevented the epileptogenesis in a kindling model (He et al., 2010). Instead of blocking the TrkB receptor, inhibiting just the coupling of PLCγ to pY816 after chemically induced seizures prevents the epileptogenesis but does not impair the TrkB-mediated promotion of survival (Gu et al., 2015).

In the PNS neurotrophin-induced activation of TrkB receptors in the nerve terminal results in endocytosis of the receptor-ligand complex and retrograde transport of the complex to the soma resulting in CREB activation and induction of immediate-early gene *c-fos* (Watson et al., 1999b). The retrograde transport of activated Trk receptors recruits specific signaling pathways involving ERK5 to mediate the survival promoting effects in the soma, suggesting that the location of TrkB activation also controls the downstream signaling pathways (Watson et al., 2001). Clathrin- and dynamin-dependent endocytosis

of TrkB receptors after BDNF stimulation has been shown to be important for BDNF-induced PI3K-Akt signaling (Zheng et al., 2008).

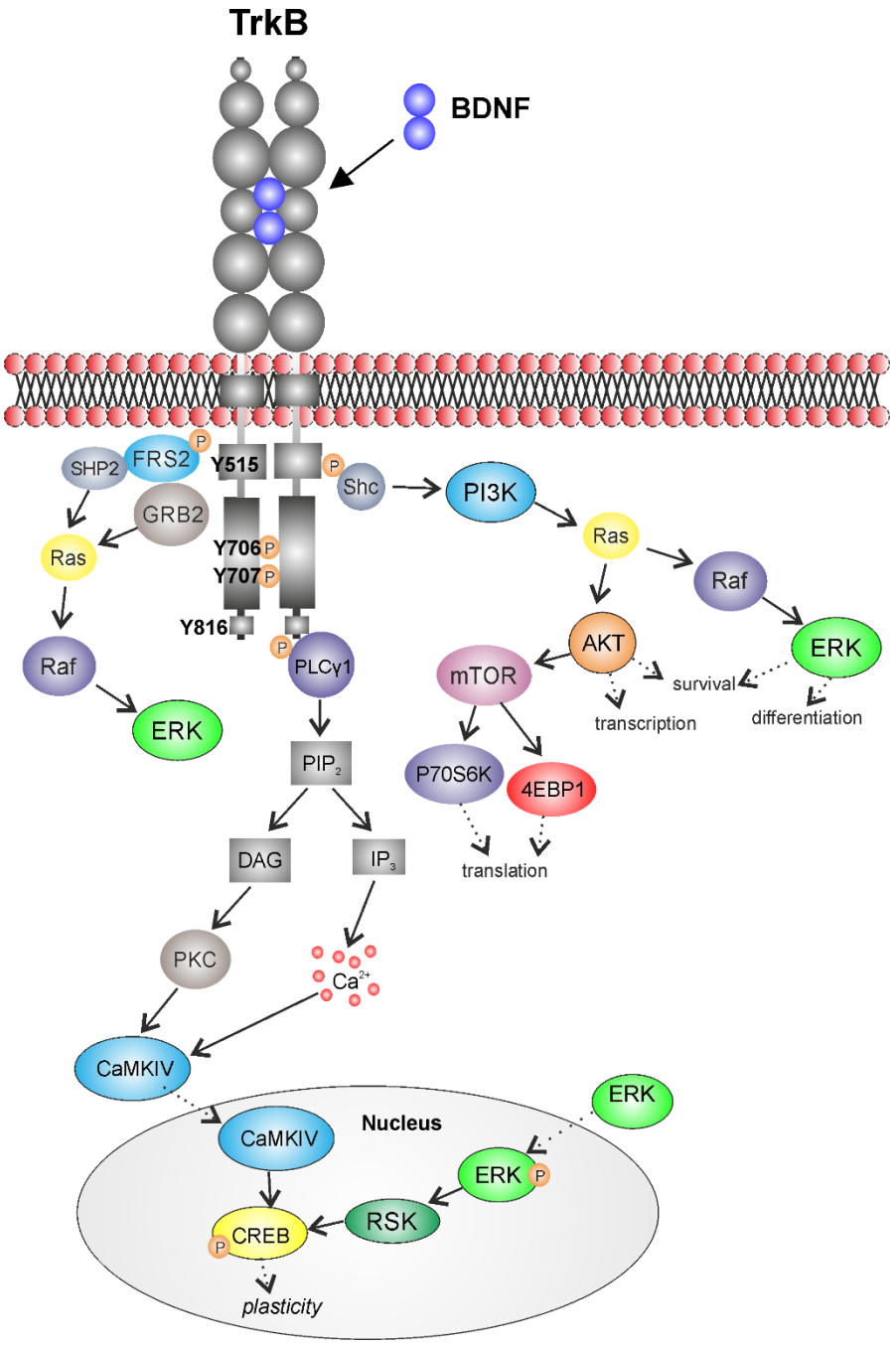

Fig 3. The main signaling pathways of the TrkB neurotrophin receptor. The TrkB receptor can be phosphorylated upon BDNF binding to the catalytic domain Y706/7, the Shc binding site Y515, and the PLCγ1 binding site Y816. The main pathways include Shc-PI3K-Akt, Shc-PI3K-Ras-Raf-ERK and PLCγ1-IP_3–PKC-CaMKIV that promote survival, differentiation, calcium release and initiation of transcription and translation.

2.1.9 Signaling of the truncated TrkB receptor

Alternative splicing of the TrkB transcript results in the truncated receptors TrkB.T1 and TrkB.T2 that lack the intracellular tyrosine kinase domain but have small intracellular domains of 23 and 21 amino acids, respectively (Baxter et al., 1997; Klein et al., 1990). The truncated TrkB receptors can bind and internalize BDNF, however, due to the lack of tyrosine kinase domain the canonical neurotrophin signaling responses cannot be activated. BDNF stimulation of *Xenopus* oocytes transfected with TrkB.FL, TrkB.T1 or TrkB.T2 increased calcium efflux in a phosphatidylinositol dependent manner only in TrkB.FL transfected cells indicating that BDNF is able to activate this signaling only via tyrosine kinase containing TrkB receptors (Eide et al., 1996).

When discovered, the truncated Trk receptors were thought to act as "simple" scavengers which limit the diffusion of neurotrophins since they are expressed widely in non-neuronal tissues and can internalize BDNF after binding (Biffo et al., 1995). However, when the truncated receptor is expressed together with the full-length receptor it can form a heterodimer and inhibit the tyrosine kinase activity of TrkB.FL in a dominant-negative manner (Eide et al., 1996; Haapasalo et al., 2001). The truncated receptors can reduce the surface expression of the full-length TrkB and thus regulate the availability of TrkB receptors to its ligands (Haapasalo et al., 2002). Because of their dominant negative function truncated receptors can negatively affect the survival role of BDNF (Ninkina et al., 1996). *In vivo* deletion of truncated TrkB receptors could partially rescue the phenotype of BDNF heterozygous knockout mice suggesting that the truncated TrkB receptor negatively regulates the full-length TrkB signaling when expressed at physiological levels (Carim-Todd et al., 2009). Moreover, overexpression of TrkB.T1 reduced TrkB phosphorylation *in vivo* (Saarelainen et al., 2000).

The effects of the truncated TrkB receptor are not limited to the regulation of the TrkB.FL signaling; they can also initiate intracellular signaling themselves. The small intracellular domains of the truncated receptors are required for this signaling (Baxter et al., 1997). BDNF stimulation can induce release of calcium from intracellular storages through TrkB.T1-activated IP3 signaling in the absence of TrkB.FL (Rose et al., 2003). More precisely, Rho GDI1 can bind the intracellular domain of TrkB.T1 and upon ligand binding dissociates from TrkB.T1 and activates other Rho GTPases that initiate changes in astrocytic function (Fenner, 2012).

2.1.10 TrkB transactivation

Receptor transactivation occurs independently of the ligand binding via activation of intracellular signaling events. Studies concerning the transactivation of Trk receptors have been mostly done *in vitro* and it is yet to be confirmed whether the same phenomena exists in physiological conditions *in vivo*. The first demonstrations of Trk receptor transactivation came from studies done in PC12 cells and primary hippocampal neurons, where G-protein coupled receptor (GPCR) ligands adenosine and pituitary adenylate cyclase-activating polypeptide (PACAP) were shown to activate Trk receptors without direct binding to the receptors or effects mediated via neurotrophins (Lee and Chao, 2001; Lee et al., 2002). The activation was mediated by G-protein coupled adenosine A2 or PAC1 receptors and occurred relatively slowly, requiring a minimum of 90 minutes. Adenosine and PACAP can also activate the PI3k-Akt-pathway via Trk receptors and to mediate cell survival. The effects of transactivation on ERK

phosphorylation are somewhat controversial since PACAP stimulation resulted in a sustained phosphorylation of ERK whereas adenosine stimulation did not. Intracellular calcium chelator EGTA and protein phosphatase 1 (PP1) (and PP2, Rajagopal and Chao 2006) blocked the effects, suggesting that intracellular calcium and Src family members could be involved in the transactivation mechanism. The specific member of the Src family kinases involved in Trk transactivation is suggested to be Fyn (Rajagopal and Chao, 2006). Phosphorylation of Fyn is seen with similar temporal pattern as Trk activation, and it also requires increases in intracellular calcium concentration and can be blocked by PP1. Fyn was also shown to interact with Trk receptor juxtamembrane region, but this interaction is not dependent on Trk kinase activity. Also inhibiting transcription and translation with actinomycin D and cycloheximide, respectively, abolished the effect of GPCR ligands on Trk phosphorylation (Rajagopal et al., 2004). The ability of adenosine A_{2A} receptor agonist to transactivate TrkB receptors was later demonstrated *in vivo* and the TrkB transactivation was crucial for the survival promoting effects of A_{2A} agonist (Wiese et al., 2007).

In addition to GPCR ligands, zinc has been reported to activate TrkB receptors and its downstream signaling pathways in a BDNF independent manner (Huang et al., 2008). In contrast to GPCR-mediated activation, the zinc-induced TrkB activation occurs quickly (in 5 minutes), thus, with similar kinetics as BDNF-induced TrkB activation. Interestingly, also reactive oxygen species (ROS) can activate TrkB receptor via an intracellular mechanism requiring zinc (Huang and McNamara, 2012). Zinc can enter the cell via the ionotropic NMDA-receptors and activate the Src family kinases similarly to other transactivators. Zinc was also shown to increase LTP of mossy fiber-CA3 synapses via transactivation of TrkB receptors. However, later a study from the same group failed to show that vesicular zinc is required for basal TrkB activation in adult mouse brain (Helgager et al., 2014). There is some controversy surrounding the role of zinc to transactivate TrkB receptors in cell culture, because it has been suggested that TrkB activation by zinc requires rapid processing of proBDNF to BDNF, suggesting that TrkB activation is mediated by matrix metalloproteinase -facilitated BDNF cleavage and not by transactivation (Hwang et al., 2005).

In addition to GPCR ligands and zinc, low-density lipoprotein receptor-related protein 1 (LRP1) agonists transactivate TrkB receptors in PC12 cells quickly (10 minutes) (Shi et al., 2009). Src family kinases were required also for the LRP1-induction of TrkB activation.

During embryonic development, TrkB transactivation has been shown to regulate the migration of cortical precursor cells and developing interneurons (Berghuis et al., 2005; Puehringer et al., 2013). At an early embryonic stage (E11) TrkB receptors in cortical precursor cells do not respond to BDNF stimulation but instead are activated by epidermal growth factor (EGF) (Puehringer et al., 2013). At this developmental timepoint the TrkB receptors are located mainly intracellularly and cannot be activated by BDNF. However, EGF can transactivate an intracellular pool of Trk receptors that can then subsequently be inserted to the cell membrane. Trk receptor transactivation by EGF is required for the proper migration of the cortical precursor cells, but does not affect the survival of the cells (Puehringer et al., 2013). TrkB also regulates the migration of interneurons via transactivation by endocannabinoid anandamide (Berghuis et al., 2005). TrkB transactivation by EGF and anandamide occurs much faster than the

adenosine or PACAP induced transactivation, but Src family kinases are essential for the transactivation in all the experiments.

Interestingly, transactivation of Trk receptors seems to induce a strong phosphorylation of a different form of Trk receptors of 110 kDa in size in addition to the normal 145 kDa protein and the regulation of this protein can occur even faster than that of the full-length form (Lee and Chao, 2001; Lee et al., 2002; Rajagopal et al., 2004). The transactivation has been shown to preferentially activate intracellular Trk receptors (Rajagopal et al., 2004). Because transactivation occurs intracellularly, immaturely glycosylated Trk receptors in trans-golgi can be phosphorylated to produce the phosphorylated 110 kDa protein (Rajagopal et al., 2004; Schecterson and Bothwell, 2010). In fact, treatment with Brefeldin A, an inhibitor of protein transport from endoplasmic reticulum to Golgi apparatus, increased the amount of 110 kDa protein (Rajagopal et al., 2004). Interestingly PACAP-induced TrkB transactivation resulted in reversible Golgi fragmentation (Schecterson et al., 2010). It is not clear what the consequences of the Golgi fragmentation induced by TrkB transactivation are, however, it is hypothesized that it could lead to redistribution of Golgi fragments to the dendrites and affect post-translational processing of dendrite targeted mRNAs. On the other hand, Golgi fragmentation can play a role in the pathophysiology of neurodegenerative disorders, thus suggesting that it may be harmful (Schecterson et al., 2010).

It has been suggested that TrkB receptors are expressed as dimers intracellularly before ligand binding, which could facilitate transactivation (Shen and Maruyama, 2012). In addition to transactivation ligand independent TrkB activation can be experimentally achieved with light stimulation using genetically modified TrkB receptors that have a light-activatable domain inserted into the intracellular domain (Chang et al., 2014). Light-induced TrkB dimerization and activation led to activation of the ERK signaling and increase in neurite outgrowth.

2.1.11 ProBDNF and p75^{NTR} signaling

In addition to Trk receptors all neurotrophins bind to and activate the p75^{NTR} (Rodriguez-Tébar et al., 1990, 1992). As discussed in preceding chapters these two receptors can have synergistic, distinct or even opposite effects on neuronal function. The p75^{NTR} receptor belongs to the tumor necrosis factor receptor family and can interact with multiple co-receptors and depending on the interaction partner initiate signaling via different signaling pathways (Dechant and Barde, 2002). P75^{NTR} has been shown to increase the specificity and the sensitivity of the Trk receptors to neurotrophins (Bibel et al., 1999; Davies et al., 1993). Upon neurotrophin binding p75^{NTR} can inactivate RhoA resulting in neurite growth. Some factors, such as NOGO, however, can promote the interaction between p75^{NTR} and Rho-GDI leading to increased RhoA activity and inhibition of neurite growth (Yamashita and Tohyama, 2003). Moreover, neurotrophin binding to p75^{NTR} in the absence of the Trk receptors can induce neuronal death via activation of JUN kinase (Bamji et al., 1998; Friedman, 2000).

Most importantly, proneurotrophins bind to p75^{NTR} with much higher affinity than mature neurotrophins (Lee et al., 2001b). Indeed, proBDNF, but not mature BDNF, can activate apoptotic signaling in the neurons via binding to a preformed complex of p75^{NTR} and sortilin (Nykjaer et al., 2004; Teng et al., 2005). Activation of survival signaling via Trk receptor, however, can rescue the cells from the apoptosis in a competitive manner.

Thus, pro and mature neurotrophins are able to elicit completely opposing functions and the cleavage of neurotrophins importantly determines the effects that follow the release of neurotrophins. Naturally, the signaling effects of proneurotrophins require that they have to be secreted and not cleaved extracellularly. However, it is still not certain to which extent proBDNF is released *in vivo*.

In *Xenopus* nerve-muscle co-cultures the activity-dependent increase in the cleavage of proBDNF to BDNF supports the establishment of the active synapse via TrkB and $p75^{NTR}$, while in the non-active synapse proBDNF stimulates the retraction of the synaptic terminal (Je et al., 2012). Similar results were obtained from *in vivo* experiments using mouse neuromuscular junctions, namely that proBDNF promotes the synapse elimination through $p75^{NTR}$ and sortilin and mature BDNF supports the survival of the synapses via TrkB (Je et al., 2013). Synaptic depression is important in the excitatory neuron synaptic clustering, which is considered to be involved in the regulation of computational power of the neuron. ProBDNF signaling via the $p75^{NTR}$ mediates the decreased transmission of synapses that are active in an asynchronous manner compared to their neighboring synapses (Winnubst et al., 2015).

The role of proBDNF cleavage to BDNF has been studied also in electrophysiological experiments. Pang et al. (2004) demonstrated that the cleavage of proBDNF to BDNF after secretion is required for the late phase LTP (L-LTP) in hippocampal slices. The levels of hippocampal proBDNF in tPA or plasminogen knock out mice are increased indicating that these enzymes are involved in the processing of proBDNF. Indeed, tPA and plasminogen deficient mice show impaired LTP that can be rescued by application of exogenous BDNF (Pang et al. 2004). The cleavage of proBDNF by plasmin is important for the LTP since cleavage resistant proBDNF cannot rescue LTP in plasminogen or tPA deficient mice. ProBDNF itself had no effect on LTP, however, it enhanced LTD. Later it was shown that the facilitation of the LTD by proBDNF depends on the $p75^{NTR}$ (Woo et al., 2005). High frequency stimulation (HFS) is required for LTP and in hippocampal neuronal cultures it has been shown that HFS results in release of tPA together with BDNF/proBDNF (Nagappan et al., 2009). In the presence of tPA more mBDNF is formed, which is important for the L-LTP. In contrast low frequency stimulation (required for LTD) increases the extracellular ratio of proBDNF to BDNF since it does not increase secretion of tPA. Thus, the extracellular cleavage of proBDNF to BDNF determines also postsynaptic effects of BDNF.

In BDNF conditional knockout mice the LTD was not affected in hippocampal slices (but LTP was impaired), suggesting that proBDNF is not necessary for LTD (Matsumoto et al. 2008). In $p75^{NTR}$ knockout mice, however, especially the NMDA receptor-dependent LTD was impaired (Woo et al., 2005). Also mice expressing proconvertase/furin cleavage-resistant proBDNF have decreased dendritic complexity in the hippocampus, impaired theta burst stimulation-induced LTP and enhanced LTD in Schaffer collaterals (Yang et al., 2014).

2.2 Role of BDNF and TrkB in CNS development and function

2.2.1 Cell survival and differentiation

The neurotrophins are essential for the development of the nervous system, and are known to regulate physiological functions also in the adulthood. During development neurotrophins are required to support the survival of a limited number of neurons that compete for target innervation. This applies mostly to the PNS and has been extensively studied in relation to survival of peripheral neurons. The applicability of the neurotrophin hypothesis to the CNS has not been reliably proven but according to current data it does appear to apply to certain populations of neurons. Notably, significant proportion of CNS neurons also undergoes apoptosis during early development. An interesting finding suffests that in contrast to TrkA and TrkC receptors TrkB receptor does not seem to be a dependence receptor that would induce apoptosis in the absence of ligand (Nikoletopoulou et al., 2010).

In general, neurons of the PNS are more dependent on certain neurotrophin and/or certain neurotrophin receptor compared to neurons in the CNS. Mice with complete deletion of BDNF or TrkB do not survive after birth because of deficits in peripheral nervous system innervation (Ernfors et al., 1994; Klein et al., 1993). Specific deficits in the nodose-petrosal ganglion, which brings gastrointestinal, cardiac and respiratory information to the CNS, results in problems with breathing and cardiac function that may underlie the fatal phenotype of the BDNF and TrkB knockout mice (Conover et al., 1995; Erickson et al., 1996; Jones et al., 1994; Silos-Santiago et al., 1997). TrkB knockout mice also have reduced amounts of vestibular neurons and loss of semicircular canal innervation causing defects in balance. TrkB or BDNF deletion does not cause gross deficits in the CNS, but decreases the amount of interneuron markers suggesting that BDNF-TrkB signaling is specifically involved in interneuron maturation and function (Alcántara et al., 1997). Parvalbumin expression is delayed in the TrkB knockout mouse hippocampus and cortex and cell death is increased during the first postnatal weeks compared to wild type mice in the cortex, hippocampus (especially dentate gyrus), striatum, septum, thalamus and olfactory bulb with specific temporal patterns (striatum earlier, dentate gyrus later) (Alcántara et al., 1997; Minichiello and Klein, 1996). Proper parvalbumin expression in interneurons and formation of GABAergic inhibitory synapses requires BDNF, TrkB and neuronal activity (Patz et al., 2004; Rico et al., 2002). BDNF and TrkB are involved in the regulation of dendritic branching, adjusting the number of dendritic spines and in synapse formation for example in some neuronal populations of the hippocampus and cortical layer 4 (Luikart et al., 2005; Martınez et al., 1998; McAllister et al., 1995; Xu et al., 2000a). *In vivo* some striatal neurons require BDNF and TrkB for survival during development and for dendritic complexity in adulthood (Baydyuk et al., 2011; Rauskolb et al., 2010). Also migration of neurons in the developing neocortex is delayed in mice lacking TrkB receptor affecting the stratification of the cortex and differentiation of neurons (Medina et al., 2004).

The effects of BDNF-TrkB signaling on the differentiation and survival of neurons have been mainly studied *in vitro*. BDNF increases the differentiation of neuronal precursor cells and promotes neurite outgrowth and survival of cultured neurons (Ahmed et al., 1995; Baj et al., 2011; Kirschenbaum and Goldman, 1995; Vicario-Abejón et al., 1998). BDNF stimulation also increases the number of excitatory and inhibitory synapses in

cultured hippocampal neurons and seems to affect especially the neurite outgrowth of GABAergic cells supporting the *in vivo* findings that BDNF is required for the normal development of inhibitory interneurons. Reduction in markers for GABAergic synapses could also be found when TrkB kinase function was inhibited for 20 days suggesting that the maintenance of the GABAergic synapses requires TrkB signaling also during adulthood (Chen et al., 2011). Signaling via the PLCγ binding site of the TrkB receptor seems to be important for these effects.

2.2.2 Plasticity

The interest to the possible plasticity promoting effects of neurotrophins were raised by the findings that seizure activity and sensory stimulation led to increased expression of neurotrophins (Thoenen, 1995). Neuronal plasticity is the ability of the brain to change in response to experience, environment and intrinsic activity. The processes regulating brain plasticity on a molecular or structural level involve the strengthening or weakening of synapses and the formation or elimination of synaptic connections. Experimental approaches to elucidate plasticity include analysis of neurogenesis, dendritic spines and long-term potentiation.

Neurogenesis

Formation of new neurons and their integration into existing neural networks is an important mechanism of neuronal plasticity. Neurogenesis in the adult brain was a matter of debate for decades but now it is well accepted that adult neurogenesis takes place in the subventricular zone (SVZ) of lateral ventricles and subgranular zone (SGZ) of dentate gyrus (Zhao et al., 2008). Mice that lack BDNF have increased apoptosis around the subventricular zone and in the dentate gyrus after the first two postnatal weeks (Linnarsson et al., 2000). Voluntary exercise on a running wheel or an enriched environment are well-known means to enhance neurogenesis in laboratory animals (Zhao et al., 2008). The main factors that are known to negatively affect neurogenesis are stress (glucocorticoids) and aging.

In dentate gyrus SGZ there are neural progenitor cells that continue to divide also in the adult brain producing neurons that migrate to the granule cell layer and extend dendrites to the molecular layer and axons to the CA3 region (van Praag et al., 2002). The basal level of bromodeoxyuridine (BrdU) -labeled adult-born neurons is normal in BDNF conditional knockout mice (BrdU incorporates into newly synthesized DNA), however, following environmental enrichment and voluntary exercise the amount of BrdU labeled cells increased more in the hippocampus of wild type mice compared to BDNF conditional knockout mice (Choi et al., 2009). BDNF deletion affected the survival of adult-born neurons, resulting in significantly reduced levels of BrdU-labeled cells measured 4 weeks after the BrdU injection when the mice were housed in a standard or enriched environmental conditions. BDNF heterozygous knockout mice also show reduced survival of hippocampal new-born neurons after environmental enrichment or chronic antidepressant treatment (Rossi et al., 2006; Sairanen et al., 2005). Intrahippocampal infusion of BDNF increased the amount of new-born neurons, but unexpectedly more in the contralateral side of the infusion, suggesting that BDNF does not directly affect these cells since BDNF presumably does not diffuse widely from the injection site (Scharfman et al., 2005).

Type B and ependymal cells in the SVZ express high levels of TrkB and low levels of BDNF (Galvão et al., 2008). The TrkB receptor expressed in SVZ is, however, mainly the truncated form of the receptor (TrkB.T1). TrkB expression co-localized with BrdU suggesting that stem cells of the SVZ express TrkB (Galvão et al., 2008). When the stem cells from TrkB knockout mice were grafted to adult wild type mice they did not show any difference when compared to grafts from wild type mice in regards to their ability to give rise to new neurons or in the migration or differentiation of the olfactory bulb neurons; suggesting that TrkB-mediated signaling is not critical for these effects (Galvão et al., 2008). However, similarly to hippocampus, TrkB signaling may be necessary for the maturation or survival of the cells in SVZ.

Intraventricular administration of BDNF has been reported to increase the amount of BrdU labeled olfactory bulb neurons in the adult rat brain (Benraiss et al., 2001; Zigova et al., 1998). However, an osmotic minipump releasing BDNF for 14 days to the lateral ventricle had no effect on the amount of new cells in the olfactory bulb of mice and, in fact, decreased the BrdU-labeled cells in the olfactory bulb of rats likely due to decreased proliferation of SVZ cells (Galvão et al., 2008). Infection of anterior SVZ cells with BDNF-expressing virus increased proliferation and/or survival of neural progenitor cells (Henry et al., 2007). Also in both of these studies increased BrdU-labeling was noticed in the striatum. Striatal neurogenesis has not been fully observed in the adult brain, yet, from human studies there is some evidence that new neurons can be found in adult striatum (Ernst et al., 2014). Damage to some brain areas can result in increased neurogenesis, and BDNF has been shown to have controversial effects in these situations. In a study by Henry et al. (2007) BDNF increased the amount of BrdU positive cells in chemically lesioned striatum. In another study, however, long-term hippocampal viral expression of BDNF in dentate gyrus prevented the formation of new-born after ischemic injury (Larsson et al., 2002).

Long-term potentiation

Long-term potentiation, LTP, is a persistent increase in the synaptic strength induced by brief high frequency electrical stimulation or coincident activation of pre- and postsynaptic neurons (Minichiello, 2009). LTP is considered as a cellular mechanism underlying learning and memory.

Application of BDNF directly to hippocampal slices enhances synaptic strength in the Schaffer collateral-CA1 synapse in a TrkB-dependent manner (Kang and Schuman, 1995). In contrast, LTP in Schaffer collaterals is impaired in BDNF heterozygous knockout mice (Korte et al., 1995). However, BDNF knockout and BDNF heterozygous knockout mice had similar impairments in LTP, with LTP being induced in some slices also in the absence of BDNF, indicating that in all conditions normal levels of BDNF are not necessary for LTP. Viral expression of BDNF in CA1 neurons or application of recombinant BDNF can mostly rescue impaired LTP, suggesting that BDNF itself is required for LTP and the deficits are not caused by abnormalities due to the lack of BDNF during the development (Korte et al., 1995; Patterson et al., 1996). The late-phase LTP in particular seemed to be affected in the absence of BDNF (Korte et al., 1996). Activation of presynaptic NMDA receptors by neuronal activity results in increased intracellular calcium concentration that triggers the release of BDNF from presynaptic terminals (Park et al., 2014). BDNF is released from the presynaptic neurons presumably together with glutamate to induce LTP and in this manner seems to specifically potentiate the

highly active synapses (Gottschalk et al., 1998; Zakharenko et al., 2003). This is further supported by the finding that application of BDNF to dendrites, but not to axons, evoked a calcium response and induced LTP when combined with brief electrical stimulation (Kovalchuk et al., 2002). Long-term spine enlargement related to LTP requires that BDNF is secreted specifically in the active synapses (Tanaka et al., 2008). BDNF promotes spine enlargement via activation of cofilin and p21 activated kinase that are involved in regulation of spine morphogenesis (Rex et al., 2007).

The effects of BDNF on LTP are mediated through TrkB receptor and requires especially the signaling initiated by PLCγ binding to Y816 of TrkB (Minichiello et al., 2002). In addition to experiments using slices, the impairment of LTP in mice with point mutation in TrkB PLCγ binding site was also demonstrated by *in vivo* electrophysiological recordings (Gruart et al., 2007). It has also been suggested that BDNF-induced activation of presynaptic TrkB receptors is required for LTP (Xu et al., 2000b). To support the requirement for both presynaptic and postsynaptic TrkB, intact presynaptic and postsynaptic PLCγ signaling is required for hippocampal LTP (Gärtner et al., 2006). Interestingly, mice overexpressing TrkB show increased PLCγ activation and enhanced spatial learning but impaired LTP (Koponen et al., 2004). It was suggested that the baseline synaptic activity in these mice is enhanced, which could lead to occlusion of LTP.

BDNF-TrkB in visual cortex plasticity

Basic organization of the brain occurs during early development independently of external stimuli. Molecular factors defined by genetics and intrinsic activity in the brain result in the formation of basic neuronal networks (Levelt and Hübener, 2012). A critical period is a well-defined time window during which environmental sensory stimulus is required for proper wiring and fine-tuning of neuronal networks in the brain. During the critical period the sensory stimulus, or lack there of, permanently affects the network in question. Sensory stimuli have less impact on the neuronal wiring before and after the critical period. In addition to sensory systems, it has been suggested that critical period exists in fear extinction (Gogolla et al., 2009). The molecular mechanisms of critical periods have been extensively studied and understanding how to enhance plasticity of the adult brain could be beneficial in the treatment of psychiatric disorders, which often have a developmental origin possibly caused by abnormal critical period and are difficult to treat when symptoms appear in adulthood.

Visual cortex is a generally-used model for studying critical period plasticity in the rodent brain. During the critical period of binocular vision formation the local inhibitory neurons in the primary visual cortex mature (Hensch, 2005). During the critical period it is possible to permanently alter the physical microstructure and organization of the visual cortex that may result in amblyopia. Apparently the columnar organization of the ocular dominance columns is well-established before the critical period but the sensory nformation during the critical period is able to modify the size of the columns and individualize the ocular dominance maps (Hensch, 2005).

Axons projecting from the lateral geniculate nucleus of the thalamus form eye-specific ocular dominance columns in the layer IV of the visual cortex during development (Cabelli et al., 1995). BDNF expression is not necessary for the formation of the eye-specific columns (Lyckman et al., 2005), however, infusion of TrkB-IgG, which binds to BDNF and possible other TrkB-binding molecules, can block the formation of the ocular

dominance columns suggesting that BDNF together with other endogenous TrkB ligands mediate this effect (Cabelli et al., 1997). Interestingly, BDNF infusion to the visual cortex can prevent the ocular dominance column formation by possibly disturbing activity-dependent competition of target innervation.

Visual input and light exposure regulates *bdnf* mRNA expression in the visual cortex (Castrén et al., 1992). If adult rats are kept in total darkness for one week *bdnf* mRNA expression in the visual cortex is reduced but can be restored quickly by one hour exposure to light. Also the normally occurring reduction in the sizes of receptive fields and improvement of visual acuity is impaired if rats are reared in darkness from birth (Fagiolini et al., 1994). In transgenic mice overexpressing BDNF in CaMKII-positive neurons the reduction in visual acuity by dark rearing is prevented (Gianfranceschi et al., 2003). Interestingly, visual experience is not required for visual acuity in these mice. Also, the receptive fields of dark-reared BDNF-overexpressing mice were not increased similarly to dark-reared wild type mice. Dark rearing in wild type mice prolonged the critical period so that monocular deprivation at P40 still produced a shift in ocular dominance. In BDNF-overexpressing mice the critical period for visual cortex occurs and closes earlier in development than in wild type mice and it cannot be delayed by dark rearing (Gianfranceschi et al., 2003; Hanover et al., 1999; Huang et al., 1999). BDNF-overexpressing mice show faster maturation of inhibitory interneurons, e.g. parvalbumin neurons, in the visual cortex (Huang et al., 1999). In contrast, dark rearing delays the emergence of GABAergic inhibition in the visual cortex in wild type mice, whereas BDNF overexpression allows for normal emergence of the GABAergic inhibition in the dark-reared animals (Gianfranceschi et al., 2003). Reduced BDNF levels do not seem to affect the critical period of the visual system, since BDNF heterozygous knockout mice have normal critical period (Bartoletti et al., 2002).

2.2.3 BDNF and TrkB mutations and polymorphisms in humans

Information about the role of BDNF and TrkB in humans has been obtained from patients with genetic mutations in the *bdnf* and *trkb* genes. Patients with a rare genetic disorder, WAGR syndrome, have a chromosomal deletion that in some cases includes the whole *bdnf* gene. Patients with heterozygous *bdnf* deletion show hyperphagia (increased appetite) and impaired pain sensation and have a higher body mass index (BMI) than WAGR patients without *bdnf* deletion (Han et al., 2008). In the patients with *bdnf* deletion also the serum BDNF levels were about 50 % lower. In addition, impaired adaptive behavior, cognitive function and speech and language skills were linked to the *bdnf* deficiency (Han et al., 2013; Shinawi et al., 2011). Patients with WAGR syndrome may have deletions of large numbers of genes, making it difficult to distinguish the effects of only *bdnf* deletion. However, symptoms of subjects without WAGR syndrome who have *bdnf* deletions support these findings, since the subjects were obese, insensitive to pain and had neurodevelopmental and behavioral phenotypes (Ernst et al., 2012). Moreover, in a case study a patient with *bdnf* haploinsufficiency was obese, hyperactive, developmentally delayed especially in speech and language skills, and manifested decreased sensitivity to pain, low IQ, impaired attention and short-term memory function (Gray et al., 2006).

De novo loss-of-function mutation in *TrkB* gene was found in an 8-year old boy suffering from severe obesity and hyperphagia (Yeo et al., 2004). In addition, the boy had developmental delay especially in speech and language, deficits in short-term memory,

stereotypic behavior and impaired nociception. The mutation resulted in replacement of one tyrosine residue by cysteine in the catalytic domain of the receptor. When studied in cell culture (PC12 cells) this mutation seemed to decrease the ligand induced phosphorylation of the receptor and also impaired downstream ERK phosphorylation. The *bdnf* haploinsufficient patient basically shows similar phenotypes as the patient with the loss-of-function mutation in the *trkb* gene.

An interesting polymorphism in the *bdnf* gene results from a single amino acid substitution of valine to methionine in the prodomain of BDNF at codon 66 (BDNF val66met) (Egan et al., 2003). BDNF met polymorph impairs the episodic memory in humans. BDNF met polymorphism, however, does not seem to be a risk factor for depression in humans (Verhagen et al., 2010). In contrast to BDNF haploinsufficiency, BDNF val66met polymorphism was not associated with obesity or eating disorders (Friedel et al., 2005). The substitution does not occur in mature BDNF but in the pro-domain of proBDNF, thus, it does not seem to have a direct effect on the functions of the mature BDNF, for example on the activation of the TrkB receptor or on the ability of BDNF to induce neurite outgrowth in PC12 cells (Egan et al., 2003). However, this single amino acid substitution seems to affect the intracellular processing and activity-dependent secretion of BDNF.

It has been estimated that 20-30 % of human population is heterozygous for the BDNF val/met, which means that both Val- and Met-form of BDNF can be expressed. When co-expressed val- and met-BDNF can form heterodimers affecting the distribution of the val-BDNF in the cell that results in its increased accumulation in the cell body because of abnormal sorting from Golgi apparatus to the secretory granules in PC12 cells (Chen et al., 2004).

In a fear conditioning paradigm human BDNF met allele carriers did not differ from val/val carriers (Soliman et al., 2010). However, the met allele resulted in impaired fear extinction that was accompanied by decreased activity in the ventromedial prefrontal cortex and increased activity of the amygdala in human neuroimaging studies done during extinction training. In another human imaging study the met allele was associated with slightly impaired memory retrieval (Hariri et al., 2003). Hippocampal activity was reduced in subjects with the met allele during memory encoding and retrieval, however, it has been found that hippocampal volume of the met allele carriers is decreased (Hajek et al., 2012).

2.3 NEUROTROPHIN AND NETWORK THEORIES OF ANTIDEPRESSANT ACTION

2.3.1 Antidepressant drugs

The first antidepressant drugs (AD) were serendipitously discovered in the 1950's. The monoamine oxidase (MAO) inhibitor iproniazid was tested for treatment of tuberculosis but was found to improve the mood of depressed patients receiving the medicine (Castrén, 2005). Iproniazid was soon followed by the first tricyclic antidepressant imipramine. Because both of these drugs affect the monoamine neurotransmitters it was suggested that depression may be caused by abnormal levels of monoamines that could be normalized with antidepressant treatment (Schildkraut, 1965). MAO inhibitors and tricyclic antidepressants were followed by more selective drugs targeting noradrenergic or serotonergic systems, for example the selective serotonin reuptake inhibitors (SSRI) that are widely used today.

In general, antidepressant drugs require weeks of treatment before their therapeutic effects appear. The primary pharmacological effects of the drugs on monoamines appear almost immediately suggesting that the therapeutic effects of ADs are not directly evolving from the increased monoamine levels in the synaptic cleft. Adaptation and plasticity of the neuronal networks resulting from chronic exposure to ADs could underlie the slow manifestation of the clinical therapeutic effect of ADs (Castrén, 2013). It is also interesting that monoamine depletion, which leads to a lack of serotonin or noradrenaline/dopamine in the brain, cannot produce depressive-like symptoms in healthy volunteers and does not lower mood in depressed patients (Ruhé et al. 2007). This suggests that there is no direct correlation with the brain monoamine levels and the depressed mood. However, the depletion in experimental conditions is acute and it is possible that longer depletion is required to induce depression symptoms. In subjects who had been previously treated with antidepressant drugs but are in a remission, tryptophan depletion moderately decreased the mood whereas phenylalanine/tyrosine depletion had no significant effect (Ruhé et al., 2007). In patients, who are in remission and are currently treated with antidepressant drugs, depletion of the corresponding monoamine in which the drug exerts its main effect can reinstate the depressive symptoms (e.g. tryptophan depletion in patients using SSRIs) (Ruhé et al., 2007). These results suggest that depressed patients are more susceptible to depressive symptoms because of a possible vulnerability in their monoaminergic systems. Interestingly, in healthy volunteers with a family history of major depressive disorder a slight reduction in the mood could be achieved with monoamine depletion, suggesting a genetic component involved in the sensitivity to the effects of monoamine depletion (Ruhé et al., 2007).

2.3.2 Concept of neurotrophin theory of depression and antidepressant action

Neurotrophin theory of depression postulates that reduction of BDNF expression would impair the survival and connectivity of neurons resulting in neuronal atrophy, especially in brain areas vulnerable to stress and involved in the regulation of mood (e.g. hippocampus) (Duman et al., 1997). The expression levels of BDNF may be promoted following treatment with antidepressant drugs aiding the neurons in resisting the negative effects of stress to restore connectivity. In support of the neurotrophin theory of depression, reduced BDNF levels in the brain and serum of depressed patients, as well

as increases in BDNF expression after AD treatment has been reported (Chen et al., 2001; Karege et al., 2002, 2005; Matrisciano et al., 2009; Shimizu et al., 2003).

In the blood BDNF is found in the platelets and it can be released upon stimulation induced by stress or injury (Fujimura et al., 2002). Megakaryocytes, which produce the platelets, express *bdnf* mRNA in humans and rats but not mice (Chacón-Fernández et al., 2016). Megakaryocytes also store BDNF protein, which is then transferred to platelets. Levels of BDNF in serum from depressed patients are found to be lower compared to healthy controls, with some studies suggesting that BDNF levels are correlated with the severity of depressive-like symptoms (Karege et al., 2002, 2005; Pandey et al., 2010). Antidepressants appear to increase BDNF levels in serum since depressed patients treated with ADs have elevated levels of BDNF in serum compared to non-treated controls, however, the ability of different ADs to affect BDNF levels seem to vary (Matrisciano et al., 2009; Shimizu et al., 2003). In support of these findings, in meta-analyses BDNF concentration has been found to be lower in the serum of depressed patients than in healthy controls or antidepressant-treated depressive patients, however, no correlation between BDNF levels and symptom severity was found (Molendijk et al., 2014; Sen et al., 2008). Low serum BDNF concentration is thought to result from decreased release of BDNF from the platelets and not from the overall decrease in blood BDNF levels (Karege et al., 2005).

In addition to blood, changes in brain BDNF levels have been observed in *post mortem* studies of depressed patients. Specifically, patients on antidepressant medication have increased BDNF expression in the hippocampus, whereas suicide victims have reduced BDNF and TrkB expression in the hippocampus and prefrontal cortex (Chen et al., 2001; Dwivedi et al., 2003)

2.3.3 Regulation of BDNF and TrkB by stress and antidepressant drugs

The neurotrophin hypothesis of depression is supported by animal studies. Stress and glucocorticoid injections were first shown to downregulate *bdnf* mRNA expression in the hippocampus (Smith et al., 1995a). Stress did not produce changes in cortical *bdnf* levels and, in fact increased *bdnf* expression in the hypothalamus and pituitary gland (Smith et al., 1995b). The length, timing, and type of stressor affect *bdnf* expression in a complicated manner since some *bdnf* transcripts are downregulated and some upregulated in specific hippocampal subfields (Nair et al., 2006). Stress-induced downregulation of *bdnf* in the hippocampus could be prevented by chronic antidepressant treatment, electroconvulsive shocks or voluntary exercise (Dwivedi et al., 2006; Nibuya et al., 1995a; Russo-Neustadt et al., 2001). Chronic AD treatment *per se* also increased *bdnf* and *trkb* mRNA levels in the hippocampus and the frontal cortex. The combination of an antidepressant drug and voluntary exercise increased hippocampal *bdnf* expression even more than either treatment alone (Russo-Neustadt et al., 2001). Chronic antidepressant treatments can also prevent the increase in the immobility time in the forced swim test (FST) and reduction in the sucrose consumption in the sucrose preference test that can be seen in rodents after chronic stress (Haenisch et al., 2009).

Important finding linking BDNF and mood came from studies showing that BDNF could modulate monoaminergic systems that have been strongly related to depression (Mamounas et al., 1995; Siuciak et al., 1996). It was demonstrated that BDNF infusion

for 6-7 days into the midbrain produced antidepressant-like behavior in the learned helplessness model and the forced swim test in rats; suggesting that BDNF itself was sufficient to induce antidepressant-like behavioral responses (Siuciak et al., 1997). Injection of BDNF into the dentate gyrus or CA3 of the hippocampus, or intracerebroventricular infusion of BDNF also resulted in AD-like behavior in rats (Hoshaw et al., 2005; Shirayama et al., 2002). Intrahippocampal implant releasing BDNF during 2 days reduced immobility in the FST when tested 7 days after implantation (Sirianni et al., 2010). BDNF overexpression in the dorsal dentate gyrus prevented the reduction in sucrose preference after chronic mild stress paradigm suggesting that BDNF could increase the resilience to stress (Taliaz et al., 2011).

The antidepressant-like effects of BDNF are likely mediated via its receptor TrkB and it has been demonstrated that mice overexpressing TrkB show antidepressant-like behavior in FST (Koponen et al., 2005). TrkB overexpressing mice have increased baseline activation of the receptor, which could explain the behavioral phenotype. Supporting this idea, intact BDNF-TrkB signaling is necessary for the behavioral effects of antidepressant drugs in the FST since BDNF heterozygous knockout mice, BDNF conditional knockout mice, and mice overexpressing the truncated isoform of the TrkB receptor do not respond normally to antidepressant drugs (Monteggia et al., 2004; Saarelainen et al., 2003). Antidepressant drugs with unique pharmacological profiles activate TrkB receptors and signaling pathways in the mouse brain (Rantamäki et al., 2007; Saarelainen et al., 2003). Whether ADs activate TrkB through increasing the release of BDNF or through other mechanisms remains to be investigated.

An often cited paper from Eisch et al. 2003 describes that infusion of BDNF to ventral tegmental area (VTA) shortens the latency to the first immobility period in FST (Eisch et al., 2003). However, the number of the rats in the test was really small, only 4-5 animals per group and there was no effect of BDNF on the immobility time. Therefore the results generated from this paper do not fully demonstrate the role of BDNF in VTA on behavioral effects in the FST. It has been later shown that electroconvulsive shock (a model of electroconvulsive therapy (ECT)) treatment for 10 days increased BDNF expression in the dorsal dentate gyrus but decreased BDNF expression in the ventral tegmental area (Taliaz et al., 2013). Knocking down BDNF from the dentate gyrus did not, however, abolish the antidepressant-like effect of ECT whereas overexpression of BDNF in the VTA could block the behavioral effects of ECT in the FST. BDNF overexpression in the VTA reduced sucrose preference and that could be rescued by ECS. Additionally, BDNF expression in the VTA has been linked to the development of social aversion in the social defeat stress paradigm. Social defeat stress increased BDNF expression in the nucleus accumbens and BDNF deletion specifically in VTA (the most probable source for BDNF in nucleus accumbens) prevented the defeated mice from developing social aversion behavior (Berton et al., 2006). Chronic social defeat stress also downregulated *bdnf* mRNA in the hippocampus affecting especially the transcription of *bdnf* variants III and IV (Tsankova et al., 2006). Chronic treatment with AD imipramine prevented the decrease in *bdnf* mRNA and also increased *bdnf* mRNA in non-stressed mice.

2.3.4 Neurogenesis and depression

The first study showing that chronic (14-28 days), but not single treatment with antidepressant drugs and electroconvulsive shocks (10 days) increases neurogenesis

(proliferation) in the rat brain was done by Malberg et al. (2000). The effect of antidepressant drugs was specific to the hippocampus since in the subventricular zone antidepressant drugs had no effect on the neurogenesis. Then, Santarelli et al. (2003) showed the same effects in mice. X-ray irradiation of the subgranular zone abolished the behavioral effect of fluoxetine in the novelty suppressed feeding test and also in some parameters measured after chronic unpredictable stress model indicating the requirement for neurogenesis in the behavioral effects of fluoxetine. Chronic fluoxetine treatment increases proliferation, facilitates the maturation and promotes the survival of newborn cells and it can prevent the stress-induced downregulation of hippocampal neurogenesis (Alonso et al., 2003; Wang et al., 2008).

Chronic treatment with fluoxetine increases the amount of BrdU positive neurons in the hippocampus while simultaneously activating apoptosis; suggesting that antidepressants affect the turnover of newborn cells rather than cell proliferation (Sairanen et al. 2005). However, after chronic antidepressant treatment the survival of the new-born cells seems to be increased. Antidepressant-induced proliferation in the SGZ was unaffected in BDNF heterozygous knockout mice or in mice overexpressing the truncated TrkB receptor, suggesting that BDNF-TrkB signaling is not critical for regulating the effects of antidepressant drugs on proliferation of neural precursor cells. Long-term survival of newborn cells was decreased in BDNF heterozygous knockout mice or TrkB.T1 overexpressing mice and the antidepressant effect was abolished.

There exists a strong correlation between the time course of the neurogenesis and the behavioral effects of antidepressant drugs, thus supporting the hypothesis that neurogenesis could underlie some important aspects of therapeutic effects of antidepressant drugs (Castrén and Hen, 2013). This is further supported by the findings from human studies that show an increased expression of neural progenitor cells in the dentate gyrus of SSRI treated patients compared to non-treated patients or patients treated with tricyclic antidepressant drugs (Boldrini et al., 2009, 2012). Additionally, the volume of the dentate gyrus was larger in the SSRI treated patients. In baboon hippocampus chronic treatment with antidepressant fluoxetine did not affect the proliferation of adult born neurons (Wu et al., 2014). How ADs exactly promote neurogenesis is not known. In general the microenvironment around the neural stem cells (i.e. the neurogenic niche) can regulate the function of the neural progenitor cells, and it is possible that antidepressants promote expression or availability of some critical regulators of neurogenesis (Zhao et al. 2008).

Additional plasticity promoting effects are associated with antidepressant drugs. These include increases in dendritic spines of inhibitory interneurons of PFC and pyramidal cells of the CA1 and CA3 areas of the hippocampus (Guirado et al., 2014; Hajszan et al., 2005). Furthermore, antidepressants have been shown to enhance LTP in the medial perforant path-dentate gyrus pathway and in the lateral amygdala (Karpova et al., 2011; Wang et al., 2008).

2.3.5 The concept of network theory of depression and antidepressant action

The delayed onset of action of antidepressant drugs brought about the idea that the clinical effects of ADs require adaptation and changes at the neuronal network level (Castrén, 2013; Castrén and Hen, 2013). The plasticity of the adult brain is restricted, however, the molecular mechanisms of learning and memory are based on brain

plasticity that is expressed as strengthening or weakening of synapses. In addition to changes in existing synapses, new neuronal connections can be formed when adult born neurons connect to existing neural networks indicating adult neurogenesis affects neuronal plasticity (Castrén and Hen, 2013). Plastic changes on the structural or functional level can eventually result in remodeling of complete neural networks. Information processing (e.g. emotions) requires the cooperation of multiple brain regions via neural networks (Castrén, 2005). The information transmitted between brain areas can be enhanced or diminished as a result of network level plasticity. In depressed patients abnormalities in morphology and wiring of the areas involved in emotional processing have been detected (Price and Drevets, 2009). It has been reported that depressed patients have synaptic atrophy in the dorsolateral PFC and decreased volumes of PFC and hippocampus (Kang et al., 2012; Savitz and Drevets, 2009). Depression can be a manifestation of these structural and functional changes in the mood-related neural networks. Plastic processes involved in the recovery from depression likely affect the neural networks controlling mood, balancing their actions to resemble the healthy state (Castrén, 2005).

An important aspect of the network theory of depression and antidepressant action is that the shaping of the neuronal networks requires environmental guidance (Castrén, 2013). The ability of antidepressant drugs to enhance the plasticity of the brain has been shown in studies conducted on rat visual cortex and mouse fear processing circuitry (Karpova et al., 2011; Maya Vetencourt et al., 2008). Results from both studies demonstrate the requirement for environmental guidance to support antidepressant effects.

2.3.6 Plasticity models and antidepressant drug action

The antidepressant fluoxetine can restore juvenile-like plasticity in the adult rodent visual cortex (Maya Vetencourt et al. 2008). This is a crucial finding supporting the hypothesis that the effects of ADs could be mediated by enhanced plasticity of the neuronal networks. In the study Maya Vetencourt et al. used two models to examine the plasticity of the visual cortex: an amblyopia model and monocular deprivation during adulthood. In the amblyopia model one eye is closed during the critical period of visual system development, thus resulting in ocular dominance of the open eye in the visual cortex and reduction in the visual acuity of the closed eye. After the end of the critical period the connections and acuity of the closed eye cannot be restored anymore. Similarly, if monocular deprivation is done during adulthood, the connections or acuity of the closed eye are not weakening since the critical period is over and the state of the visual cortex and binocularity are already established (Hensch, 2005). However, after chronic fluoxetine treatment it was possible to modify the connections of the visual cortex similarly to what could be done during the critical period (Maya Vetencourt et al., 2008).

In adulthood closing of one eye during fluoxetine treatment resulted in a similar shift in ocular dominance that can be achieved when the eye is closed during the critical period (Maya Vetencourt et al., 2008). In the control group, no shift in ocular dominance was observed. During eye closing the response evoked by the closed eye in the visual cortex was reduced, demonstrating that the shift in ocular dominance happens because the connections from the closed eye are weakening and not because the connections from the open eye would be strengthening. In addition, when the amblyopic rats were treated

with fluoxetine the visual acuity of the closed eye improved to the level of the normal eye, however, it was necessary to close the opposite eye to cause the closed eye to recover. This is crucial, since it demonstrates the requirement for environmental guidance for the effects of fluoxetine. Another possibility is a need arising from the environment that requires adaptation of the connections. If the better eye stays open, the animal can continue to use it and there is no need to adjust to new situations, but when the better eye is closed there is a requirement for the weaker eye to try to adapt.

The mechanism underlying how chronic fluoxetine treatment could make the cortex more plastic was linked to increased expression of BDNF and reduced levels of GABA-mediated inhibition in the cortex (Maya Vetencourt et al., 2008). These effects are not dependent on the shift of the ocular dominance, but rather appear as a result of chronic fluoxetine treatment. As mentioned previously, the fluoxetine-induced shift in ocular dominance cannot occur without closing of the stronger eye. In the case of monocular deprivation during adulthood, the shift in ocular dominance occured because of weakening connections from the closed eye and not because of strengthening of connections from the open eye (Maya Vetencourt et al., 2008). The functional role of BDNF as a neurotrophic factor is to promote synaptic strengthening rather that repression, thus there may be many additional mechanisms contributing to the enhanced plasticity. BDNF has an important role as a plasticity inducer or enhancer since direct administration of BDNF via minipumps to the visual cortex at the same time with monocular deprivation resulted in an ocular dominance shift (Maya Vetencourt et al., 2008). The likely explanation would be that BDNF via TrkB signaling initiates a process which renders the synapses in a more plastic state, including the possibility to strengthen but also to weaken the synapses.

The other mechanism suggested by Maya Vetencourt et al. is reduced GABAergic inhibition in the cortex. GABAergic neurotransmission is an important regulator of cortical plasticity and increases in the inhibitory GABAergic signaling are linked to the closure of the critical period (Hensch, 2005). When diazepam (GABAA receptor positive allosteric modulator) was infused to the visual cortex during monocular deprivation it could prevent the shift in ocular dominance caused by fluoxetine (Maya Vetencourt et al., 2008). This suggests that enhanced GABAergic signaling could block the plasticity inducing effects of fluoxetine. It would be interesting to know if diazepam could also block the effects of intracortical BDNF infusion.

Chen et al. studied the effects of fluoxetine on dendritic spine dynamics and found that fluoxetine treatment, similarly to monocular deprivation alone, increased retraction and elongation of the spines but not the total spine number in the visual cortex layer 1 and layer 2/3 interneurons (Chen et al., 2011). In combination with monocular deprivation fluoxetine increased spine elongations in layer 2/3 during the first two days of monocular deprivation. This was faster than in the group treated with monocular deprivation alone without fluoxetine, in which the increase in the spine elongation was seen 4-7 days after starting the monocular deprivation. The retraction rate, however, was similar in monocular deprivation groups with or without fluoxetine. The retraction of spines co-occurred with reductions in the amount of inhibitory synapses onto excitatory cells, suggesting that monocular deprivation reduces the amount of inhibitory synapses onto excitatory neurons and could thus decrease the network inhibition. With fluoxetine these changes happen more quickly indicating that fluoxetine “primes” the visual cortex for

monocular deprivation by potentially affecting plasticity enhancing mechanisms and itself reducing the cortical inhibition (Chen et al., 2011).

The effect of fluoxetine on plasticity was also demonstrated on brain circuits involved in the fear processing, proving that the effect is not specific for the visual system only. In Karpova et al. (2011) fear-conditioning was used to study the antidepressant-induced plasticity of the fear processing circuitry. In fear conditioning paradigm the animal is exposed to unconditioned noxious stimulus (e.g. electric shock) which is combined with the conditioned stimulus (e.g. sound) (Maren, 2001). The animal learns quickly that the conditioned stimulus predisposes the noxious unconditioned stimulus and detecting the conditioned stimulus leads to freezing behavior. This behavioral response can be perturbed by exposing the animal to the conditioned stimulus without the unconditioned stimulus thereby removing the connection between stimuli. However, fear memory cannot normally be completely removed in adult animals since after a certain period of time when the animal is again exposed to the conditioned stimulus the fear is renewed and the animal freezes again after detecting the conditioned stimulus (Myers and Davis, 2006). Interestingly, in young animals fear memory can be permanently removed by extinction training (Gogolla et al., 2009). This concept was used in the study of Karpova et al. (2011) to investigate the potential "rejuvenating" effect of fluoxetine in an area different from visual cortex of adult mice. Fluoxetine indeed facilitated permanent fear extinction, since the mice treated chronically with fluoxetine showed reduced fear renewal and fear reinstatement (Karpova et al., 2011). This was repeated in two different experiments where the mice received chronic fluoxetine treatment either before starting the fear conditioning paradigm or in a more clinically relevant set up in which the mice were treated with fluoxetine after fear acquisition. In vehicle treated animals the fear renewal and reinstatement were clearly detectable.

The effects of fluoxetine were accompanied by a decreased number of parvalbumin positive cells surrounded by perineuronal nets in the basolateral amygdala and CA1 area of the hippocampus suggesting that some neurons lost the parvalbumin expression, which could be considered as a shift toward a more immature state (Karpova et al., 2011). Also other markers related to the more immature state of neurons were detected in the amygdala, namely reduction in the expression of potassium-chloride cotransporter 2 (KCC2) and increases in the expression of polysialynated neuronal cell adhesion molecule (PSA-NCAM). Long-term potentiation was enhanced in the lateral amygdala of fluoxetine treated animals demonstrating increased synaptic plasticity (Karpova et al., 2011). As in the study by Maya Vetencourt et al. (2008) BDNF was demonstrated to be sufficient to induce changes in synaptic plasticity also in this study, since overexpression of BDNF in amygdala after extinction training prevented fear renewal similarly to chronic fluoxetine treatment. To further support the BDNF's role in fear extinction, the effects of fluoxetine treatment on fear renewal were lacking in BDNF heterozygous knockout mice (Karpova et al., 2011).

Together these two studies by Maya Vetencourt et al. and Karpova et al. demonstrate that chronic fluoxetine treatment enhances plasticity in adult rodent brain. In humans the possible alterations in brain plasticity by antidepressant drugs are harder to evaluate but some studies have been done. The visual cortex has been used also in humans to study the effects of chronic antidepressant treatment. Normann et al. (2007) measured visually evoked potentials (VEPs) in healthy human subjects and in patients with major

depression, both receiving chronic treatment with sertraline. They found that the cortical plasticity induced by prolonged visual stimulus measured as early VEPs was enhanced in healthy subjects and reduced in depressed patients when compared to healthy controls. In the study there was no group of depressed patients without antidepressant treatment, so the effect of antidepressant drugs in depressed patients could not be demonstrated. According to the results, the ongoing antidepressant treatment could not increase the VEPs of the depressed patients to normal levels. Some of the patients in the study were also taking benzodiazepines at the same time with antidepressant drugs, but the average VEPs were similar in patients with or without benzodiazepines (Normann et al., 2007). In the rat study by Maya Vetencourt et al. (2008) the benzodiazepine diazepam could prevent the plastic changes in the visual cortex. Also diazepam blocked the neurogenesis-inducing effect of chronic fluoxetine treatment (Wu and Castrén, 2009), thus suggesting that simultaneous use of antidepressants and benzodiazepines may be problematic in depressed patients.

2.4 RAPID-ACTING ANTIDEPRESSANT DRUGS

2.4.1 Short history of rapid antidepressant effects

Induction of seizure activity in the brain was found to relieve symptoms of psychiatric patients in the 1930's. Pharmacologically induced shocks with insulin or pentylentetrazol were followed and soon replaced by electrically-induced seizures (Merkl et al., 2009). Electroconvulsive therapy (ECT) was found to be a surprisingly effective treatment for psychiatric disorders, especially depression. ECT, however, has side effects that include severe memory problems, thus restricting its use. The emergence of antidepressant drugs reduced the need for ECT and other shock treatments. ECT, however, is still in use and is currently the most effective treatment for the patients who do not respond to conventional antidepressant drugs or psychotherapy (Greenberg and Kellner, 2005; Group, 2003). The mechanism of action of ECT is not known, but it is thought that neurotransmitter systems, especially monoamines and GABA are targeted. For example, ECT is known to increase expression of neurotrophic factors, neurogenesis, and increase hippocampal volume and connectivity (Abbott et al., 2014; Merkl et al., 2009). Amongst other things it has been suggested that the post seizure burst suppression is an important factor determining the efficacy of ECT (Langer et al., 1985). In general, understanding the mechanism of action of ECT would be beneficial in the development of novel rapid and effective depression treatments.

2.4.2 The effects of rapid-acting antidepressant ketamine

Ketamine is a non-competitive NMDA receptor antagonist generally used as an anesthetic. Ketamine acts as an open-channel NMDA receptor blocker inhibiting the ion flux through the channel (Sanacora and Schatzberg, 2015). NMDA receptors are widely expressed in the brain and can be found almost in all excitatory synapses (Johnson et al. 2014). NMDA receptor antagonists were found to produce antidepressant-like behavior in animal models and it was discovered that conventional antidepressant drugs affect the glutamatergic system with NMDA receptor antagonists having effects that resemble those of antidepressant drugs (Papp and Moryl, 1994; Skolnick et al., 1996; Trullas and Skolnick, 1990). These findings further encouraged the testing of ketamine in depressed humans. The antidepressant effect of a subanesthetic dose of intravenous ketamine infusion in humans was first characterized by Berman et al. in a small group of depressed patients; and the findings have been replicated in several studies (Berman et al., 2000; Murrough et al., 2013; Zarate CA et al., 2006). In human patients the antidepressive effect of ketamine appears remarkably fast, within a few hours, and the effect of one administration normally lasts up to one week (McGirr et al., 2015). Ketamine has a short half-life (about 3 hours) so its long-lasting effects cannot be simply explained by "real-time" NMDA receptor antagonism.

The finding that noncompetitive NMDA receptor antagonist memantine does not produce antidepressant effects in human patients suggests that ketamine has some specific properties that underlie its antidepressive efficacy (Smith et al., 2013; Zarate CA et al., 2006). The clinical differences in ketamine and memantine could be explained by their off-target effects (other than NMDA receptors) or by their effects on distinct populations of NMDA receptors (Johnson et al., 2014). In the absence of Mg^{2+} both ketamine and memantine blocked miniature excitatory postsynaptic currents (mEPSCs) suggesting that both are able to block NMDA receptor-mediated responses, however, in

physiological conditions (in the presence of Mg^{2+}) only ketamine blocked mEPSC's (Gideons et al., 2014). The results obtained on other NMDA receptor antagonists in animal models also suggest that ketamine has unique mechanisms of action since they do not seem to produce as fast and long-lasting effects as ketamine (Autry et al., 2011; Maeng et al., 2008). It has been shown, however, that the NR2B subunit of the NMDA receptor is crucial in mediating ketamine's antidepressant-like behavioral effects in rodents (Miller et al. 2014). Recently, it was shown that a metabolite of ketamine, (2R,6R)-hydroxynorketamine (HNK) can reproduce the behavioral effects of ketamine in animal models (Zanos et al., 2016). (2R,6R)-HNK does not bind to NMDA receptors but increases AMPA-mediated currents. The behavioral effects of (2R,6R)-HNK can be blocked with pretreatment of 2,3- dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) interestingly even when the NBQX is administered 23,5 h after (2R,6R)-HNK. This indicates that AMPA receptor-mediated synaptic potentiation could be maintaining the antidepressive effects of ketamine. Pre-treatment with NBQX can abolish the antidepressant-like effects of ketamine and another NMDA receptor antagonist MK-801, but not imipramine, in FST (Autry et al., 2011; Maeng et al., 2008).

The role of AMPA receptors in regulating the effects of ketamine is further supported by the findings that ketamine increases glutamate levels and can thus promote signaling via kainate and AMPA receptors. The counterintuitive finding of increased glutamate levels in the PFC after injection of a subanesthetic dose of ketamine was first described in rats (Moghaddam et al., 1997). The effects of ketamine on glutamate and GABA concentrations in the human PFC have also been studied. Increases in GABA and glutamate/glutamine levels in the anterior cingulate cortex were found in human studies during ketamine infusion, specifically in the first 30 minutes, and soon after ketamine infusion (Milak et al., 2015; Rowland et al., 2005; Stone et al., 2012; Taylor et al., 2012). If GABA and glutamate levels were measured from occipital cortex of depressed patients 3 h or 48 h after ketamine infusion changes were no longer detected (Valentine et al., 2011). In rats 30 mg/kg ketamine increased GABA, glutamate, and glutamine in the mPFC 10 minutes after the injection, but not in hippocampus or after ketamine dose of 80 mg/kg (Chowdhury et al., 2012). With glutamate levels increased and NMDA receptors blocked glutamatergic effects are mediated by kainate and AMPA receptors. An anesthetic dose of ketamine also increased the extracellular dopamine levels in the PFC partially in an AMPA/kainate receptor-dependent manner (Moghaddam et al., 1997).

Riluzole is an NMDA receptor blocker and modulator of glutamate release that has been shown to increase the expression of AMPA receptors (Dutta et al., 2015). It has been hypothesized to be able to prolong or enhance the ketamine's antidepressant effects and to protect against relapse, especially if the mechanisms of antidepressant effects of ketamine are increased AMPA-mediated signaling and blockade of NMDA receptors. No significant change, however, in the duration of antidepressant effects of ketamine has been observed in humans if ketamine infusion has been followed by treatment with riluzole (Ibrahim et al., 2012; Mathew et al., 2010).

Increases in BDNF protein expression and TrkB signaling has been also linked to the antidepressant effects of ketamine in rodents (Autry et al., 2011). Autry et al. (2011) found in their study that ketamine (3mg/kg) rapidly increases protein translation via dephosphorylation of eukaryotic elongation factor 2 (eEF2). They show that the

translation of mBDNF and proBDNF is significantly increased 30 min after administration of ketamine or another NMDA-receptor antagonist MK-801. Furthermore, they demonstrate that blockers of the eEF2 kinase, which inhibit the phosphorylation of eEF2 by the kinase, have similar effect as ketamine in FST and with regards to BDNF protein levels. The AD effects of ketamine and eEF2 kinase inhibitor were abolished in the inducible BDNF conditional knockout mice in FST, and for ketamine also in TrkB conditional knockout mice indicating that BDNF-TrkB signaling is involved in the behavioral effects of ketamine (Autry et al., 2011). However, in BDNF heterozygous knockout mice ketamine produced an antidepressant-like effect in the FST similarly to wild type mice (Lindholm et al., 2012). In rats, acute administration of ketamine (15 mg/kg) increased BDNF protein levels in the hippocampus but chronic administration of ketamine with the same dose for 14 days did not (Garcia et al., 2008a, 2008b). Chronic administration of ketamine with 5, 10, or 15 mg/kg doses, however, decreased immobility time in FST.

The effects of ketamine in mice and humans with the BDNF val66met polymorphism indicate that the activity-dependent release of BDNF could be important in regulating the effects of ketamine. BDNF met/met mice have decreased spine density and spine head diameter in the layer V pyramidal cells of PFC and the ketamine-induced increase in spine number is abolished in these mice (Liu et al., 2012). BDNF met/met mice did not respond to ketamine when tested 24h post-injection (10 mg/kg) in FST. In a study by Laje et al. (2012) depressed human patients carrying the met allele (met/met or val/met) did not respond to ketamine as effectively as the val/val carriers (24 % reduction in the Hamilton depression rating scale scores in met group and 41 % for val/val) when measured 210-230 minutes after ketamine infusion (Laje et al., 2012). Infusion of BDNF function blocking antibody to the mPFC of rats 30 minutes before ketamine administration (10mg/kg) abolished the behavioral effects of ketamine in the FST 24 hours after the ketamine treatment (Lepack et al., 2014). Also pretreatment with L-type calcium channel blockers nifedipine or verapamil abrogated the effect of ketamine in the FST.

In addition to rodent studies, the effects of ketamine on blood BDNF levels have been assessed in humans. After single ketamine infusion the responding patients showed higher increase in blood BDNF levels and the BDNF increase correlated with slow wave activity in electroencephalography during sleep (Duncan et al., 2013). The authors hypothesized that the increased slow wave activity in ketamine responders is a consequence of BDNF-induced synaptic strengthening and enhanced plasticity. A significant negative correlation between Montgomery-Åsberg depression rating scale score and plasma BDNF concentration in ketamine treated patients, but not in patients treated with benzodiazepine midazolam, was found when studied 240 min after ketamine infusion (Haile et al., 2014). In some studies, however, the BDNF levels were not affected by the ketamine treatment (Machado-Vieira et al., 2009).

It has been demonstrated that ketamine activates the Akt/ERK-mTOR-p70S6kinase pathway rapidly and transiently and increases the levels of synaptic proteins in 2 h after the administration (Li et al., 2010). Ketamine also rapidly increased (in 24 h) dendritic spines in PFC layer V pyramidal neurons and produced antidepressant-like effects 24 h after administration in FST, novelty suppressed feeding and learned helplessness paradigms. The effects on synaptic proteins, dendritic spines and behavior could be

blocked by intracerebroventricular injection of mTOR inhibitor rapamycin, suggesting that mTOR signaling is involved in these actions of ketamine.

In addition to mTOR signaling, glycogen synthase kinase 3 beta (GSK3β)-related mechanisms have been implicated in the action of ketamine. Previously, dysregulation of GSK3β has been linked to the pathophysiology of depression and the mechanism of action of mood stabilizer lithium (Jope and Roh, 2006). Ketamine (10mg/kg) increases GSK3β phosphorylation, which results into inhibition of its function, in the hippocampus and cortex (Beurel et al., 2011). Interestingly, memantine also has similar effects on GSK3β as seen with ketamine. Using transgenic mice with mutations in the inhibitory GSK3α/β phosphorylation sites Beurel et al. (2011) were able to demonstrate that in these mice the antidepressant-like effects of ketamine in the learned helplessness model were abolished.

The ability of ketamine to affect fast synaptic transmission and activity of neuronal circuits could underlie its rapid antidepressant effects. Recently, ketamine-like effects on rat behavior in FST and NSF were achieved by optogenetic stimulation of infralimbic cortex (Fuchikami et al., 2015). Interestingly, the antidepressant-like effects achieved with the optogenetic stimulation of mPFC remained for almost three weeks in FST. The optogenetic stimulation also caused increase in the amount of dendritic spines in the infralimbic cortex. In addition the effects of ketamine were blocked by infusing the GABA agonist muscimol to the infralimbic cortex and direct injection of ketamine to infralimbic cortex could produce antidepressant-like behavior in the FST and NSF, however, only at certain dose (10 ng) (Fuchikami et al., 2015). These findings support the hypothesis that increased activity in a certain population of neurons in PFC resulting from ketamine induced disinhibition could underlie the antidepressant-like effects of ketamine. It is possible that in the case of low-dose ketamine treatment specifically the fast-spiking and tonically active GABAergic interneurons are sensitive to the NMDA receptor blockade by ketamine, which then via disinhibition produces glutamate release and neuronal excitability in pyramidal neurons. In pyramidal neurons AMPA and kainate receptors may mediate the effects of glutamate. Administration of MK-801 (0,1mg/kg) to rats reduced the firing rate of the fast-spiking interneurons and increased the firing rate of the regular spiking neurons, suggesting that the cortical excitation caused by NMDA antagonists results from disinhibition (Homayoun and Moghaddam, 2007). It is important to note, however, that blocking the NMDA receptors results in complex network effects that differ also along the timescale of the blockade.

In another recent paper projections from ventral hippocampus to PFC seemed to be important for the sustained (1 week) effects of ketamine in the FST (Carreno et al. 2015). K252a infusion to ventral hippocampus blunted the effects of ketamine at the 1 week timepoint (30 min timepoint results were not studied or shown in the article). Infusion of K252a to hippocampus blocks TrkB receptor activation in hippocampus, but how that affects the activity of the cells in PFC was not studied (Carreno et al., 2015). This demonstrates that Trk receptor activation in the hippocampus by ketamine is important for sustained antidepressant effects. Optogenetic inactivation of ventral hippocampus during the ketamine administration did not affect swimming behavior 1 week later but increased immobility if done during the FST, indicating that ventral hippocampus-PFC circuitry seems to be involved in antidepressant-like behavioral effects of ketamine (Carreno et al., 2015). There is also some support for ketamine's effects on hippocampus-

PFC circuit from human studies since immediately after ketamine infusion the functional connectivity between hippocampus and dorsolateral PFC is increased in healthy human subjects (Grimm et al., 2015).

The ketamine infusion can produce hallucinogenic effects and acutely impair verbal learning (Murrough et al., 2014). Abusers who have frequently (about 5 times a week) used ketamine for long time have decreased hippocampal function when tested on spatial navigation tasks, spatial memory tasks and pattern recognition memory tasks (Morgan et al., 2010, 2014). Chronic ketamine use can also increase the risk for cystitis and other related problems in the urinary tract (Middela and Pearce, 2011; Muetzelfeldt et al., 2008). The doses used by recreational ketamine users are, however, different than the dose of ketamine used in the treatment of depression. The most common side effects after ketamine infusion include nausea, visual disturbances and dizziness (Francois et al., 2015).

2.4.3 The effects of other rapid-acting antidepressant drugs

The concerns about the therapeutic use of ketamine in the treatment of depression emerge from the current lack of knowledge about the proper treatment protocol, long-term effects and dependence and abuse potential of ketamine (Sisti et al., 2014). Thus, the potential of other NMDA receptor antagonists and anesthetics in the treatment of depression are of interest. Sub-anesthetic doses of nitrous oxide (laughing gas), which also acts as NMDA receptor antagonist, has promoted antidepressant-like effects in a small pilot study done on treatment resistant depressed patients (Nagele et al., 2015). Also NMDA receptor glycine binding site partial agonist GLYX-13 rapidly alleviated depression symptoms in depressed patients (Preskorn et al., 2015)

Before ketamine was tested for treatment of depression, studies about antidepressant potential of isoflurane anesthesia were conducted (Langer et al., 1985, 1995). The rationale for the experiments came from finding that under isoflurane anesthesia patients demonstrate similar burst suppression in the electroencephalogram as recorded during ECT (Langer et al., 1985). The efficacy of isoflurane in treatment resistant depressed patients was found to be similar to ECT in these small clinical trials and the finding was recently replicated (Langer et al., 1985, 1995; Weeks et al., 2013). Isoflurane treatment, however, did not produce as severe cognitive side effects as seen with ECT. The potential of isoflurane in the treatment of depression has not been widely studied and there are additional studies with controversial findings about the efficacy of burst suppression anesthesia in the treatment of depression (García-Toro et al., 2001). It remains to be studied if burst suppression is the critical factor in the antidepressant effects of anesthesia or ECT and if anesthesia could be used in the treatment of depression.

In addition to anesthetics, the cholinergic muscarinic receptor antagonist scopolamine and serotonin agonist psilocybin have demonstrated rapid antidepressant effects in depressed patients (Carhart-Harris et al.; Drevets and Furey, 2010; Furey and Drevets, 2006). The pharmacological effects of these drugs differ from those of ketamine, isoflurane and nitrous oxide indicating that the mechanisms underlying rapid antidepressant effects are complex and require further characterization.

3. AIMS OF THE STUDY

Pharmacological enhancement of brain plasticity could be beneficial in the treatment of psychiatric disorders, especially depression. Since TrkB receptor is involved in regulation of brain plasticity it could be a favorable target for drug development. Our purpose was to develop a method that would be suitable for screening of novel TrkB receptor activators or inhibitors or molecules that would enhance the effects of BDNF. In addition, we aimed at characterizing further the mechanisms how the already existing TrkB activators, the monoaminergic antidepressant drugs, promote TrkB activation. Recently, TrkB receptor has been also linked to the rapid antidepressant-like effects of ketamine. In addition to ketamine, isoflurane anesthesia has been demonstrated to rapidly reduce depression symptoms of treatment resistant depressed patients. Our aim was to examine molecular, structural and behavioral effects of isoflurane in rodents to elucidate the putative neurobiological basis of the antidepressant effects of isoflurane.

The specific aims of the study were:

1. To develop novel methods to screen for drugs regulating TrkB receptor phosphorylation (I)
2. To elucidate the mechanisms of antidepressant-induced TrkB activation (II,III)
3. To investigate the neurobiological basis of antidepressant effects of isoflurane anesthesia (IV)

4. MATERIALS AND METHODS

The materials and methods personally used by the author are summarized here. Detailed information of the additional materials and methods can be found in the original publications.

4.1 Animals

Adult (2-4 months) or pup (P5-P21) C57BL/6JRccHsd mice (Harlan Laboratories, Venray, Netherland), Bdnf+/–, Bdnf–/–, B6.Cg-Tg(Thy1- YFPH)2Jrs/J (Thy1-YFP, Jackson Laboratories, Bar Harbor, ME, USA) and transgenic mice overexpressing flag-tagged TrkB or truncated TrkB.T1 and their wild type littermates were maintained in the animal facility of University of Helsinki, Finland, under standard laboratory conditions (21°C, 12-hour light-dark cycle, lights on at 6 A.M.) with free access to food and water. Littermates were randomly assigned to different treatment groups. All the experiments were carried out according to the guidelines of the Society for Neuroscience and were approved by the County Administrative Board of Southern Finland.

4.2 Drug treatments

Fluoxetine (HCl salt; Orion Pharma, dissolved in 0,9% NaCl, 30 mg/kg) was intraperitoneally injected to mice and the mice were culled 1 h later (II, SDS-PAGE zymography). Isoflurane (Vetflurane®, Virbac) anesthesia was induced with 4% isoflurane for 2 min, after which the mouse freely inhaled isoflurane (3,0 % for 1 min, then 2 % for maximum of 30 minutes; airflow: 0.3-0.5 l/min) in the chamber of mask. Halothane anesthesia was conducted with similar concentrations and protocol as isoflurane anesthesia. Body temperature was maintained with a heat pad throughout the treatment. Sham mice were kept in the induction chamber for 2 min without isoflurane. NBQX (2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide, Tocris Bioscience, Bristol, UK) was injected (i.p., 10 mg/kg; dissolved in saline) 10 min before sham/isoflurane treatment. Mice were culled by cervical dislocation while still under anesthesia or following the described recovery periods after stunning with CO_2. Control mice were culled by cervical dislocation after stunning with CO_2.

4.3 Cell culture

4.3.1 Fibroblasts

TrkB expressing MG87 fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 1% penicillin/streptomycin, 1% l-glutamine and 400 µg/ml G418. The cell line was maintained in a cell incubator (5% CO2, 37 °C).

4.3.2 Primary neuronal cultures

For the primary neuronal cultures, hippocampi or cortex was dissected from E18 rat embryos and the tissue dissociated in papain solution (in mg: 10 DL-Cysteine-HCl, 10 BSA, 250 glucose, ad 50 ml PBS (pH 7.4); 10 min, 37 °C). Next the cells were triturated and suspended in a medium containing 9.8 ml of Ca^{2+}/Mg^{2+} free HBBS, 1 mM sodium

pyruvate, 10 mM HEPES (pH 7.2) and 10 µl DNAse I. The cells were plated onto poly-L-lysine (Sigma–Aldrich) coated cell culture plates. Neurons were maintained in Neurobasal medium supplemented with 2% B27, 1% penicillin/streptomycin and 1% L-glutamine and fresh medium was added twice a week.

4.4 Enzyme-linked immunosorbent assay (ELISA)

4.4.1 Conventional pTrkB ELISA

Cell homogenates were transferred from culture plates into pre-coated (sc-11-R, Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:500–1000 in Optacoat™; overnight at +4 °C) and pre-blocked (2% bovine serum albumin (BSA)/phosphate buffered saline-Tween 20 (PBS-T); 2 h at room temperature) white 96-well Optiplate™ (PerkinElmer Oy, Espoo, Finland) plates and 3% BSA/PBS-T (+2 mM Na_3VO_4 tyrosine phosphatase inhibitor) added ad 200 µl. After overnight incubation at +4 °C the wells were washed with PBS-T (4 × 300 µl) and anti-phosphotyrosine antibody was added to the wells (in house biotinylated PY20; AbD Serotec, Kidlington, UK; 1:1000 in 2% BSA/PBS-T; overnight at +4 °C). Following sequential washes and horseradish peroxidase (HRP)-coupled streptavidin antibody incubation (1:10,000 in 2% BSA/PBS-T; O/N at 4 °C) 100 µl of enhanced chemiluminescence (ECL) substrate mix was added to the wells and luminescence measured with Varioskan Flash plate reader (Thermo Fisher Scientific Oy).

4.4.2 In situ TrkB ELISA

For the in situ phospho-Trk ELISA, dissociated cells were directly plated onto UV-sterilized ELISA plates pre-coated (sterile filtered sc-11-R, Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:500–1000 in Optacoat™; overnight at +4 °C) and pre-blocked (sterile filtered 2% BSA/PBS-T; 2 h at room temperature). After drug and/or BDNF treatment, the plates were put on ice, medium discarded and lysis buffer applied (10–25 µl). Next, the plates were rigorously shaken in a cold room for 30–60 min (800 rpm, Labsystems Wellmix, Thermo Fisher Scientific Oy) after which 3% BSA/PBS-T (+2 mM Na_3VO_4 tyrosine phosphatase inhibitor) was applied ad 200 µl (96-multiwell) or ad 50 µl (384-multiwell). After overnight incubation at +4 °C, the wells were washed and ELISA assay continued as described above.

4.5 Proof-of-concept small molecule screening

For the small molecule screening, MG87-trkB fibroblasts were cultured directly on 96-well ELISA plates. Next day, the cells (at ~80 % confluency) were pre-stimulated with vehicle or with Spectrum Collection compounds for 15 min (5 µM; n = 1; +37 °C) using Biomek FXp workstation (Beckman Coulter Finland Oy, Helsinki, Finland) and then post-stimulated with vehicle or with BDNF (5 ng/ml; +37 °C) for another 15 min using Multidrop Combi (Thermo Fisher Scientific Oy). After the stimulations, the medium was discarded, cells were lysed in ProteoJET™ membrane protein extraction buffer and ELISA analyses run as described. Compounds regarded as hits (response to BDNF ± 3× standard deviation (STDEV)) were re-tested in triplicates and the compounds that still passed the hit criteria were analyzed in a dose-response assay (0.25, 1, 5, 25 µM; n = 3) (inhibitors with and activators without BDNF post-treatment).

4.6 Brain sample collection

The brain samples were dissected on a cooled dish and homogenized in NP buffer (137mM NaCl, 20mM Tris, 1% NP-40, 10% glycerol, 48mM NaF, H_2O, Complete inhibitor mix (Roche), 2mM Na_3VO_4). After minimum of 15 min incubation on ice, samples were centrifuged (16000 x g, 15 min, +4 °C) and the resulting supernatant was collected for further analysis. Sample protein concentrations were measured using Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA).

4.7 *Ex vivo* stimulations

The *ex vivo* BDNF stimulation assay was performed according to Knüsel et al (1994) with slight modifications. After dissection the samples were chopped to microslices with scalpel. The slices were transferred in fresh tubes with 10% FCS in supplemented neurobasal medium and centrifuged (6000 x g, 1 min, +4 °C). Fresh medium was added to the slices according to their weight and the slices were resuspended to the medium and divided to stimulation tubes. The medium was removed and 300 µl of prewarmed (+ 37 °C) neurobasal medium +10% FCS with or without BDNF (Peprotech) or NGF (Promega) was added. The tubes were closed and incubated at +37°C for 15 minutes gently shaking. Finally the tubes were put on ice, spun down, the medium removed, the pellet was rinsed once with PBS and then the samples were homogenized in NP++ buffer.

4.8 Western blot

Western blotting analysis was conducted from medial prefrontal cortex (including prelimbic and infralimbic cortices), somatosensory cortex and the whole hippocampus. Immunoprecipitation (500 µg protein) was carried out using anti-Flag M2 antibody (F1804; Sigma-Aldrich). For immunoprecipitation the samples (500 µg protein) were rotated overnight with 5 µl of the antibody at +4°C, then 30 µl 50% G-sepharose slurry was added (rotation 2 h at +4°C) and the precipitated samples were washed 3x500µl PBS +2 mM Na_3VO_4). The samples were heated in Laemmli buffer for 5 min at +100 °C. Immunoprecipitated or unprocessed samples (50 µg protein) were separated with SDS-PAGE under reducing conditions and blotted onto a PVDF (polyvinylidene difluoride) membrane (300mA, 1 hour, 4°C). Membranes were incubated with the following primary antibodies: anti-p-TrkB^{Y816} (1:1000; from Dr. Moses Chao, Skirball Institute, NY, USA), anti-p-TrkB^{Y816} (#4168; 1:1000; Cell signaling technology (CST)), anti-p-TrkA/B$^{Y490/Y515}$ (#9141; 1:1000;CST), anti-p-TrkA/B$^{Y674\text{-}5/Y706\text{-}7}$ (#4621S; 1:1000; CST), anti-TrkB (1:2000; BD Transduction Laboratories, San Jose, CA, USA), anti-p-CREB^{S133} (#9191S; 1:1000; CST), anti-p-Akt^{Thr308} (#4056S; 1:1000; CST), anti-p-mTOR^{S2481} (#2974S; 1:1000; CST), anti-p-p70S6K$^{T421/S424}$ (#9204S; 1:1000; CST), anti-p-4E-BP1$^{T37/46}$ (#2855; 1:1000; CST), anti-p-GSK3β^{S9} (#9336; 1:1000; CST), anti-p-eEF2^{T56} (#2331; 1:1000; CST), anti-Trk (sc-11; 1:1000; Santa Cruz Biotechnology (SCB)) and anti-GAPDH (sc-25778; 1:10000; SCB). Further, the membranes were washed with TBS/0.1% Tween (TBST) and incubated with horseradish peroxidase conjugated secondary antibodies (1:10000 in non-fat dry milk, 1 hour at room temperature; Bio-Rad). After subsequent washes, secondary antibodies were visualized using enhanced chemiluminescence (ECL Plus, ThermoScientific, Vantaa, Finland) and detected by Fuji LAS-3000 camera (Tamro Medlabs, Vantaa, Finland).

4.9 SDS-PAGE zymography

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) -zymographty was used to examine the activity of tissue plasminogen activator (tPA). For tPA activity assay, freshly dissected brain samples were homogenized into buffer consisting of 0.1 M Tris-HCl (pH 8.0), 2.5% Triton-X-100, 10 μM leupeptin, 10 μg/ml aprotinin, 1 mM phenylmethanesulfonylfluoride (PMSF). Samples and controls (human recombinant tPA) were loaded under non-reducing conditions in SDS-PAGE gels ±human plasminogen (Sigma-Aldrich) and pre-heated non-fat dry milk at run at low current (~15–20 mA) overnight (O/N) at cold bath. Next the gels were rinsed thoroughly with 2.5% Triton X-100 to remove SDS and allow proteins to renaturate. Then the gels were rinsed thoroughly with 10 mM $CaCl_2$ in 50 mM Tris-HCl (pH 7.6) to remove Triton X-100 and the caseinolysis was allowed to occur by incubating the gels at +37°C for 16–24 h in the same solution. Caseinolytic areas were shown as translucent areas when the gels were stained with Coomassie Brilliant Blue.

4.10 Immunohistochemistry and dendritic spine analysis

The density of dendritic spines was analyzed in fixed cortical sections from B6.Cg-Tg(Thy1- YFPH)2Jrs/J (Thy1-YFP, Jackson Laboratories, Bar Harbor, ME, USA) mice. Animals were transcardially perfused at 24 hours after isoflurane/sham treatment under deep pentobarbital anesthesia with 4% paraformaldehyde in phosphate buffer (PB) 0.1M and post-fixed for 1 hour. Floating sections (50 μm thick) were cut using a vibratome (VT 1000S, Leica, Germany) and processed for immunohistochemistry as follows: PBS wash, blocking for 1 h (10% normal goat serum in PBS/0,2% Triton-X100), anti-YFP antibody overnight (chicken polyclonal 1:1000; Abcam), PBS wash, Alexa-488-conjugated goat secondary antibody for 2 h (1:200; Invitrogen), PB wash and mounting using fluorescence medium (Dako). Images were obtained using a confocal microscope (Leica TCS SP5II HCS), and pyramidal neurons from the medial prefrontal cortex (mPFC) and somatosensory cortex (SSCx) were selected with the following criteria: intense fluorescence, soma located in layer V, and primary apical dendrite >200 μm long. We imaged the dendrites in three ~65 μm long segments (proximal/medial/distal). We distinguished different types of dendritic spines: (i) stubby (protrusion length <1.5 μm); (ii) mushroom (clearly visible head with a diameter >1.5 times the average length of the neck, and the total length of the protrusion <3 μm) and; (iii) filopodia/thin (the length of the protrusion >3 μm or non-headed 1.5-3 μm protrusion).

4.11 Quantitative real-time polymerase chain reaction (qPCR)

RNA was extracted using Trizol® reagent (Thermo Scientific) and treated with DNAse I mix (Fermentas GmbH) and then reverse transcribed using oligo(dT) primer and RevertAid First Strand cDNA synthesis kit (Thermo Scientific). The control reactions without reverse transcriptase were also performed. The amount of cDNA was quantified using Maxima SYBR green qPCR master mix (Thermo Scientific) by real-time PCR. Total *Bdnf* cDNA was amplified using the following primers: 5´-GAAGGCTGCAGGGGCATAGACAAA-3´ and 5´-TACACAGGAAGTGTCTATCCTTATG-3´. For normalization GAPDH cDNA levels were analyzed with the following primers 5 ´-GGTGAAGGTCGGTGTGAACGG-3´ and 5 ´-CATGTAGTTGAGGTCAATGAAGGG-3´. Ct

values from each sample were obtained using the LightCycler 480 software (Roche Diagnostics Ltd.).

4.12 Behavioral experiments

4.12.1 Forced swim test

For the forced swim test a mouse was placed into a glass cylinder (diameter 19 cm, height 24 cm) filled with water (21±1°C) to the height of 14 cm. The latency to the first immobility period (passive floating, when the animal was motionless or doing only slight movements with tail or one hind limb) and immobility during the last 4 minutes of the 6 min test was measured.

4.12.2 Open field test

The open field test was performed for 30 min in an illuminated (300 lux) transparent acrylic cage (length 28.5 × height 8.5 × width 20 cm) (Med Associates). Interruptions of infrared photo beams were used to calculate the overall distance traveled (cm) and time spent in the center of the arena.

4.12.3 Water maze

Noldus EthoVision XT 10 program (Noldus Information Technology, The Netherlands) was used for monitoring the mouse behavior in the water maze test. The system consisted of a black circular swimming pool (Ø 120 cm) and a transparent escape platform (Ø 10 cm) submerged 0.5 cm under the water surface in the centre of one of four imaginary quadrants. The animals were released to swim (in random positions) from all three other quadrants facing the wall and the time to reach the escape platform (maximum time 60 s) and the swimming distance were measured in every trial. Two training blocks consisting of three trials each were conducted daily. The interval between trials was 4-5 min and between training blocks about 4 hours. The hidden platform remained in a constant location for 3 days (6 initial training sessions) and was thereafter moved to the opposite quadrant for 2 days (4 reverse training sessions).

4.13 Statistical tests

The statistical analyses were conducted with unpaired two-tailed Student's t-test, Mann Whitney U test (non-normally distributed data), one-way analysis of variance (ANOVA) or two-way ANOVA. Tukey HSD, Newmann-Keuls or Dunnett's test were used for post hoc analysis. Statistically significant p value was set to ≤ 0.05. The results are represented as mean ± SEM (standard error of mean).

5.RESULTS

5.1 Development of phospho-Trk ELISAs (I)

Enzyme-linked immunosorbent assay (ELISA) is a widely used method in molecular biology. Sandwich ELISA is a method conducted on a multiwell plate where capture antibody is immobilized to the bottom of the plate. After sample incubation the non-specifically bound proteins are washed away and a secondary antibody coupled to an enzyme is added. The amount of the protein of interest captured by the primary antibody can then be measured by adding a substrate to the enzyme and detecting the enzymatic reaction by colorimetric or luminometric assays. We set up a conventional sandwich ELISA where we use Trk receptor antibody as a capturing antibody and biotinylated phosphotyrosine antibody as a secondary antibody. A HRP-coupled streptavidin antibody is used as a tertiary antibody and after adding substrate to the HRP the amount of tyrosine phosphorylated Trk receptors can be measured. This conventional phospho-Trk ELISA was used in publications I,II and III in several experimental conditions to detect the phosphorylated Trk receptors from cultured neurons and MG87-TrkB cells.

To optimize the ELISA protocol we tested different coating conditions, primary antibodies and lysis buffers and found that Optacoat buffer, SC-11 rabbit polyclonal Trk antibody and ProteoJet lysis buffer significantly increased the signal to noise ratio when compared to other buffers or antibodies (I).

Next, we modified the standard sandwich pTrk ELISA to an *in situ* format where all the steps including cell culture, stimulation, antibody incubation and detection can be done in the same plate (I). Transferring cell lysate from the cell culture plate to the ELISA plate is time consuming and laborious and can be avoided in the *in situ* ELISA. In the *in situ* pTrk ELISA MG87 fibroblasts overexpressing TrkB receptors are cultured on UV-sterilized Optiplate™ HB 96 or 384 well plates that are previously coated with SC-11 Trk antibody and blocked. The cells are incubated 24 hours after which they can be stimulated. After the stimulation lysis buffer is added to the wells and the plate is vigorously shaken at + 4 ^{O}C for 1 h. When the cells have been lysed the ELISA protocol is continued similarly to the conventional pTrk ELISA.

We also showed that the in situ ELISA can be upscaled from 96 to 384 well plate making it suitable for high throughput screening (I). As a proof of concept we performed a screening of a library of 2000 compounds and identified several compounds that were able to induce TrkB phosphorylation or inhibit or potentiate BDNF-induced TrkB activation. Among the TrkB activators was betamethasone valerate and epoxygedunin and among the inhibitors for example tomatine, gramicidin and fenbendazole.

5.2 Mechanisms of antidepressant induced TrkB activation - BDNF and serotonin transporter (SERT) are dispensable for TrkB activation by antidepressant drugs (II)

Saarelainen et al. (2003) first described that antidepressant drug imipramine can induce TrkB receptor phosphorylation in the mouse hippocampus and frontal cortex and later it was demonstrated that TrkB activation appears to be a common feature of ADs with different pharmacological properties (Rantamäki et al., 2007). How ADs promote TrkB activation was, however, unknown and especially it remained unclear whether BDNF is required for the effect. It is impossible to directly measure BDNF release in the brain and thus TrkB phosphorylation has been used as a surrogate measure for BDNF release. TrkB activation can occur, however, independently of BDNF via transactivation (Lee and Chao, 2001). It has been also suggested that antidepressant amitriptyline and 7,8—dihydroxyflavone are direct TrkB receptor agonists (Jang et al., 2009, 2010).

In addition to the full-length TrkB receptor (145 kDa), ADs seem to induce phosphorylation of a protein ~105 kDa in size (II). It has been suggested to be an immaturely glycosylated form of the TrkB receptor and indeed we found that digestion with endoglycosidase H, which deglycosylates proteins, reduced the molecular weight of the 105 kDa protein (II). To support the assumption that the 105 kDa protein is an isoform of TrkB, in TrkB overexpressing mice also the baseline phosphorylation of this protein is increased. Furthermore, 1NaPP1 inhibitor treatment of $TrkB^{F616A}$ mutant mice decreased also the imipramine-induced phosphorylation of the 105 kDa protein.

The TrkB activation occurs in 30 minutes after imipramine injection but no changes have been detected in the BDNF protein or mRNA levels after acute AD treatment, suggesting that local release of BDNF could mediate the effect (Nibuya et al., 1995b; Rantamäki et al., 2007; Saarelainen et al., 2003). It has been shown in hippocampal slices that proBDNF and BDNF can be released together with tPA in response to increased neuronal activity (Pang et al., 2004). tPA cleaves proBDNF to BDNF and thus could increase the BDNF concentration in the synaptic cleft resulting in increased TrkB phosphorylation. We studied with SDS-PAGE zymography if fluoxetine treatment (1h) increases the enzymatic activity of tPA in the hippocampus but no difference in the tPA activity or BDNF protein levels were found between saline and fluoxetine treated animals (II). More importantly, imipramine activated TrkB in the brains of BDNF cKO animals whose hippocampal BDNF levels are below detection limits. This indicates that antidepressants can activate TrkB receptor also in the absence of BDNF.

Since the TrkB receptor activation by ADs occurred independently of BDNF we investigated if ADs would facilitate adenosine receptor –mediated TrkB transactivation (II). Previously, adenosine A_{2A} receptors have been shown to transactivate TrkB receptors *in vitro* and *in vivo* (Lee and Chao, 2001; Wiese et al., 2007). Blocking adenosine A_{2A} receptors, however, did not affect the ability of imipramine to induce TrkB phosphorylation suggesting that adenosine mediated transactivation is not involved in the antidepressant-induced TrkB activation (II). To study the possibility that ADs act as direct agonists of TrkB receptor and can induce the TrkB phosphorylation similarly to BDNF, we stimulated hippocampal and cortical neuron cultures with imipramine or

amitriptyline but no increase in TrkB phosphorylation was detected (II). These results indicate that antidepressants apparently are not able to directly bind to TrkB receptors.

SERT is the main pharmacological target for fluoxetine and we examined if the effects of fluoxetine on TrkB phosphorylation require binding to SERT. In the SERT knockout mice fluoxetine induced TrkB phosphorylation similarly to wild type mice, indicating that SERT is not required for fluoxetine to activate TrkB receptors (II). Moreover, the extracellular 5-HT concentration in SERT knockout mice is significantly increased but no change at baseline TrkB phosphorylation was detected, suggesting that 5-HT does not directly induce TrkB activation. We further examined the effects of monoamines on TrkB phosphorylation in cortical neuron cultures but 15 minutes incubations with 10 nM-10 μM concentrations of noradrenaline or 5-HT did not affect TrkB phosphorylation.

5.3 Developmental regulation of TrkB activation by antidepressant drugs and BDNF (III)

Previously it has been shown that BDNF-induced TrkB phosphorylation is developmentally regulated (Knusel et al., 1994). We were able to reproduce these findings using BDNF *ex vivo* stimulation in microslices prepared from mouse hippocampus of different developmental stages (P5-P21) demonstrating that responsiveness of TrkB to exogenous BDNF decreases dramatically after P12 (III). Intriguingly, we further discovered that at this particular developmental time point the antidepressant drug imipramine starts to activate TrkB receptor in the mouse PFC and hippocampus when injected intraperitoneally (30 mg/kg), whereas at the earlier timepoints imipramine did not increase TrkB phosphorylation from the baseline. Also imipramine-induced phosphorylation of CREB, which is activated downstream of the TrkB-PLCγ pathway, appeared at P12. To demonstrate the role of BDNF in the TrkB activation *in vivo*, we measured the baseline TrkB phosphorylation levels in BDNF heterozygous knockout mice and BDNF knockout mice and found that at P11 the TrkB phosphorylation was significantly lower in BDNF heterozygous mice and knockout mice when compared to wild type mice. Intriguingly, in the adulthood there was no difference in TrkB phosphorylation levels in BDNF conditional knockout or heterozygous knockout mice and wild type mice, supporting the finding that BDNF is critical for the baseline phosphorylation of TrkB at early postnatal age but dispensable in the adulthood.

To examine what could underlie the developmental switch in the TrkB responsiveness to BDNF and ADs we conducted several experiments. The expression of the truncated isoform of TrkB increases at the same developmental timepoint when the responsiveness of TrkB to BDNF diminishes (Fryer et al., 1996). We used transgenic mice lacking the TrkB.T1 receptor to examine the role of TrkB.T1 receptor in the TrkB responsiveness to BDNF and ADs (III). The phosphorylation of TrkB in response to BDNF decreased similarly in the TrkB.T1 KO mice microslices as in wild type mice microslices, indicating that TrkB.T1 receptor expression does not regulate the activation of the full-length TrkB receptor by exogenous BDNF. Also in adult TrkB.T1 KO mice imipramine induced TrkB phosphorylation normally.

Chronic treatment with fluoxetine has been shown to promote neuronal plasticity and restore more immature developmental stage in the adult rodent brain (Karpova et al., 2011; Maya Vetencourt et al., 2008). We tested if chronic fluoxetine treatment (21 days)

could restore the TrkB responsiveness to BDNF in adult mice but no difference between fluoxetine-treated and saline-treated animals was observed, implicating that the juvenating effects of fluoxetine were not enough to restore the responsiveness of TrkB to *ex vivo* BDNF (III).

By using a cell-free kinase assay we investigated if TrkB receptor itself had undergone some structural modifications during development that could explain the decrease in BDNF responsiveness (III). BDNF stimulation of cell-free lysates from adult brain in the presence of ATP induced TrkB phosphorylation, demonstrating that the receptor can still be activated by BDNF. In addition, NGF *ex vivo* stimulation could readily induce TrkA activation at P24 suggesting that the ability of neurotrophins to penetrate the tissue is not the reason for the reduced responsiveness of TrkB to BDNF and that the developmental regulation may be a specific trait for BDNF.

5.4 Isoflurane activates TrkB signaling, enhances synaptic plasticity and induces antidepressant-like behavior (IV)

Volatile anesthetic isoflurane has previously been shown to relieve depression symptoms in treatment-resistant depressed patients (Langer et al., 1985, 1995; Weeks et al., 2013). We examined the ability of isoflurane to affect BDNF-TrkB signaling and found that isoflurane anesthesia readily activates TrkB receptor by inducing phosphorylation of the catalytic domain Y706/7 and PLCγ binding site Y816 (but not Shc binding site Y515) of TrkB (IV). In the prefrontal cortex and hippocampus of anesthetized mice phosphorylations of CREB, Akt (not hippocampus), mTOR (not hippocampus), P70S6K, 4EBP-1 and GSK3beta were significantly increased. These TrkB downstream signaling molecules have been previously shown to be phosphorylated by conventional antidepressant drugs and especially by rapid-acting antidepressant ketamine (Li et al., 2010; Rantamäki et al., 2007; Saarelainen et al., 2003). Interestingly, similarly to antidepressant drugs, isoflurane does not induce TrkB phosphorylation in the hippocampus of mouse pups at P8 (Fig. 5).

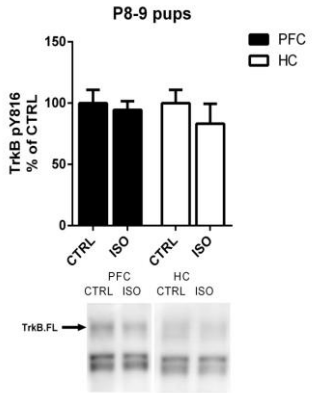

Fig 4. Isoflurane anesthesia did not induce TrkB activation in the prefrontal cortex or hippocampus of P8-9 wild type mice pups. Abbreviations: CTRL, control treatment; ISO, isoflurane treatment 15 min; PFC, prefrontal cortex; HC, hippocampus; TrkB.FL, full-length TrkB receptor.

The activation of TrkB by isoflurane occurs quickly, within few minutes (IV). When measured 15 minutes after the end of the anesthesia the phosphorylation of TrkB has returned close to the baseline indicating that the phosphorylation is transient. Activation of the signaling molecules seems to be regulated in a similar manner, since the activation of the mTOR and CREB occurs already in 2 minutes. Pretreatment with AMPA receptor antagonist NBQX did not affect the ability of isoflurane to activate TrkB receptor and the downstream signaling molecules, suggesting that the effects of isoflurane on TrkB signaling are not mediated via AMPA receptors.

Importantly, isoflurane activated TrkB receptors also in the hippocampus of BDNF conditional knockout mice, suggesting that TrkB activation by isoflurane occurs via transactivation and is not mediated by BDNF (IV). To investigate the putative mechanisms underlying the isoflurane-induced TrkB transactivation we conducted a mass spectrometry analysis of proteins interacting with TrkB in the adult brain. We found that PSD93, a synaptic scaffolding protein, interacts with TrkB. Since volatile anesthetics have been shown to promote disruption of PSD93 and PSD95 interaction from NR2B-subunits of NMDA receptors *in vitro* (Fang et al., 2003), we investigated if isoflurane regulates the interaction between PSD93 and TrkB. We stimulated RN33 (immortalized raphe nuclei neuronal precursor cell line) cells with isoflurane and found that isoflurane dose-dependently promoted disruption of TrkB-PSD93 interaction. Moreover, reducing PSD93 expression in RN33 cells with siRNA increased the basal TrkB phosphorylation levels, suggesting that releasing TrkB from the PSD93 protein complex facilitates its activation. Interestingly, naïve PSD93 knockout animals showed antidepressant-like behavioral effect in the FST.

To further elucidate the mechanisms that could be involved in the TrkB activation by isoflurane we examined the Src family kinases. Previously, the Src family kinases have been shown to be involved in the TrkB transactivation (Rajagopal and Chao, 2006). *In vitro,* isoflurane treatment increased association of the activated Fyn with TrkB and pretreatment with PP1 (protein phosphatase 1; Src inhibitor) abolished the effects of isoflurane on TrkB phosphorylation (IV).

Since the signaling pathways activated by isoflurane have been linked with synaptogenesis, cell survival and plasticity we examined the effects of isoflurane anesthesia on LTP in Schaffer collaterals in hippocampus. Indeed, we found that in slices from mice that were anesthetized for 30 minutes 24 hours before the electrophysiological recordings the LTP was enhanced (IV). Interestingly, the LTP enhancement could be blocked with picrotoxin pretreatment, suggesting that GABAergic component is involved. Moreover, we found that GABAergic excitability is increased at this time point and this finding was supported by increased FosB immunoreactivity (marker of neuronal activity) in GABAergic cells of CA1 area of the hippocampus.

The increase in LTP was not, however, accompanied by an increase in hippocampal dendritic spine number (IV). In addition, even though signaling pathways linked with synaptogenesis are clearly activated in the PFC during the anesthesia, no significant differences were seen in dendritic spine number or morphology in the PFC either.

TrkB signaling has been implicated in the antidepressant-like behavioral effects in the FST. Since isoflurane robustly activates TrkB signaling, we tested if isoflurane produces a behavioral response in the FST (IV). Indeed we found that isoflurane decreased the

immobility time indicating an antidepressant-like phenotype. Interestingly, this behavioral response was abolished in the TrkB.T1 overexpressing mice suggesting that TrkB signaling is necessary for the behavioral effects of isoflurane in the rodents.

To further study the behavioral effects of isoflurane we used the learned helplessness model. Isoflurane produced a decrease in the escape latency if it was administered 24h after the pretest and the actual test was conducted 6 days after the anesthesia indicating an antidepressant-like effect (IV). We also examined the ability of isoflurane to counteract the effects of chronic neuropathic pain on behavior of sciatic nerve cuffed mice. After 8 weeks of sciatic nerve cuffing the mice showed increased latency to eat the pellet in the novelty suppressed feeding test. Markedly, the anxiodepressive behavioral phenotype was normalized with a single isoflurane anesthesia. These data implicate that isoflurane produces antidepressant-like behavioral phenotype and may have rapid and long-lasting antidepressant effects similar to ketamine.

6. DISCUSSION

TrkB neurotrophin receptor is an interesting target for drug development. The receptor is able to activate downstream signaling cascades that facilitate neuronal excitability and regulate translation and transcription of proteins promoting neuronal survival, proliferation, differentiation and plasticity (Huang and Reichardt, 2003). Impaired brain plasticity has been linked to many brain disorders, including depression, stroke and neurodegenerative disorders, and promoting TrkB activity could be beneficial in these conditions (Castrén and Rantamäki, 2010). Delivering exogenous BDNF to the patients is difficult, since the neurotrophin cannot reach the brain if taken orally and even injecting it directly to the brain does not guarantee its efficacy because of its poor tissue penetrance (Mufson et al., 1994). Developing drugs that directly target the TrkB receptor would be a possible approach to circumvent these issues.

We developed an *in situ* ELISA method that is suitable for screening of TrkB activators and inhibitors (I). The *in situ* ELISA allows skipping of some laborious parts of the conventional ELISA protocol and provides a possibility for high throughput screening, also for industrial purposes. We showed that the phosphoTrk ELISA works in 384 well plate format and applying it even to 1536 well plate format could be possible. Previously, primary sensory neurons have been cultivated on ELISA plate (Balkowiec and Katz, 2000), and our purpose was to further develop the *in situ* ELISA to be suitable for primary neurons, however, finding optimal conditions to support the survival of the primary neurons in the ELISA plate for long enough was not yet possible.

The *in situ* ELISA method can also be used for discovering compounds that facilitate the effects of BDNF. A compound that would enhance the effects of endogenous BDNF would be physiologically relevant and allow more specific targeting of the drug effect. Activating TrkB signaling randomly all over the brain could promote risk for epileptogenic activity and randomly strengthen synapses creating more "noise" in the neuronal networks. Yet, activation of TrkB receptor independently of BDNF could be useful especially in the situations where the expression or release of BDNF is reduced, such as in patients with BDNF met/met polymorphism.

Intriguingly, TrkB activating drugs already exist and are widely used by millions of people – the antidepressant drugs. It has been previously demonstrated that antidepressant drugs can activate TrkB receptor, at least in the rodent brain (Rantamäki et al., 2007; Saarelainen et al., 2003). The clinical efficacy of different ADs is fairly similar and their primary target in the brain are the widely projecting monoaminergic systems. Even though the ADs primarily target the monoamines, they have effects that are not directly mediated via the monoaminergic system. Especially the fact that ADs require weeks before their antidepressive effects in humans appear, even though their effects on the monoamines are acute, has evoked interest in examining the effects of these drugs on targets outside the monoaminergic systems. The neurotrophin and network theories of depression and antidepressant action suggest that brain plasticity is involved in the pathophysiology of depression and in antidepressant action (Castrén and Hen, 2013; Duman and Monteggia, 2006). One of the mediators of the plasticity-inducing effects of ADs is BDNF, which acts through TrkB (Karpova et al., 2011; Maya Vetencourt et al., 2008). We examined the mechanism of antidepressant-induced TrkB

activation and showed that it occurs independently of BDNF and monoamines, suggesting that ADs transactivate TrkB receptor (II).

Neuronal activity is an important regulator of TrkB expression and localization on cell surface (Du et al., 2000; Merlio et al., 1993) but transactivation of the receptor could occur also in the absence of increased neuronal activity. In addition to receptors on the cell surface also receptors residing inside the cell could be transactivated (Schecterson and Bothwell, 2010). Activation of the intracellular pool of the TrkB receptors could theoretically produce stronger response than the BDNF-dependent activation of limited amount of cell surface receptors. Transactivation of the intracellular receptors could also promote activation of immature Trk receptors. AD (and isoflurane) treatment constantly induced phosphorylation of an unknown 105 kDa protein that we assume to be an immaturely glycosylated form of TrkB. Glycosylation of the receptor can inhibit ligand-independent activation of the receptor, and it has been shown that ERK pathway is not activated via the immaturely glycosylated TrkA receptor (Watson et al., 1999a). Since ADs transactivate TrkB receptor and do not induce signaling via ERK pathway, this further supports the possibility that immaturely glycosylated form of the receptor is activated by these drugs. Furthermore, in humans an N-terminal truncated form of TrkB receptor is expressed and it is not targeted to the membrane but can be phosphorylated (Luberg et al., 2010).

In addition, it is not known if TrkB transactivation promotes retrograde transport of the activated receptors toward the soma. The retrograde transport has been implicated to be important for the survival-promoting effects of neurotrophins in the PNS (Watson et al., 2001). Nonetheless, increased CREB phosphorylation indicates that proteins in the soma are also activated by ADs and isoflurane (II, IV). Transactivation of the TrkB receptor by ADs does not activate the Shc binding site (Y515) of the receptor (II) that is readily phosphorylated upon BDNF binding to the receptor (Segal et al., 1996). In addition, ADs did not induce Akt or ERK activation that are in the canonical neurotrophin signaling pathway mediated via the Shc binding site (II). The lack of Y515 phosphorylation could be related to the conformational properties of the receptor when transactivated or to the possibly different subcellular compartment where the transactivated receptors are localized. Antidepressant drugs, thus, do not induce completely similar TrkB activation as BDNF.

In addition to conventional ADs we found that isoflurane anesthesia can induce TrkB receptor phosphorylation in the adult rodent brain (IV). The TrkB phosphorylation was induced also by other inhalation anesthetics, sevoflurane and halothane, suggesting that the effect may be common to all anesthetics. Similarly to ADs, TrkB and CREB are activated during isoflurane anesthesia (Rantamäki et al., 2007). The TrkB activation by isoflurane occurs already in couple of minutes, which is more quickly than by ADs (II,IV). The more rapid effect of isoflurane is probably related to the different kinetics of volatile, inhalation anesthetic when compared to intraperitoneally injected drugs. Interestingly, in contrast to ADs (Li et al., 2010), isoflurane can activate Akt and mTOR-P70S6K-4EBP1 –signaling in the PFC (IV). This signaling pathway has been previously shown to be involved in the antidepressant-like behavioral effects and the rapid synaptogenesis enhancing effects of ketamine (Li et al., 2010). Even though ADs and isoflurane are able to activate the TrkB receptors, the differences in the activated signaling cascades could explain some of the disparities in their effects. Isoflurane for example did not regulate

the activity of EEF2 that has been previously shown to be involved in the behavioral effects of ketamine (Autry et al., 2011). Moreover, AMPA receptor blockade by NBQX did not prevent the isoflurane-induced signaling effects, suggesting that AMPA receptor-mediated effects are not critically involved in the regulation of TrkB signaling by isoflurane (IV). However, AMPA receptor has been crucially linked to the antidepressant-like effects of ketamine (Li et al., 2010; Maeng et al., 2008; Zanos et al., 2016).

The ability of ketamine to induce rapid increase in the number of dendritic spines has been suggested to underlie its rapid antidepressant effects (Li et al., 2010, 2011). Even though the synaptogenesis-related signaling is robustly activated by isoflurane, we were not able to demonstrate any increase in dendritic spine number in isoflurane treated mice when examined 24 hours after the anesthesia (IV). Moreover, activity-dependent secretion of BDNF has been linked to the synaptogenesis-promoting effects of ketamine, since the ketamine-induced increase in dendritic spines was abolished in BDNF heterozygous knockout mice (Liu et al., 2012). The ability of isoflurane to transactivate TrkB independently of BDNF could be involved in the differerential effects of isoflurane and ketamine on spines. Futhermore, it would have been important to include ketamine as a positive control in the experiments, since the effects of ketamine on dendritic spines in previous studies have been demonstrated mainly in rats (Li et al., 2010, 2011) and we used mice. In addition, stress pretreatment could have been required, since ketamine has been shown to counteract the stress-induced downregulation in the spine number (Li et al., 2011). Previously, however, general anesthesia has been shown to promote synaptogenesis in rats only at certain developmental stage (~P16) but not in the adulthood, supporting our findings on naïve adult animals (Briner et al., 2010, 2011; Roo et al., 2009). The negative effects of anesthetics on dendritic spine density have been demonstrated in rodents at early postnatal age (<P10) (Briner et al., 2011) and since the developmental shift also in TrkB activation by isoflurane occurs around this same time point, it is tempting to speculate that TrkB signaling could be involved in the synaptogenesis-promoting effects of isoflurane.

The developmental shift in the TrkB response to BDNF and ADs occurs around P12 (III). Even though in the adult rodent brain ADs induce TrkB activation, they do not activate TrkB receptors when injected to mice at early postnatal age (III). This same applies also for isoflurane, since isoflurane anesthesia at P8-9 does not produce TrkB activation (Fig 5.). The developmental shift in the TrkB activation is not related to changes in the structure of the receptor itself but could be explained by changes in the TrkB interacting partners capable of regulating the activation and localization of the receptor (III). We have not been able to demonstrate TrkB activation by ADs in cultured hippocampal or cortical neurons and it is possible that the lack of proper interacting partners at the neuronal cells derived from embryonic rat brain could be an explanation. Also the TrkB transactivation by ADs may require functional neuronal network that would respond to AD treatment in a complex manner recruiting e.g. G-protein coupled receptors, reducing the activity of protein phosphatases or affecting general neuronal activity that would then eventually lead to TrkB activation. Thus, TrkB transactivation by ADs may require processes and mediators that emerge only after certain developmental stage.

The lack of TrkB activation in cell culture by drugs that can promote TrkB activation *in vivo* complicates the study of the mechanisms of AD-induced TrkB activation in detail

using cultured cells. This also has to be taken into account when using the cell-culture based *in situ* ELISA for screening of TrkB regulating drugs. Nonetheless, the maturation of the cultured neurons (or other cells), the cell line used and general culture conditions can affect the ability of different compounds to activate TrkB receptor. Previously e.g. amitriptyline and 7,8-DHF have been shown to activate TrkB receptor *in vitro* (Jang et al., 2009, 2010) and also in our studies we were able to detect isoflurane-induced TrkB activation in RN33 cells (IV).

In addition to the developmental shift in the TrkB activation by ADs, the ability of exogenous BDNF to activate TrkB receptor in *ex vivo* context is developmentally regulated (III). Importantly, the reduction in BDNF-induced TrkB activation occurs at the same time point when the AD effect appears (~P12). Changes in the interactome could also explain the lack of BDNF effect if there are interacting proteins capable of preventing the conformational changes of the receptor upon BDNF binding or the autophosphorylation of the TrkB receptors. We did not examine in this study whether BDNF injection directly to hippocampus of adult mice would increase TrkB activation. The baseline TrkB activation in the brain of BDNF conditional knockout or heterozygous knockout mice is comparable to wild type mice suggesting that BDNF is not regulating the basal level of TrkB phosphorylation in the adulthood (III). In studies where BDNF is injected to the brain, its ability to activate TrkB receptor is not normally investigated, however, in the study by Guo et al. (2014) BDNF injection to adult rat hippocampus could promote phosphorylation of the tyrosine 515 of TrkB. Thus, it is not known for sure if the reduction in TrkB activation by BDNF is a special feature of the *ex vivo* treatment of brain microslices and if it applies or not *in vivo*.

In addition to the activation of molecules linked to synaptogenesis, the enhanced hippocampal LTP 24 hours after isoflurane anesthesia could be accompanied by increased mushroom spine number. Yet, we did not find any difference in the spine morphology between isoflurane and sham treated animals (IV). Interestingly, we found that picrotoxin pretreatment could block the enhanced LTP by isoflurane, which indicates that changes in the GABAergic system are involved in the effects of isoflurane. This was further supported by the finding that the GABAergic excitability was enhanced and the activity of the inhibitory interneurons was increased 24 hours after isoflurane treatment. The explanation for the lack of changes in the excitatory spines could be that the changes occur rather in the amount and/or strength of the inhibitory synapses than in the excitatory synapses.

The changes in the neuronal network excitability could underlie the long-lasting behavioral effects of isoflurane. The long-lasting effects of isoflurane on rodent behavior were demonstrated in the learned helplessness model, where a single isoflurane treatment produced an antidepressant-like effect when the test was conducted 6 days after the treatment (IV). To show that isoflurane has more rapid effects than conventional ADs we used novelty suppressed feeding test. The conventional ADs require weeks of administration before they produce an effect in the novelty suppressed feeding test (David et al., 2009), but a single isoflurane treatment only 12 hours before the test was enough to normalize the behavior of the mice in the neuropathic pain model of depression (IV). Probably already at this time point changes occur in the network function of the isoflurane-treated animals that could underlie the behavioral phenotype.

Ketamine has also been suggested to activate TrkB receptor at subanesthetic dose and the behavioral effect of ketamine in FST was abolished in TrkB conditional knockout mice (Autry et al., 2011). We found that TrkB signaling is required also for the behavioral effects of isoflurane in the forced swim test. If TrkB receptor activation is an important mediator of the rapid antidepressant effects of ketamine and isoflurane, it is interesting why conventional antidepressant drugs then require weeks before their effect arises even though they induce TrkB phosphorylation already after single injection. Differences in the downstream signaling and the possible rapid increase in BDNF translation have been suggested to underlie the differential effects of conventional and rapid-acting ADs (Autry et al., 2011; Li et al., 2010). In addition to antidepressant drugs and anesthetics, also anticholinesterases galantamine and donepezil, used in the treatment of Alzheimer's disease, can induce TrkB, Akt and CREB activation in mouse hippocampus (Autio et al., 2011). Thus, drugs with completely different mechanism of action and different therapeutic effects produce TrkB activation. All these drugs do not produce antidepressant effects in humans, suggesting that TrkB activation solely is not sufficient to guarantee antidepressant efficacy.

We examined the behavioral effects of isoflurane in paradigms that are widely used to elucidate the potential antidepressant-like effects of drugs. In general, it is impossible to model depression thoroughly in rodents and the models are able to dissect only some specific aspects of depression, e.g. anhedonia or coping in stressful situation. The ability of drugs to alter the behavior of rodents in these tests allows examination of the critical factors that underlie the drug-induced behavioral phenotype in question. These factors can then be determined to participate in the effects that the drug mediates. However, it is important to be cautious when claiming that the factors regulating rodent behavior are crucial for the antidepressant effects in human patients. In addition, even though isoflurane anesthesia produces similar changes in rodent behavior as antidepressant drugs, to prove that isoflurane relieves depression in humans, and that the effect is rapid and long-lasting, requires clinical studies.

One limitation of our study is that we examined the effects of isoflurane anesthesia only on selected proteins downstream of TrkB receptor and on proteins previously linked with the rapid-antidepressant effects of ketamine. Apparently, isoflurane can induce phosphorylation of a wide range of proteins as was demonstrated by phosphoproteomic analysis from isoflurane treated animals (Kohtala et al., 2016) suggesting that all the effects are not specific to TrkB signaling pathways, but may be related to a more general increase in protein phosphorylation. The induction of protein phosphorylation may be dose-dependent, since subanesthetic dose of isoflurane did not induce similar increase in protein phosphorylation (IV).

Most probably, the antidepressant effects of isoflurane are not induced only by BDNF or TrkB but require co-expression of other molecules involved in the regulation of neuronal function and plasticity. The effects of conventional ADs and rapid-acting antidepressants on these molecules probably differ significantly. Ketamine and isoflurane for example robustly affect the NMDA and GABA receptors that directly regulate the neuronal network excitability whereas the effects of conventional antidepressant drugs, mediated via e.g. elevated levels of serotonin, are modulatory and slower in action. The ability of ketamine to rapidly affect synaptic function and plasticity has been suggested to underlie its rapid antidepressant effects (Duman et al., 2016). In general, drugs that would

produce rapid effects on synaptic functions in neuronal circuits involved in mood regulation could theoretically have antidepressant potential. For example psilocybin, which induces psychedelic effects and acutely modulates the functional connectivity of the brain, was recently shown to have antidepressant effects in human patients (Carhart-Harris et al., 2013, 2016). One of the problems related to the therapeutic use of ketamine is that the antidepressant effect in human patients is often transient (Krystal et al., 2013). Even though acute and rapid regulation of the mood is possible by ketamine, stabilizing the functions of the mood networks into a beneficial state appears to be difficult.

The ability of single isoflurane anesthesia to produce robust effects on the signaling pathways and to induce changes in the neuronal excitability, synaptic function and rodent behavior may cause problems in experimental procedures where isoflurane anesthesia is used. This is important to take into account when planning experiments where anesthesia is required.

The effects of antidepressants and isoflurane on TrkB activation are especially interesting in the context of neuronal plasticity. Chronic treatment with antidepressant drugs can promote brain plasticity via BDNF–dependent mechanism (Karpova et al., 2011; Maya Vetencourt et al., 2008). ADs induce TrkB activation already after single injection, however, increase in BDNF protein requires chronic treatment (Nibuya et al., 1995; Rantamäki et al., 2007; Saarelainen et al., 2003). It is possible that the TrkB transactivation is not enough to promote the critical period like plasticity demonstrated with fluoxetine, since in the study of Karpova et al. (2011) the effects of ADs and extinction training on fear removal were abolished in BDNF heterozygous knockout mice, even though in these mice ADs induce TrkB receptor activation similarly to wild type mice (II). The possible explanation could be that TrkB receptors are activated differentially by ADs when compared to BDNF, which could result in lack of activation of some important downstream mediators required for the induction of plasticity. BDNF can also have off-target effects that are not yet characterized. Currently, the role of TrkB receptor in the mechanism of action of antidepressant drugs in humans is not known, and solid evidence about the ability of antidepressant drugs to promote brain plasticity in humans is lacking.

7. CONCLUSIONS

In this thesis we aimed to examine the mechanisms of antidepressant-induced TrkB activation and elucidate the neurobiological basis for the antidepressant effects of isoflurane anesthesia. In our experiments we were able to demonstrate that TrkB receptor activation by antidepressant drugs can occur independently of BDNF *in vivo*. The exact mechanism of how antidepressant drugs promote TrkB activation, however, requires further characterization. The transactivation of TrkB receptor by antidepressant drugs seems to differ from BDNF-induced TrkB activation since there are differences in the phosphorylation of the tyrosines of TrkB and activation of the downstream signaling molecules (Fig 5). Moreover, the TrkB receptor transactivation by ADs appears only after certain developmental stage (P12 in mice), when TrkB responsiveness to BDNF is reduced, indicating that the effects of ADs during development may differ from those in the adult. The ability of isoflurane to promote brain plasticity and to activate signaling pathways linked to the mechanisms of action of conventional antidepressant drugs and ketamine supports the possibility that isoflurane anesthesia could be a potential treatment option for depressed patients.

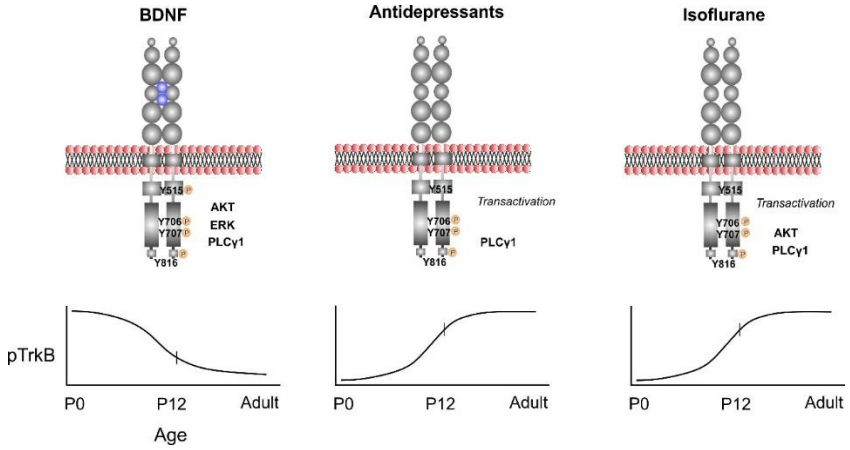

***FIG 5*. Activation of TrkB receptors by BDNF, antidepressant drugs and isoflurane.** BDNF induces phosphorylations of Y515, Y706/7 and Y816 of TrkB, whereas antidepressant drugs and isoflurane do not phosphorylate Y515. BDNF activates TrkB downstream signaling pathways including Akt, ERK and PLCγ1, whereas antidepressant drugs activate only the PLCγ1 signaling. Isoflurane induces signaling via PLCγ1 and Akt. Antidepressant drugs and isoflurane activate the TrkB receptor via transactivation. The TrkB responsiveness to BDNF (*ex vivo*) and antidepressant drugs/isoflurane (*in vivo*) is developmentally regulated so that the responsiveness to BDNF decreases around postnatal day 12 and at this same timepoint the TrkB responsiveness to antidepressant drugs/isoflurane appears. Abbreviations: BDNF, brain-derived neurotrophic factor; AKT, protein kinase B; ERK, extracellular signal-regulated kinase; PLCγ1, phospholipase C gamma 1; pTrkB, phosphorylated TrkB receptor; P0, postnatal day 0; P12, postnatal day 12; Y, tyrosine.

The main conclusions are:

1. Antidepressant drugs transactivate TrkB receptor.
2. TrkB receptor activation by antidepressant drugs is developmentally regulated.
3. Isoflurane anesthesia induces molecular, functional and behavioral effects similar to those of conventional ADs and ketamine.

ACKNOWLEDGEMENTS

The work of this thesis was carried out in the Neuroscience Center during years 2010-2016 and has been financially supported by Doctoral Programme Brain & Mind, Orion-Farmos Research Foundation, Sigrid Jusélius foundation, Academy of Finland and European Research Council.

My deepest gratitude goes to my supervisors docent Tomi Rantamäki and professor Eero Castrén. I always remember the first time I contacted Tomi by email to ask about a possibility to do my Master's thesis in the Trophin Lab. I thought he will probably never answer to my email, but he did - in half an hour. In couple of hours we had already agreed that I will come to meet him and possibly start my Master's thesis project in the lab. When we met, he talked about TrkB, tPA, proBDNF, BDNF, MMPs, western blot, ELISA, zymography, and I understood nothing. During these years, however, I have learnt something (well, a lot) and that is mainly because of Tomi. I am grateful to him that he has supported me all the time during these years and believed in me and, importantly, made me believe in myself. I wish I have absorbed at least a bit of Tomi's enthusiasm, intelligence, and diligence during these years. From a graduate student's point of view I have been very lucky since as a supervisor Tomi has always been available for discussion - and for beers! I am as grateful to my other supervisor, Eero. Eero is a visionary and his vast knowledge about neuroscience (and many other things) has always impressed me. It has been such a privilege to be able to work in his lab and learn from him. In the darkest moments of the lab work, Eero has an incredible ability to make a person feel again enthusiastic and ambitious about science. It is impossible to leave his office without feeling that the things that I'm doing actually make sense.

I want to thank the reviewers of my thesis, associate professor Annakaisa Haapasalo and docent Mikko Airavaara, who critically evaluated the thesis during the summer. They made me think many things from a different point of view and their comments were of great importance in significantly improving the thesis manuscript. In addition, I want to sincerely thank Giuseppe Cortese, who revised the English language of the thesis.

I am grateful to Professor Moses Chao for accepting the invitation to act as my opponent in the public defense of this thesis.

Definitely, I have not done alone the work presented in this thesis, so all the co-authors of the publications and the isoflurane manuscript are thanked for the great work that they have done. Without you I could not have finished my PhD.

The people in the Trophin lab have changed completely during my thesis project, only Outi, our irreplaceable lab technician has been here longer than me. I want to thank Outi for always being interested in how I am doing. I think that a modified sentence from Tomi's thesis acknowledgements fits here well: "Your effort in the lab can only be underestimated". I cannot even imagine the amount of suffering that I would have needed to survive if you had not been there ordering reagents, helping me to find things in the lab and teaching me how to use ultracentrifuge or pH meter or electronic pipette or… Also our other technician, Sulo, is thanked for always offering his help to me and especially for his dark humor that has cheered me up - although I'm sure that was not his intention! Our previous lab technician Hanna Jr. is also thanked for all the help and all the laughs.

All the past and present members of the Trophin lab are warmly thanked for all the help, discussions, ass-kicking, peer-support and great moments in and outside the lab. Ettore, Yumiko, Marie, Henri, Liisa, Jesse and Ramon – I still miss you all. The two marvelous people sitting next to me in the office, Anna and Juzoh, are thanked for sharing with me in addition to the office also science, laughs, desperate moments, happiness, chocolate, cookies, and lunch. I'm thankful of course also to the other current members of our lab Frederike, Merve, Plinio, Carol, Madhu and all the students, for making this such a pleasant place to work, we have a great team! In addition, I want to thank all the other members of the Neuroscience Center and the laboratory animal facilities that I have worked and/or partied with!

I am grateful to the members of HKV:n aikuisten pikajuoksuryhmä for the Tuesday evening sprints that allow me to think about something else than TrkB - namely Trk & field – for a while! The members of my extended family in Crossfit Herttoniemi are thanked for sharing with me the sweatiest, and some of the funniest, moments of my life. Especially I want to thank Nyytit (Maija, Miia, Mikko, Perttu, Tatu and Pauli) for all the bönthö and "support" during the thesis writing. My dearest and oldest friends, Paula, Annemari and Heli, are thanked for always being there for me.

Jussi is thanked for everything. Without your help, support and encouragement this thesis would still be in preparation. I have learnt so much from you about myself, life and science that I would be completely different person if I had not known you.

I want to warmly thank my family, isä, äiti, JP and Ville, for all the love and support.

August 2016, couple of days after back squatting 120 kg, just before the thesis should be sent to print,

Hanna

REFERENCES

Abbott, C.C., Jones, T., Lemke, N.T., Gallegos, P., McClintock, S.M., Mayer, A.R., Bustillo, J., and Calhoun, V.D. (2014). Hippocampal structural and functional changes associated with electroconvulsive therapy response. Transl. Psychiatry *4*, e483.

Adachi, N., Kohara, K., and Tsumoto, T. (2005). Difference in trafficking of brain-derived neurotrophic factor between axons and dendrites of cortical neurons, revealed by live-cell imaging. BMC Neurosci. *6*, 42.

Ahmed, S., Reynolds, B.A., and Weiss, S. (1995). BDNF enhances the differentiation but not the survival of CNS stem cell-derived neuronal precursors. J. Neurosci. *15*, 5765–5778.

Aid, T., Kazantseva, A., Piirsoo, M., Palm, K., and Timmusk, T. (2007). Mouse and rat BDNF gene structure and expression revisited. J. Neurosci. Res. *85*, 525–535.

Alcántara, S., Frisén, J., del Río, J.A., Soriano, E., Barbacid, M., and Silos-Santiago, I. (1997). TrkB signaling is required for postnatal survival of CNS neurons and protects hippocampal and motor neurons from axotomy-induced cell death. J. Neurosci. *17*, 3623–3633.

Alonso, M., Medina, J.H., and Pozzo-Miller, L. (2004). ERK1/2 Activation Is Necessary for BDNF to Increase Dendritic Spine Density in Hippocampal CA1 Pyramidal Neurons. Learn. Mem. *11*, 172–178.

Alonso, R., Griebel, G., Pavone, G., Stemmelin, J., Le Fur, G., and Soubrié, P. (2003). Blockade of CRF1 or V1b receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. Mol. Psychiatry *9*, 278–286.

Altar, C.A., Cai, N., Bliven, T., Juhasz, M., Conner, J.M., Acheson, A.L., Lindsay, R.M., and Wiegand, S.J. (1997). Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature *389*, 856–860.

An, J.J., Gharami, K., Liao, G.-Y., Woo, N.H., Lau, A.G., Vanevski, F., Torre, E.R., Jones, K.R., Feng, Y., Lu, B., et al. (2008). Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell *134*, 175–187.

Atwal, J.K., Massie, B., Miller, F.D., and Kaplan, D.R. (2000). The TrkB-Shc site signals neuronal survival and local axon growth via MEK and P13-kinase. Neuron *27*, 265–277.

Autio, H., Mätlik, K., Rantamäki, T., Lindemann, L., Hoener, M.C., Chao, M., Arumäe, U., and Castrén, E. (2011). Acetylcholinesterase inhibitors rapidly activate Trk neurotrophin receptors in the mouse hippocampus. Neuropharmacology *61*, 1291–1296.

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

Baj, G., Leone, E., Chao, M.V., and Tongiorgi, E. (2011). Spatial segregation of BDNF transcripts enables BDNF to differentially shape distinct dendritic compartments. Proc. Natl. Acad. Sci. *108*, 16813–16818.

Balkowiec, A., and Katz, D.M. (2000). Activity-Dependent Release of Endogenous Brain-Derived Neurotrophic Factor from Primary Sensory Neurons Detected by ELISAIn Situ. J. Neurosci. *20*, 7417–7423.

Bamji, S.X., Majdan, M., Pozniak, C.D., Belliveau, D.J., Aloyz, R., Kohn, J., Causing, C.G., and Miller, F.D. (1998). The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. J. Cell Biol. *140*, 911–923.

Barde, Y.A., Edgar, D., and Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. EMBO J. *1*, 549–553.

von Bartheld, C.S., Williams, R., Lefcort, F., Clary, D.O., Reichardt, L.F., and Bothwell, M. (1996). Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and trkB receptors. J. Neurosci. *16*, 2995–3008.

Bartoletti, A., Cancedda, L., Reid, S.W., Tessarollo, L., Porciatti, V., Pizzorusso, T., and Maffei, L. (2002). Heterozygous Knock-Out Mice for Brain-Derived Neurotrophic Factor Show a Pathway-Specific Impairment of Long-Term Potentiation But Normal Critical Period for Monocular Deprivation. J. Neurosci. *22*, 10072–10077.

Baxter, G.T., Radeke, M.J., Kuo, R.C., Makrides, V., Hinkle, B., Hoang, R., Medina-Selby, A., Coit, D., Valenzuela, P., and Feinstein, S.C. (1997). Signal Transduction Mediated by the Truncated trkB Receptor Isoforms, trkB.T1 and trkB.T2. J. Neurosci. *17*, 2683–2690.

Baydyuk, M., Russell, T., Liao, G.-Y., Zang, K., An, J.J., Reichardt, L.F., and Xu, B. (2011). TrkB receptor controls striatal formation by regulating the number of newborn striatal neurons. Proc. Natl. Acad. Sci. U. S. A. *108*, 1669–1674.

Benraiss, A., Chmielnicki, E., Lerner, K., Roh, D., and Goldman, S.A. (2001). Adenoviral brain-derived neurotrophic factor induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. J. Neurosci. *21*, 6718–6731.

Berghuis, P., Dobszay, M.B., Wang, X., Spano, S., Ledda, F., Sousa, K.M., Schulte, G., Ernfors, P., Mackie, K., Paratcha, G., et al. (2005). Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. Proc. Natl. Acad. Sci. U. S. A. *102*, 19115–19120.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., and Krystal, J.H. (2000). Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry *47*, 351–354.

Berton, O., McClung, C.A., DiLeone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanos, C.A., Rios, M., et al. (2006). Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress. Science *311*, 864–868.

Beurel, E., Song, L., and Jope, R. (2011). Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice. Mol. Psychiatry *16*, 1068–1070.

Bhattacharyya, A., Watson, F.L., Bradlee, T.A., Pomeroy, S.L., Stiles, C.D., and Segal, R.A. (1997). Trk receptors function as rapid retrograde signal carriers in the adult nervous system. J. Neurosci. *17*, 7007–7016.

Bibel, M., Hoppe, E., and Barde, Y.A. (1999). Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR. EMBO J. *18*, 616–622.

Biffo, S., Offenhäuser, N., Carter, B.D., and Barde, Y.A. (1995). Selective binding and internalisation by truncated receptors restrict the availability of BDNF during development. Dev. Camb. Engl. *121*, 2461–2470.

Boldrini, M., Underwood, M.D., Hen, R., Rosoklija, G.B., Dwork, A.J., John Mann, J., and Arango, V. (2009). Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology *34*, 2376–2389.

Boldrini, M., Hen, R., Underwood, M.D., Rosoklija, G.B., Dwork, A.J., Mann, J.J., and Arango, V. (2012). Hippocampal Angiogenesis and Progenitor Cell Proliferation Are Increased with Antidepressant Use in Major Depression. Biol. Psychiatry *72*, 562–571.

Bonni, A., Brunet, A., West, A.E., Datta, S.R., Takasu, M.A., and Greenberg, M.E. (1999). Cell Survival Promoted by the Ras-MAPK Signaling Pathway by Transcription-Dependent and -Independent Mechanisms. Science *286*, 1358–1362.

Bothwell, M. (2014). NGF, BDNF, NT3, and NT4. Handb. Exp. Pharmacol. *220*, 3–15.

Brigadski, T., Hartmann, M., and Lessmann, V. (2005). Differential Vesicular Targeting and Time Course of Synaptic Secretion of the Mammalian Neurotrophins. J. Neurosci. *25*, 7601–7614.

Briner, A., De Roo, M., Dayer, A., Muller, D., Habre, W., and Vutskits, L. (2010). Volatile anesthetics rapidly increase dendritic spine density in the rat medial prefrontal cortex during synaptogenesis. Anesthesiology *112*, 546–556.

Briner, A., Nikonenko, I., De Roo, M., Dayer, A., Muller, D., and Vutskits, L. (2011). Developmental Stage-dependent persistent impact of propofol anesthesia on dendritic spines in the rat medial prefrontal cortex. Anesthesiology *115*, 282–293.

Cabelli, R.J., Hohn, A., and Shatz, C.J. (1995). Inhibition of ocular dominance column formation by infusion of NT-4/5 or BDNF. Science *267*, 1662–1666.

Cabelli, R.J., Shelton, D.L., Segal, R.A., and Shatz, C.J. (1997). Blockade of Endogenous Ligands of TrkB Inhibits Formation of Ocular Dominance Columns. Neuron *19*, 63–76.

Canossa, M., Griesbeck, O., Berninger, B., Campana, G., Kolbeck, R., and Thoenen, H. (1997). Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. Proc. Natl. Acad. Sci. U. S. A. *94*, 13279–13286.

Canossa, M., Gärtner, A., Campana, G., Inagaki, N., and Thoenen, H. (2001). Regulated secretion of neurotrophins by metabotropic glutamate group I (mGluRI) and Trk receptor activation is mediated via phospholipase C signalling pathways. EMBO J. *20*, 1640–1650.

Carhart-Harris, R.L., Leech, R., Erritzoe, D., Williams, T.M., Stone, J.M., Evans, J., Sharp, D.J., Feilding, A., Wise, R.G., and Nutt, D.J. (2013). Functional Connectivity Measures After Psilocybin Inform a Novel Hypothesis of Early Psychosis. Schizophr. Bull. *39*, 1343–1351.

Carhart-Harris, R.L., Bolstridge, M., Rucker, J., Day, C.M.J., Erritzoe, D., Kaelen, M., Bloomfield, M., Rickard, J.A., Forbes, B., Feilding, A., et al. Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. Lancet Psychiatry.

Carim-Todd, L., Bath, K.G., Fulgenzi, G., Yanpallewar, S., Jing, D., Barrick, C.A., Becker, J., Buckley, H., Dorsey, S.G., Lee, F.S., et al. (2009). Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function in vivo. J. Neurosci. *29*, 678–685.

Carmignoto, G., Pizzorusso, T., Tia, S., and Vicini, S. (1997). Brain-derived neurotrophic factor and nerve growth factor potentiate excitatory synaptic transmission in the rat visual cortex. J. Physiol. *498*, 153–164.

Carpenter, G., and Ji, Q. (1999). Phospholipase C-γ as a Signal-Transducing Element. Exp. Cell Res. *253*, 15–24.

Carreno, F.R., Donegan, J.J., Boley, A.M., Shah, A., DeGuzman, M., Frazer, A., and Lodge, D.J. (2015). Activation of a ventral hippocampus–medial prefrontal cortex pathway is both necessary and sufficient for an antidepressant response to ketamine. Mol. Psychiatry.

Castrén, E. (2005). Is mood chemistry? Nat. Rev. Neurosci. *6*, 241–246.

Castrén, E. (2013). Neuronal network plasticity and recovery from depression. JAMA Psychiatry *70*, 983–989.

Castrén, E., and Hen, R. (2013). Neuronal plasticity and antidepressant actions. Trends Neurosci. *36*, 259–267.

Castrén, E., and Rantamäki, T. (2010). Role of brain-derived neurotrophic factor in the aetiology of depression: implications for pharmacological treatment. CNS Drugs *24*, 1–7.

Castrén, E., Zafra, F., Thoenen, H., and Lindholm, D. (1992). Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. Proc. Natl. Acad. Sci. U. S. A. *89*, 9444–9448.

Chacón-Fernández, P., Säuberli, K., Colzani, M., Moreau, T., Ghevaert, C., and Barde, Y.-A. (2016). Brain-derived Neurotrophic Factor in Megakaryocytes. J. Biol. Chem. *291*, 9872–9881.

Chang, K.-Y., Woo, D., Jung, H., Lee, S., Kim, S., Won, J., Kyung, T., Park, H., Kim, N., Yang, H.W., et al. (2014). Light-inducible receptor tyrosine kinases that regulate neurotrophin signalling. Nat. Commun. *5*, 4057.

Chen, M.J., and Russo-Neustadt, A.A. (2009). Running exercise-induced up-regulation of hippocampal brain-derived neurotrophic factor is CREB-dependent. Hippocampus *19*, 962–972.

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

Chen, J.L., Lin, W.C., Cha, J.W., So, P.T., Kubota, Y., and Nedivi, E. (2011). Structural basis for the role of inhibition in facilitating adult brain plasticity. Nat. Neurosci. *14*, 587–594.

Chen, Z.-Y., Patel, P.D., Sant, G., Meng, C.-X., Teng, K.K., Hempstead, B.L., and Lee, F.S. (2004). Variant Brain-Derived Neurotrophic Factor (BDNF) (Met66) Alters the

Intracellular Trafficking and Activity-Dependent Secretion of Wild-Type BDNF in Neurosecretory Cells and Cortical Neurons. J. Neurosci. *24*, 4401–4411.

Choi, S.H., Li, Y., Parada, L.F., and Sisodia, S.S. (2009). Regulation of hippocampal progenitor cell survival, proliferation and dendritic development by BDNF. Mol. Neurodegener. *4*, 52.

Chowdhury, G.M.I., Behar, K.L., Cho, W., Thomas, M.A., Rothman, D.L., and Sanacora, G. (2012). 1H-[13C]-Nuclear Magnetic Resonance Spectroscopy Measures of Ketamine's Effect on Amino Acid Neurotransmitter Metabolism. Biol. Psychiatry *71*, 1022–1025.

Cohen, M.S., Orth, C.B., Kim, H.J., Jeon, N.L., and Jaffrey, S.R. (2011). Neurotrophin-mediated dendrite-to-nucleus signaling revealed by microfluidic compartmentalization of dendrites. Proc. Natl. Acad. Sci. *108*, 11246–11251.

Conner, J.M., Lauterborn, J.C., Yan, Q., Gall, C.M., and Varon, S. (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J. Neurosci. *17*, 2295–2313.

Conover, J.C., Erickson, J.T., Katz, D.M., Bianchi, L.M., Poueymirou, W.T., McClain, J., Pan, L., Helgren, M., Ip, N.Y., and Boland, P. (1995). Neuronal deficits, not involving motor neurons, in mice lacking BDNF and/or NT4. Nature *375*, 235–238.

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.-W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A., Guiard, B.P., Guilloux, J.-P., et al. (2009). Behavioral effects of fluoxetine in an animal model of anxiety/depression are mediated by both neurogenesis-dependent and independent mechanisms. Neuron *62*, 479–493.

Davies, A.M., Lee, K.F., and Jaenisch, R. (1993). p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. Neuron *11*, 565–574.

Dechant, G., and Barde, Y.-A. (2002). The neurotrophin receptor p75NTR: novel functions and implications for diseases of the nervous system. Nat. Neurosci. *5*, 1131–1136.

Dieni, S., Matsumoto, T., Dekkers, M., Rauskolb, S., Ionescu, M.S., Deogracias, R., Gundelfinger, E.D., Kojima, M., Nestel, S., Frotscher, M., et al. (2012). BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. J. Cell Biol. *196*, 775–788.

Drevets, W.C., and Furey, M.L. (2010). Replication of Scopolamine's Antidepressant Efficacy in Major Depressive Disorder: a Randomized, Placebo-Controlled Clinical Trial. Biol. Psychiatry *67*, 432–438.

Du, J., Feng, L., Yang, F., and Lu, B. (2000). Activity- and Ca2+-Dependent Modulation of Surface Expression of Brain-Derived Neurotrophic Factor Receptors in Hippocampal Neurons. J. Cell Biol. *150*, 1423–1434.

Du, J., Feng, L., Zaitsev, E., Je, H.-S., Liu, X., and Lu, B. (2003). Regulation of TrkB receptor tyrosine kinase and its internalization by neuronal activity and Ca2+ influx. J. Cell Biol. *163*, 385–395.

Duman, R.S., and Monteggia, L.M. (2006). A neurotrophic model for stress-related mood disorders. Biol. Psychiatry *59*, 1116–1127.

Duman, R.S., Aghajanian, G.K., Sanacora, G., and Krystal, J.H. (2016). Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat. Med. *22*, 238–249.

Duman RS, Heninger GR, and Nestler EJ (1997). A molecular and cellular theory of depression. Arch. Gen. Psychiatry *54*, 597–606.

Duncan, W.C., Sarasso, S., Ferrarelli, F., Selter, J., Riedner, B.A., Hejazi, N.S., Yuan, P., Brutsche, N., Manji, H.K., Tononi, G., et al. (2013). Concomitant BDNF and sleep slow wave changes indicate ketamine-induced plasticity in major depressive disorder. Int. J. Neuropsychopharmacol. Off. Sci. J. Coll. Int. Neuropsychopharmacol. CINP *16*, 301–311.

Dutta, A., McKie, S., and Deakin, J.F.W. (2015). Ketamine and other potential glutamate antidepressants. Psychiatry Res. *225*, 1–13.

Dwivedi, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, and Pandey GN (2003). ALtered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase b in postmortem brain of suicide subjects. Arch. Gen. Psychiatry *60*, 804–815.

Dwivedi, Y., Rizavi, H.S., and Pandey, G.N. (2006). Antidepressants reverse corticosterone-mediated decrease in brain-derived neurotrophic factor expression: Differential regulation of specific exons by antidepressants and corticosterone. Neuroscience *139*, 1017–1029.

Easton, J.B., Royer, A.R., and Middlemas, D.S. (2006). The protein tyrosine phosphatase, Shp2, is required for the complete activation of the RAS/MAPK pathway by brain-derived neurotrophic factor. J. Neurochem. *97*, 834–845.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., et al. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell *112*, 257–269.

Eide, F.F., Vining, E.R., Eide, B.L., Zang, K., Wang, X.-Y., and Reichardt, L.F. (1996). Naturally Occurring Truncated trkB Receptors Have Dominant Inhibitory Effects on Brain-Derived Neurotrophic Factor Signaling. J. Neurosci. *16*, 3123–3129.

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

Erickson, J.T., Conover, J.C., Borday, V., Champagnat, J., Barbacid, M., Yancopoulos, G., and Katz, D.M. (1996). Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing. J. Neurosci. *16*, 5361–5371.

Ernfors, P., Merlio, J.-P., and Persson, H. (1992). Cells Expressing mRNA for Neurotrophins and their Receptors During Embryonic Rat Development. Eur. J. Neurosci. *4*, 1140–1158.

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

Ernst, A., Alkass, K., Bernard, S., Salehpour, M., Perl, S., Tisdale, J., Possnert, G., Druid, H., and Frisén, J. (2014). Neurogenesis in the Striatum of the Adult Human Brain. Cell *156*, 1072–1083.

Ernst C, Marshall CR, Shen Y, and et al (2012). HIghly penetrant alterations of a critical region including bdnf in human psychopathology and obesity. Arch. Gen. Psychiatry *69*, 1238–1246.

Fagiolini, M., Pizzorusso, T., Berardi, N., Domenici, L., and Maffei, L. (1994). Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark rearing and monocular deprivation. Vision Res. *34*, 709–720.

Fang, M., Tao, Y.-X., He, F., Zhang, M., Levine, C.F., Mao, P., Tao, F., Chou, C.-L., Sadegh-Nasseri, S., and Johns, R.A. (2003). Synaptic PDZ Domain-mediated Protein Interactions Are Disrupted by Inhalational Anesthetics. J. Biol. Chem. *278*, 36669–36675.

Fawcett, J.P., Aloyz, R., McLean, J.H., Pareek, S., Miller, F.D., McPherson, P.S., and Murphy, R.A. (1997). Detection of brain-derived neurotrophic factor in a vesicular fraction of brain synaptosomes. J. Biol. Chem. *272*, 8837–8840.

Fawcett, J.P., Bamji, S.X., Causing, C.G., Aloyz, R., Ase, A.R., Reader, T.A., McLean, J.H., and Miller, F.D. (1998). Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. J. Neurosci. *18*, 2808–2821.

Fenner, B.M. (2012). Truncated TrkB: Beyond a dominant negative receptor. Cytokine Growth Factor Rev. *23*, 15–24.

Finkbeiner, S., Tavazoie, S.F., Maloratsky, A., Jacobs, K.M., Harris, K.M., and Greenberg, M.E. (1997). CREB: A Major Mediator of Neuronal Neurotrophin Responses. Neuron *19*, 1031–1047.

Francois, J., Grimm, O., Schwarz, A.J., Schweiger, J., Haller, L., Risterucci, C., Böhringer, A., Zang, Z., Tost, H., Gilmour, G., et al. (2015). Ketamine Suppresses the Ventral Striatal Response to Reward Anticipation: A Cross-Species Translational Neuroimaging Study. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.

Friedel, S., Horro, F.F., Wermter, A.K., Geller, F., Dempfle, A., Reichwald, K., Smidt, J., Brönner, G., Konrad, K., Herpertz-Dahlmann, B., et al. (2005). Mutation screen of the brain derived neurotrophic factor gene (BDNF): identification of several genetic variants and association studies in patients with obesity, eating disorders, and attention-deficit/hyperactivity disorder. Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. *132B*, 96–99.

Friedman, W.J. (2000). Neurotrophins induce death of hippocampal neurons via the p75 receptor. J. Neurosci. *20*, 6340–6346.

Fryer, R.H., Kaplan, D.R., Feinstein, S.C., Radeke, M.J., Grayson, D.R., and Kromer, L.F. (1996). Developmental and mature expression of full-length and truncated TrkB, receptors in the rat forebrain. J. Comp. Neurol. *374*, 21–40.

Fuchikami, M., Thomas, A., Liu, R., Wohleb, E.S., Land, B.B., DiLeone, R.J., Aghajanian, G.K., and Duman, R.S. (2015). Optogenetic stimulation of infralimbic PFC reproduces ketamine's rapid and sustained antidepressant actions. Proc. Natl. Acad. Sci. U. S. A.

Fujimura, H., Altar, C.A., Chen, R., Nakamura, T., Nakahashi, T., Kambayashi, J., Sun, B., and Tandon, N.N. (2002). Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb. Haemost. *87*, 728–734.

Furey ML, and Drevets WC (2006). Antidepressant efficacy of the antimuscarinic drug scopolamine: A randomized, placebo-controlled clinical trial. Arch. Gen. Psychiatry *63*, 1121–1129.

Galvão, R.P., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (2008). Brain-derived neurotrophic factor signaling does not stimulate subventricular zone neurogenesis in adult mice and rats. J. Neurosci. *28*, 13368–13383.

Garcia, L.S., Comim, C.M., Valvassori, S.S., Réus, G.Z., Andreazza, A.C., Stertz, L., Fries, G.R., Gavioli, E.C., Kapczinski, F., and Quevedo, J. (2008a). Chronic Administration of Ketamine Elicits Antidepressant-Like Effects in Rats without Affecting Hippocampal Brain-Derived Neurotrophic Factor Protein Levels. Basic Clin. Pharmacol. Toxicol. *103*, 502–506.

Garcia, L.S.B., Comim, C.M., Valvassori, S.S., Réus, G.Z., Barbosa, L.M., Andreazza, A.C., Stertz, L., Fries, G.R., Gavioli, E.C., Kapczinski, F., et al. (2008b). Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. Prog. Neuropsychopharmacol. Biol. Psychiatry *32*, 140–144.

García-Toro, M., Segura, C., González, A., Perelló, J., Valdivia, J., Salazar, R., Tarancón, G., Campoamor, F., Salva, J., De La Fuente, L., et al. (2001). Inefficacy of burst-suppression anesthesia in medication-resistant major depression: a controlled trial. J. ECT *17*, 284–288.

Gärtner, A., Polnau, D.G., Staiger, V., Sciarretta, C., Minichiello, L., Thoenen, H., Bonhoeffer, T., and Korte, M. (2006). Hippocampal Long-Term Potentiation Is Supported by Presynaptic and Postsynaptic Tyrosine Receptor Kinase B-Mediated Phospholipase Cγ Signaling. J. Neurosci. *26*, 3496–3504.

Gianfranceschi, L., Siciliano, R., Walls, J., Morales, B., Kirkwood, A., Huang, Z.J., Tonegawa, S., and Maffei, L. (2003). Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. Proc. Natl. Acad. Sci. *100*, 12486–12491.

Gideons, E.S., Kavalali, E.T., and Monteggia, L.M. (2014). Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses. Proc. Natl. Acad. Sci. *111*, 8649–8654.

Gogolla, N., Caroni, P., Lüthi, A., and Herry, C. (2009). Perineuronal Nets Protect Fear Memories from Erasure. Science *325*, 1258–1261.

Gomes, R.A., Hampton, C., El-Sabeawy, F., Sabo, S.L., and McAllister, A.K. (2006). The dynamic distribution of TrkB receptors before, during, and after synapse formation between cortical neurons. J. Neurosci. *26*, 11487–11500.

Gorba, T., and Wahle, P. (1999). Expression of TrkB and TrkC but not BDNF mRNA in neurochemically identified interneurons in rat visual cortex in vivo and in organotypic cultures. Eur. J. Neurosci. *11*, 1179–1190.

Gottschalk, W., Pozzo-Miller, L.D., Figurov, A., and Lu, B. (1998). Presynaptic Modulation of Synaptic Transmission and Plasticity by Brain-Derived Neurotrophic Factor in the Developing Hippocampus. J. Neurosci. *18*, 6830–6839.

Gray, J., Yeo, G.S.H., Cox, J.J., Morton, J., Adlam, A.-L.R., Keogh, J.M., Yanovski, J.A., Gharbawy, A.E., Han, J.C., Tung, Y.C.L., et al. (2006). Hyperphagia, Severe Obesity, Impaired Cognitive Function, and Hyperactivity Associated With Functional Loss of One Copy of the Brain-Derived Neurotrophic Factor (BDNF) Gene. Diabetes *55*, 3366–3371.

Greenberg, R.M., and Kellner, C.H. (2005). Electroconvulsive Therapy: A Selected Review. Am. J. Geriatr. Psychiatry *13*, 268–281.

Greenberg, M.E., Xu, B., Lu, B., and Hempstead, B.L. (2009). New Insights in the Biology of BDNF Synthesis and Release: Implications in CNS Function. J. Neurosci. *29*, 12764–12767.

Grimm, O., Gass, N., Weber-Fahr, W., Sartorius, A., Schenker, E., Spedding, M., Risterucci, C., Schweiger, J.I., Böhringer, A., Zang, Z., et al. (2015). Acute ketamine challenge increases resting state prefrontal-hippocampal connectivity in both humans and rats. Psychopharmacology (Berl.) *232*, 4231–4241.

Group, T.U.E.R. (2003). Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis. The Lancet *361*, 799–808.

Gruart, A., Sciarretta, C., Valenzuela-Harrington, M., Delgado-García, J.M., and Minichiello, L. (2007). Mutation at the TrkB PLCγ-docking site affects hippocampal LTP and associative learning in conscious mice. Learn. Mem. *14*, 54–62.

Gu, B., Huang, Y.Z., He, X.-P., Joshi, R.B., Jang, W., and McNamara, J.O. (2015). A Peptide Uncoupling BDNF Receptor TrkB from Phospholipase Cγ1 Prevents Epilepsy Induced by Status Epilepticus. Neuron *88*, 484–491.

Guirado, R., Perez-Rando, M., Sanchez-Matarredona, D., Castrén, E., and Nacher, J. (2014). Chronic fluoxetine treatment alters the structure, connectivity and plasticity of cortical interneurons. Int. J. Neuropsychopharmacol. Off. Sci. J. Coll. Int. Neuropsychopharmacol. CINP *17*, 1635–1646.

Guo, W., Ji, Y., Wang, S., Sun, Y., and Lu, B. (2014). Neuronal activity alters BDNF–TrkB signaling kinetics and downstream functions. J. Cell Sci. *127*, 2249–2260.

Haapasalo, A., Koponen, E., Hoppe, E., Wong, G., and Castrén, E. (2001). Truncated trkB.T1 Is Dominant Negative Inhibitor of trkB.TK+-Mediated Cell Survival. Biochem. Biophys. Res. Commun. *280*, 1352–1358.

Haapasalo, A., Sipola, I., Larsson, K., Akerman, K.E.O., Stoilov, P., Stamm, S., Wong, G., and Castren, E. (2002). Regulation of TRKB surface expression by brain-derived neurotrophic factor and truncated TRKB isoforms. J. Biol. Chem. *277*, 43160–43167.

Haenisch, B., Bilkei-Gorzo, A., Caron, M.G., and Bönisch, H. (2009). Knockout of the norepinephrine transporter and pharmacologically diverse antidepressants prevent behavioral and brain neurotrophin alterations in two chronic stress models of depression. J. Neurochem. *111*, 403–416.

Haile, C.N., Murrough, J.W., Iosifescu, D.V., Chang, L.C., Jurdi, R.K.A., Foulkes, A., Iqbal, S., Mahoney, J.J., Garza, R.D.L., Charney, D.S., et al. (2014). Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression. Int. J. Neuropsychopharmacol. *17*, 331–336.

Hajek, T., Kopecek, M., and Höschl, C. (2012). Reduced hippocampal volumes in healthy carriers of brain-derived neurotrophic factor Val66Met polymorphism: meta-analysis. World J. Biol. Psychiatry Off. J. World Fed. Soc. Biol. Psychiatry *13*, 178–187.

Hajszan, T., MacLusky, N.J., and Leranth, C. (2005). Short-term treatment with the antidepressant fluoxetine triggers pyramidal dendritic spine synapse formation in rat hippocampus. Eur. J. Neurosci. *21*, 1299–1303.

Hallberg, B., Ashcroft, M., Loeb, D.M., Kaplan, D.R., and Downward, J. (1998). Nerve growth factor induced stimulation of Ras requires Trk interaction with Shc but does not involve phosphoinositide 3-OH kinase. Oncogene *17*, 691.

Hallböök, F., Ibáñez, C.F., and Persson, H. (1991). Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in xenopus ovary. Neuron *6*, 845–858.

Han, J.C., Liu, Q.-R., Jones, M., Levinn, R.L., Menzie, C.M., Jefferson-George, K.S., Adler-Wailes, D.C., Sanford, E.L., Lacbawan, F.L., Uhl, G.R., et al. (2008). Brain-derived neurotrophic factor and obesity in the WAGR syndrome. N. Engl. J. Med. *359*, 918–927.

Han, J.C., Thurm, A., Golden Williams, C., Joseph, L.A., Zein, W.M., Brooks, B.P., Butman, J.A., Brady, S.M., Fuhr, S.R., Hicks, M.D., et al. (2013). Association of brain-derived neurotrophic factor (BDNF) haploinsufficiency with lower adaptive behaviour and reduced cognitive functioning in WAGR/11p13 deletion syndrome. Cortex J. Devoted Study Nerv. Syst. Behav. *49*, 2700–2710.

Haniu, M., Talvenheimo, J., Le, J., Katta, V., Welcher, A., and Rohde, M.F. (1995). Extracellular Domain of Neurotrophin Receptor trkB: Disulfide Structure, N-Glycosylation Sites, and Ligand Binding. Arch. Biochem. Biophys. *322*, 256–264.

Hanover, t J.L., Huang, Z.J., Tonegawa, S., and Stryker, M.P. (1999). t Brain-Derived Neurotrophic Factor Overexpression Induces Precocious Critical Period in Mouse Visual Cortex. J. Neurosci. *19*, RC40-RC40.

Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F., and Weinberger, D.R. (2003). Brain-Derived Neurotrophic Factor val66met Polymorphism Affects Human Memory-Related Hippocampal Activity and Predicts Memory Performance. J. Neurosci. *23*, 6690–6694.

He, X.P., Pan, E., Sciarretta, C., Minichiello, L., and McNamara, J.O. (2010). Disruption of TrkB-Mediated Phospholipase Cγ Signaling Inhibits Limbic Epileptogenesis. J. Neurosci. *30*, 6188–6196.

Heerssen, H.M., Pazyra, M.F., and Segal, R.A. (2004). Dynein motors transport activated Trks to promote survival of target-dependent neurons. Nat. Neurosci. *7*, 596–604.

Helgager, J., Huang, Y.Z., and Mcnamara, J.O. (2014). Brain-derived neurotrophic factor but not vesicular zinc promotes TrkB activation within mossy fibers of mouse hippocampus in vivo. J. Comp. Neurol.

Henry, R.A., Hughes, S.M., and Connor, B. (2007). AAV-mediated delivery of BDNF augments neurogenesis in the normal and quinolinic acid-lesioned adult rat brain. Eur. J. Neurosci. *25*, 3513–3525.

Hensch, T.K. (2005). Critical period plasticity in local cortical circuits. Nat. Rev. Neurosci. *6*, 877–888.

Hofer, M., Pagliusi, S.R., Hohn, A., Leibrock, J., and Barde, Y.A. (1990). Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J. *9*, 2459–2464.

Hollis, E.R., Jamshidi, P., Löw, K., Blesch, A., and Tuszynski, M.H. (2009). Induction of corticospinal regeneration by lentiviral trkB-induced Erk activation. Proc. Natl. Acad. Sci. *106*, 7215–7220.

Homayoun, H., and Moghaddam, B. (2007). NMDA Receptor Hypofunction Produces Opposite Effects on Prefrontal Cortex Interneurons and Pyramidal Neurons. J. Neurosci. *27*, 11496–11500.

Hong, E.J., McCord, A.E., and Greenberg, M.E. (2008). A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. Neuron *60*, 610–624.

Horger, B.A., Iyasere, C.A., Berhow, M.T., Messer, C.J., Nestler, E.J., and Taylor, J.R. (1999). Enhancement of Locomotor Activity and Conditioned Reward to Cocaine by Brain-Derived Neurotrophic Factor. J. Neurosci. *19*, 4110–4122.

Hoshaw, B.A., Malberg, J.E., and Lucki, I. (2005). Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. Brain Res. *1037*, 204–208.

Huang, E.J., and Reichardt, L.F. (2003). Trk Receptors: Roles in Neuronal Signal Transduction *. Annu. Rev. Biochem. *72*, 609–642.

Huang, Y.Z., and McNamara, J.O. (2012). Neuroprotective effects of reactive oxygen species mediated by BDNF-independent activation of TrkB. J. Neurosci. *32*, 15521–15532.

Huang, S.-H., Zhao, L., Sun, Z.-P., Li, X.-Z., Geng, Z., Zhang, K.-D., Chao, M.V., and Chen, Z.-Y. (2009). Essential role of Hrs in endocytic recycling of full-length TrkB receptor but not its isoform TrkB.T1. J. Biol. Chem. *284*, 15126–15136.

Huang, Y.Z., Pan, E., Xiong, Z.-Q., and 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., and Tonegawa, S. (1999). BDNF Regulates the Maturation of Inhibition and the Critical Period of Plasticity in Mouse Visual Cortex. Cell *98*, 739–755.

Hwang, J.J., Park, M.-H., Choi, S.-Y., and Koh, J.-Y. (2005). Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. J. Biol. Chem. *280*, 11995–12001.

Ibrahim, L., DiazGranados, N., Franco-Chaves, J., Brutsche, N., Henter, I.D., Kronstein, P., Moaddel, R., Wainer, I., Luckenbaugh, D.A., Manji, H.K., et al. (2012). Course of Improvement in Depressive Symptoms to a Single Intravenous Infusion of Ketamine vs Add-on Riluzole: Results from a 4-Week, Double-Blind, Placebo-Controlled Study. Neuropsychopharmacology *37*, 1526–1533.

IJzendoorn, S.C.D. van (2006). Recycling endosomes. J. Cell Sci. *119*, 1679–1681.

Jakawich, S.K., Nasser, H.B., Strong, M.J., McCartney, A.J., Perez, A.S., Rakesh, N., Carruthers, C.J.L., and Sutton, M.A. (2010). Local Presynaptic Activity Gates

Homeostatic Changes in Presynaptic Function Driven by Dendritic BDNF Synthesis. Neuron *68*, 1143–1158.

Jang, S.-W., Liu, X., Chan, C.-B., Weinshenker, D., Hall, R.A., Xiao, G., and Ye, K. (2009). Amitriptyline is a TrkA and TrkB Receptor Agonist that Promotes TrkA/TrkB Heterodimerization and Has Potent Neurotrophic Activity. Chem. Biol. *16*, 644–656.

Jang, S.-W., Liu, X., Yepes, M., Shepherd, K.R., Miller, G.W., Liu, Y., Wilson, W.D., Xiao, G., Blanchi, B., Sun, Y.E., et al. (2010). A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. Proc. Natl. Acad. Sci. *107*, 2687–2692.

Je, H.S., Yang, F., Ji, Y., Nagappan, G., Hempstead, B.L., and Lu, B. (2012). Role of pro-brain-derived neurotrophic factor (proBDNF) to mature BDNF conversion in activity-dependent competition at developing neuromuscular synapses. Proc. Natl. Acad. Sci. U. S. A. *109*, 15924–15929.

Je, H.S., Yang, F., Ji, Y., Potluri, S., Fu, X.-Q., Luo, Z.-G., Nagappan, G., Chan, J.P., Hempstead, B., Son, Y.-J., et al. (2013). ProBDNF and mature BDNF as punishment and reward signals for synapse elimination at mouse neuromuscular junctions. J. Neurosci. *33*, 9957–9962.

Johnson, J.W., Glasgow, N.G., and Povysheva, N.V. (2014). Recent insights into the mode of action of memantine and ketamine. Curr. Opin. Pharmacol. *20C*, 54–63.

Jones, K.R., Fariñas, I., Backus, C., and Reichardt, L.F. (1994). Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. Cell *76*, 989–999.

Kang, H., and Schuman, E.M. (1995). Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. Science *267*, 1658–1662.

Kang, H.J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C.A., Licznerski, P., Lepack, A., Majik, M.S., Jeong, L.S., Banasr, M., et al. (2012). Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. Nat. Med. *18*, 1413–1417.

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

Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J.-M., and 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. Biol. Psychiatry *57*, 1068–1072.

Karpova, N.N., Pickenhagen, A., Lindholm, J., Tiraboschi, E., Kulesskaya, N., Agústsdóttir, A., Antila, H., Popova, D., Akamine, Y., Bahi, A., et al. (2011). Fear erasure in mice requires synergy between antidepressant drugs and extinction training. Science *334*, 1731–1734.

Kirschenbaum, B., and Goldman, S.A. (1995). Brain-derived neurotrophic factor promotes the survival of neurons arising from the adult rat forebrain subependymal zone. Proc. Natl. Acad. Sci. U. S. A. *92*, 210–214.

Klein, R., Parada, L.F., Coulier, F., and Barbacid, M. (1989). trkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. EMBO J. *8*, 3701–3709.

Klein, R., Conway, D., Parada, L.F., and Barbacid, M. (1990). The trkB tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain. Cell *61*, 647–656.

Klein, R., Nanduri, V., Jing, S., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K.R., Reichardt, L.F., and Barbacid, M. (1991a). The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. Cell *66*, 395–403.

Klein, R., Jing, S., Nanduri, V., O'Rourke, E., and Barbacid, M. (1991b). The trk proto-oncogene encodes a receptor for nerve growth factor. Cell *65*, 189–197.

Klein, R., Lamballe, F., Bryant, S., and Barbacid, M. (1992). The trkB tyrosine protein kinase is a receptor for neurotrophin-4. Neuron *8*, 947–956.

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

Knusel, B., Rabin, S.J., Hefti, F., and Kaplan, D.R. (1994). Regulated neurotrophin receptor responsiveness during neuronal migrationand early differentiation. J. Neurosci. *14*, 1542–1554.

Kohara, K., Kitamura, A., Morishima, M., and Tsumoto, T. (2001). Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. Science *291*, 2419–2423.

Kohtala, S., Theilmann, W., Suomi, T., Wigren, H.-K., Porkka-Heiskanen, T., Elo, L.L., Rokka, A., and Rantamäki, T. (2016). Brief Isoflurane Anesthesia Produces Prominent Phosphoproteomic Changes in the Adult Mouse Hippocampus. ACS Chem. Neurosci.

Kolbeck, R., Bartke, I., Eberle, W., and Barde, Y.A. (1999). Brain-derived neurotrophic factor levels in the nervous system of wild-type and neurotrophin gene mutant mice. J. Neurochem. *72*, 1930–1938.

Koponen, E., Võikar, V., Riekki, R., Saarelainen, T., Rauramaa, T., Rauvala, H., Taira, T., and Castrén, E. (2004). Transgenic mice overexpressing the full-length neurotrophin receptor trkB exhibit increased activation of the trkB–PLCγ pathway, reduced anxiety, and facilitated learning. Mol. Cell. Neurosci. *26*, 166–181.

Koponen, E., Rantamäki, T., Voikar, V., Saarelainen, T., MacDonald, E., and Castrén, E. (2005). Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. Cell. Mol. Neurobiol. *25*, 973–980.

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., and Bonhoeffer, T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc. Natl. Acad. Sci. U. S. A. *92*, 8856–8860.

Korte, M., Griesbeck, O., Gravel, C., Carroll, P., Staiger, V., Thoenen, H., and Bonhoeffer, T. (1996). Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. Proc. Natl. Acad. Sci. U. S. A. *93*, 12547–12552.

Korte, M., Minichiello, L., Klein, R., and Bonhoeffer, T. (2000). Shc-binding site in the TrkB receptor is not required for hippocampal long-term potentiation. Neuropharmacology *39*, 717–724.

Kovalchuk, Y., Hanse, E., Kafitz, K.W., and Konnerth, A. (2002). Postsynaptic Induction of BDNF-Mediated Long-Term Potentiation. Science *295*, 1729–1734.

Krystal, J.H., Sanacora, G., and Duman, R.S. (2013). Rapid-Acting Glutamatergic Antidepressants: The Path to Ketamine and Beyond. Biol. Psychiatry *73*, 1133–1141.

Kuczewski, N., Porcher, C., Lessmann, V., Medina, I., and Gaiarsa, J.-L. (2009). Activity-Dependent Dendritic Release of BDNF and Biological Consequences. Mol. Neurobiol. *39*, 37–49.

Kumar, V., Zhang, M.-X., Swank, M.W., Kunz, J., and Wu, G.-Y. (2005). Regulation of Dendritic Morphogenesis by Ras–PI3K–Akt–mTOR and Ras–MAPK Signaling Pathways. J. Neurosci. *25*, 11288–11299.

Laje, G., Lally, N., Mathews, D., Brutsche, N., Chemerinski, A., Akula, N., Kelmendi, B., Simen, A., McMahon, F.J., Sanacora, G., et al. (2012). Brain-derived neurotrophic factor Val66Met polymorphism and antidepressant efficacy of ketamine in depressed patients. Biol. Psychiatry *72*, e27-28.

Lamballe, F., Klein, R., and Barbacid, M. (1991). trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3. Cell *66*, 967–979.

Langer, G., Neumark, J., Koinig, G., Graf, M., and Schönbeck, G. (1985). Rapid psychotherapeutic effects of anesthesia with isoflurane (ES narcotherapy) in treatment-refractory depressed patients. Neuropsychobiology *14*, 118–120.

Langer, G., Karazman, R., Neumark, J., Saletu, B., Schönbeck, G., Grünberger, J., Dittrich, R., Petricek, W., Hoffmann, P., and Linzmayer, L. (1995). Isoflurane narcotherapy in depressive patients refractory to conventional antidepressant drug treatment. A double-blind comparison with electroconvulsive treatment. Neuropsychobiology *31*, 182–194.

Larsson, E., Mandel, R.J., Klein, R.L., Muzyczka, N., Lindvall, O., and Kokaia, Z. (2002). Suppression of Insult-Induced Neurogenesis in Adult Rat Brain by Brain-Derived Neurotrophic Factor. Exp. Neurol. *177*, 1–8.

Lazo, O.M., Gonzalez, A., Ascaño, M., Kuruvilla, R., Couve, A., and Bronfman, F.C. (2013). BDNF regulates Rab11-mediated recycling endosome dynamics to induce dendritic branching. J. Neurosci. *33*, 6112–6122.

Lee, F.S., and Chao, M.V. (2001). Activation of Trk neurotrophin receptors in the absence of neurotrophins. Proc. Natl. Acad. Sci. *98*, 3555–3560.

Lee, F.S., Kim, A.H., Khursigara, G., and Chao, M.V. (2001a). The uniqueness of being a neurotrophin receptor. Curr. Opin. Neurobiol. *11*, 281–286.

Lee, F.S., Rajagopal, R., Kim, A.H., Chang, P.C., and Chao, M.V. (2002). Activation of Trk Neurotrophin Receptor Signaling by Pituitary Adenylate Cyclase-activating Polypeptides. J. Biol. Chem. *277*, 9096–9102.

Lee, R., Kermani, P., Teng, K.K., and Hempstead, B.L. (2001b). Regulation of Cell Survival by Secreted Proneurotrophins. Science *294*, 1945–1948.

Lepack, A.E., Fuchikami, M., Dwyer, J.M., Banasr, M., and Duman, R.S. (2014). BDNF Release Is Required for the Behavioral Actions of Ketamine. Int. J. Neuropsychopharmacol. Off. Sci. J. Coll. Int. Neuropsychopharmacol. CINP.

Levelt, C.N., and Hübener, M. (2012). Critical-Period Plasticity in the Visual Cortex. Annu. Rev. Neurosci. *35*, 309–330.

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. Science *237*, 1154–1162.

Levine, E.S., Dreyfus, C.F., Black, I.B., and Plummer, M.R. (1995). Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. Proc. Natl. Acad. Sci. U. S. A. *92*, 8074–8077.

Li, N., Lee, B., Liu, R.-J., Banasr, M., Dwyer, J.M., Iwata, M., Li, X.-Y., Aghajanian, G., and Duman, R.S. (2010). mTOR-Dependent Synapse Formation Underlies the Rapid Antidepressant Effects of NMDA Antagonists. Science *329*, 959–964.

Li, N., Liu, R.-J., Dwyer, J.M., Banasr, M., Lee, B., Son, H., Li, X.-Y., Aghajanian, G., and Duman, R.S. (2011). Glutamate N-methyl-D-aspartate Receptor Antagonists Rapidly Reverse Behavioral and Synaptic Deficits Caused by Chronic Stress Exposure. Biol. Psychiatry *69*, 754–761.

Li, Y.X., Zhang, Y., Lester, H.A., Schuman, E.M., and Davidson, N. (1998). Enhancement of neurotransmitter release induced by brain-derived neurotrophic factor in cultured hippocampal neurons. J. Neurosci. *18*, 10231–10240.

Lindholm, J.S.O., Autio, H., Vesa, L., Antila, H., Lindemann, L., Hoener, M.C., Skolnick, P., Rantamäki, T., and 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.

Linnarsson, S., Willson, C.A., and Ernfors, P. (2000). Cell death in regenerating populations of neurons in BDNF mutant mice. Brain Res. Mol. Brain Res. *75*, 61–69.

Liu, R.-J., Lee, F.S., Li, X.-Y., Bambico, F., Duman, R.S., and Aghajanian, G.K. (2012). Brain-Derived Neurotrophic Factor Val66Met Allele Impairs Basal and Ketamine-Stimulated Synaptogenesis in Prefrontal Cortex. Biol. Psychiatry *71*, 996–1005.

Lu, Y., Ji, Y., Ganesan, S., Schloesser, R., Martinowich, K., Sun, M., Mei, F., Chao, M.V., and Lu, B. (2011). TrkB as a Potential Synaptic and Behavioral Tag. J. Neurosci. *31*, 11762–11771.

Luberg, K., Wong, J., Weickert, C.S., and Timmusk, T. (2010). Human TrkB gene: novel alternative transcripts, protein isoforms and expression pattern in the prefrontal cerebral cortex during postnatal development. J. Neurochem. *113*, 952–964.

Luikart, B.W., Nef, S., Virmani, T., Lush, M.E., Liu, Y., Kavalali, E.T., and Parada, L.F. (2005). TrkB Has a Cell-Autonomous Role in the Establishment of Hippocampal Schaffer Collateral Synapses. J. Neurosci. *25*, 3774–3786.

Lyckman, A.W., Fan, G., Rios, M., Jaenisch, R., and Sur, M. (2005). Normal eye-specific patterning of retinal inputs to murine subcortical visual nuclei in the absence of brain-derived neurotrophic factor. Vis. Neurosci. *22*, 27–36.

Machado-Vieira, R., Yuan, P., Brutsche, N., DiazGranados, N., Luckenbaugh, D., Manji, H.K., and Zarate, C.A. (2009). Brain-derived neurotrophic factor and initial antidepressant response to an N-methyl-D-aspartate antagonist. J. Clin. Psychiatry *70*, 1662–1666.

Maeng, S., Zarate, C.A., Du, J., Schloesser, R.J., McCammon, J., Chen, G., and Manji, H.K. (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biol. Psychiatry *63*, 349–352.

Maisonpierre, P.C., Belluscio, L., Squinto, S., Ip, N.Y., Furth, M.E., Lindsay, R.M., and Yancopoulos, G.D. (1990a). Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. Science *247*, 1446–1451.

Maisonpierre, P.C., Belluscio, L., Friedman, B., Alderson, R.F., Wiegand, S.J., Furth, M.E., Lindsay, R.M., and Yancopoulos, G.D. (1990b). NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron *5*, 501–509.

Malberg, J.E., Eisch, A.J., Nestler, E.J., and Duman, R.S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J. Neurosci. *20*, 9104–9110.

Mamounas, L.A., Blue, M.E., Siuciak, J.A., and Altar, C.A. (1995). Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J. Neurosci. *15*, 7929–7939.

Maren, S. (2001). Neurobiology of Pavlovian Fear Conditioning. Annu. Rev. Neurosci. *24*, 897–931.

Martı´nez, A., Alcántara, S., Borrell, V., Rı´o, J.A.D., Blasi, J., Otal, R., Campos, N., Boronat, A., Barbacid, M., Silos-Santiago, I., et al. (1998). TrkB and TrkC Signaling Are Required for Maturation and Synaptogenesis of Hippocampal Connections. J. Neurosci. *18*, 7336–7350.

Mathew, S.J., Murrough, J.W., aan het Rot, M., Collins, K.A., Reich, D.L., and Charney, D.S. (2010). Riluzole for relapse prevention following intravenous ketamine in treatment-resistant depression: a pilot randomized, placebo-controlled continuation trial. Int. J. Neuropsychopharmacol. Off. Sci. J. Coll. Int. Neuropsychopharmacol. CINP *13*, 71–82.

Matrisciano, F., Bonaccorso, S., Ricciardi, A., Scaccianoce, S., Panaccione, I., Wang, L., Ruberto, A., Tatarelli, R., Nicoletti, F., Girardi, P., et al. (2009). Changes in BDNF serum levels in patients with major depression disorder (MDD) after 6 months treatment with sertraline, escitalopram, or venlafaxine. J. Psychiatr. Res. *43*, 247–254.

Matsuda, N., Lu, H., Fukata, Y., Noritake, J., Gao, H., Mukherjee, S., Nemoto, T., Fukata, M., and Poo, M. (2009). Differential Activity-Dependent Secretion of Brain-Derived Neurotrophic Factor from Axon and Dendrite. J. Neurosci. *29*, 14185–14198.

Matsumoto, T., Rauskolb, S., Polack, M., Klose, J., Kolbeck, R., Korte, M., and Barde, Y.-A. (2008). Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. Nat. Neurosci. *11*, 131–133.

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

Maynard, K.R., Hill, J.L., Calcaterra, N.E., Palko, M.E., Kardian, A., Paredes, D., Sukumar, M., Adler, B.D., Jimenez, D.V., Schloesser, R.J., et al. (2015). Functional Role of BDNF Production from Unique Promoters in Aggression and Serotonin Signaling. Neuropsychopharmacology.

McAllister, A.K., Lo, D.C., and Katz, L.C. (1995). Neurotrophins regulate dendritic growth in developing visual cortex. Neuron *15*, 791–803.

McGirr, A., Berlim, M.T., Bond, D.J., Fleck, M.P., Yatham, L.N., and Lam, R.W. (2015). A systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials of ketamine in the rapid treatment of major depressive episodes. Psychol. Med. *45*, 693–704.

Meakin, S.O., MacDonald, J.I.S., Gryz, E.A., Kubu, C.J., and Verdi, J.M. (1999). The Signaling Adapter FRS-2 Competes with Shc for Binding to the Nerve Growth Factor Receptor TrkA - A model for discriminating proliferation and differentiation. J. Biol. Chem. *274*, 9861–9870.

Medina, D.L., Sciarretta, C., Calella, A.M., Von Bohlen Und Halbach, O., Unsicker, K., and Minichiello, L. (2004). TrkB regulates neocortex formation through the Shc/PLCgamma-mediated control of neuronal migration. EMBO J. *23*, 3803–3814.

Merkl, A., Heuser, I., and Bajbouj, M. (2009). Antidepressant electroconvulsive therapy: Mechanism of action, recent advances and limitations. Exp. Neurol. *219*, 20–26.

Merlio, J.P., Ernfors, P., Kokaia, Z., Middlemas, D.S., Bengzon, J., Kokaia, M., Smith, M.L., Siesjö, B.K., Hunter, T., and Lindvall, O. (1993). Increased production of the TrkB protein tyrosine kinase receptor after brain insults. Neuron *10*, 151–164.

Meyer-Franke, A., Wilkinson, G.A., Kruttgen, A., Hu, M., Munro, E., Hanson Jr., M.G., Reichardt, L.F., and Barres, B.A. (1998). Depolarization and cAMP Elevation Rapidly Recruit TrkB to the Plasma Membrane of CNS Neurons. Neuron *21*, 681–693.

Middela, S., and Pearce, I. (2011). Ketamine-induced vesicopathy: a literature review. Int. J. Clin. Pract. *65*, 27–30.

Middlemas, D.S., Lindberg, R.A., and Hunter, T. (1991). trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors. Mol. Cell. Biol. *11*, 143–153.

Middlemas, D.S., Meisenhelder, J., and Hunter, T. (1994). Identification of TrkB autophosphorylation sites and evidence that phospholipase C-gamma 1 is a substrate of the TrkB receptor. J. Biol. Chem. *269*, 5458–5466.

Milak, M.S., Proper, C.J., Mulhern, S.T., Parter, A.L., Kegeles, L.S., Ogden, R.T., Mao, X., Rodriguez, C.I., Oquendo, M.A., Suckow, R.F., et al. (2015). A pilot in vivo proton magnetic resonance spectroscopy study of amino acid neurotransmitter response to ketamine treatment of major depressive disorder. Mol. Psychiatry.

Minichiello, L. (2009). TrkB signalling pathways in LTP and learning. Nat. Rev. Neurosci. *10*, 850–860.

Minichiello, L., and Klein, R. (1996). TrkB and TrkC neurotrophin receptors cooperate in promoting survival of hippocampal and cerebellar granule neurons. Genes Dev. *10*, 2849–2858.

Minichiello, L., Casagranda, F., Tatche, R.S., Stucky, C.L., Postigo, A., Lewin, G.R., Davies, A.M., and Klein, R. (1998). Point mutation in trkB causes loss of NT4-dependent neurons without major effects on diverse BDNF responses. Neuron *21*, 335–345.

Minichiello, L., Calella, A.M., Medina, D.L., Bonhoeffer, T., Klein, R., and Korte, M. (2002). Mechanism of TrkB-mediated hippocampal long-term potentiation. Neuron *36*, 121–137.

Moghaddam, B., Adams, B., Verma, A., and Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J. Neurosci. *17*, 2921–2927.

Molendijk, M.L., Spinhoven, P., Polak, M., Bus, B. a. A., Penninx, B.W.J.H., and Elzinga, B.M. (2014). Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). Mol. Psychiatry *19*, 791–800.

Monteggia, L.M., Barrot, M., Powell, C.M., Berton, O., Galanis, V., Gemelli, T., Meuth, S., Nagy, A., Greene, R.W., and Nestler, E.J. (2004). Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proc. Natl. Acad. Sci. U. S. A. *101*, 10827–10832.

Morgan, C.J.A., Muetzelfeldt, L., and Curran, H.V. (2010). Consequences of chronic ketamine self-administration upon neurocognitive function and psychological wellbeing: a 1-year longitudinal study. Addict. Abingdon Engl. *105*, 121–133.

Morgan, C.J.A., Dodds, C.M., Furby, H., Pepper, F., Fam, J., Freeman, T.P., Hughes, E., Doeller, C., King, J., Howes, O., et al. (2014). Long-Term Heavy Ketamine Use is Associated with Spatial Memory Impairment and Altered Hippocampal Activation. Front. Psychiatry *5*, 149.

Mowla, S.J., Pareek, S., Farhadi, H.F., Petrecca, K., Fawcett, J.P., Seidah, N.G., Morris, S.J., Sossin, W.S., and Murphy, R.A. (1999). Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. J. Neurosci. *19*, 2069–2080.

Mowla, S.J., Farhadi, H.F., Pareek, S., Atwal, J.K., Morris, S.J., Seidah, N.G., and Murphy, R.A. (2001). Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. J. Biol. Chem. *276*, 12660–12666.

Muetzelfeldt, L., Kamboj, S.K., Rees, H., Taylor, J., Morgan, C.J.A., and Curran, H.V. (2008). Journey through the K-hole: phenomenological aspects of ketamine use. Drug Alcohol Depend. *95*, 219–229.

Mufson, E.J., Kroin, J.S., Sobreviela, T., Burke, M.A., Kordower, J.H., Penn, R.D., and Miller, J.A. (1994). Intrastriatal infusions of brain-derived neurotrophic factor: retrograde transport and colocalization with dopamine containing substantia nigra neurons in rat. Exp. Neurol. *129*, 15–26.

Murrough, J.W., Iosifescu, D.V., Chang, L.C., Al Jurdi, R.K., Green, C.E., Perez, A.M., Iqbal, S., Pillemer, S., Foulkes, A., Shah, A., et al. (2013). Antidepressant Efficacy of Ketamine in Treatment-Resistant Major Depression: A Two-Site Randomized Controlled Trial. Am. J. Psychiatry *170*, 1134–1142.

Murrough, J.W., Wan, L.-B., Iacoviello, B., Collins, K.A., Solon, C., Glicksberg, B., Perez, A.M., Mathew, S.J., Charney, D.S., Iosifescu, D.V., et al. (2014). Neurocognitive effects of ketamine in treatment-resistant major depression: association with antidepressant response. Psychopharmacology (Berl.) *231*, 481–488.

Myers, K.M., and Davis, M. (2006). Mechanisms of fear extinction. Mol. Psychiatry *12*, 120–150.

Nagappan, G., Zaitsev, E., Senatorov, V.V., Yang, J., Hempstead, B.L., and Lu, B. (2009). Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. Proc. Natl. Acad. Sci. U. S. A. *106*, 1267–1272.

Nagele, P., Duma, A., Kopec, M., Gebara, M.A., Parsoei, A., Walker, M., Janski, A., Panagopoulos, V.N., Cristancho, P., Miller, J.P., et al. (2015). Nitrous Oxide for Treatment-Resistant Major Depression: A Proof-of-Concept Trial. Biol. Psychiatry *78*, 10–18.

Nair, A., Vadodaria, K.C., Banerjee, S.B., Benekareddy, M., Dias, B.G., Duman, R.S., and Vaidya, V.A. (2006). Stressor-Specific Regulation of Distinct Brain-Derived Neurotrophic Factor Transcripts and Cyclic AMP Response Element-Binding Protein Expression in the Postnatal and Adult Rat Hippocampus. Neuropsychopharmacology *32*, 1504–1519.

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

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

Nikoletopoulou, V., Lickert, H., Frade, J.M., Rencurel, C., Giallonardo, P., Zhang, L., Bibel, M., and Barde, Y.-A. (2010). Neurotrophin receptors TrkA and TrkC cause neuronal death whereas TrkB does not. Nature *467*, 59–63.

Ninkina, N., Adu, J., Fischer, A., Piñón, L.G., Buchman, V.L., and Davies, A.M. (1996). Expression and function of TrkB variants in developing sensory neurons. EMBO J. *15*, 6385–6393.

Ninkina, N., Grashchuck, M., Buchman, V.L., and Davies, A.M. (1997). TrkB Variants with Deletions in the Leucine-rich Motifs of the Extracellular Domain. J. Biol. Chem. *272*, 13019–13025.

Normann, C., Schmitz, D., Fürmaier, A., Döing, C., and Bach, M. (2007). Long-term plasticity of visually evoked potentials in humans is altered in major depression. Biol. Psychiatry *62*, 373–380.

Nykjaer, A., Lee, R., Teng, K.K., Jansen, P., Madsen, P., Nielsen, M.S., Jacobsen, C., Kliemannel, M., Schwarz, E., Willnow, T.E., et al. (2004). Sortilin is essential for proNGF-induced neuronal cell death. Nature *427*, 843–848.

Obermeier, A., Lammers, R., Wiesmüller, K.H., Jung, G., Schlessinger, J., and Ullrich, A. (1993). Identification of Trk binding sites for SHC and phosphatidylinositol 3'-kinase and formation of a multimeric signaling complex. J. Biol. Chem. *268*, 22963–22966.

Obermeier, A., Bradshaw, R.A., Seedorf, K., Choidas, A., Schlessinger, J., and Ullrich, A. (1994). Neuronal differentiation signals are controlled by nerve growth factor receptor/Trk binding sites for SHC and PLC gamma. EMBO J. *13*, 1585–1590.

Pandey, G.N., Dwivedi, Y., Rizavi, H.S., Ren, X., Zhang, H., and Pavuluri, M.N. (2010). Brain-derived neurotrophic factor gene and protein expression in pediatric and adult depressed subjects. Prog. Neuropsychopharmacol. Biol. Psychiatry *34*, 645–651.

Pang, P.T., Teng, H.K., Zaitsev, E., Woo, N.T., Sakata, K., Zhen, S., Teng, K.K., Yung, W.-H., Hempstead, B.L., and Lu, B. (2004). Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science *306*, 487–491.

Papp, M., and Moryl, E. (1994). Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression. Eur. J. Pharmacol. *263*, 1–7.

Park, H., Popescu, A., and Poo, M. (2014). Essential Role of Presynaptic NMDA Receptors in Activity-Dependent BDNF Secretion and Corticostriatal LTP. Neuron *84*, 1009–1022.

Parkhurst, C.N., Yang, G., Ninan, I., Savas, J.N., Yates III, J.R., Lafaille, J.J., Hempstead, B.L., Littman, D.R., and Gan, W.-B. (2013). Microglia Promote Learning-Dependent Synapse Formation through Brain-Derived Neurotrophic Factor. Cell *155*, 1596–1609.

Patterson, S.L., Abel, T., Deuel, T.A., Martin, K.C., Rose, J.C., and Kandel, E.R. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. Neuron *16*, 1137–1145.

Patterson, S.L., Pittenger, C., Morozov, A., Martin, K.C., Scanlin, H., Drake, C., and Kandel, E.R. (2001). Some Forms of cAMP-Mediated Long-Lasting Potentiation Are Associated with Release of BDNF and Nuclear Translocation of Phospho-MAP Kinase. Neuron *32*, 123–140.

Patz, S., Grabert, J., Gorba, T., Wirth, M.J., and Wahle, P. (2004). Parvalbumin expression in visual cortical interneurons depends on neuronal activity and TrkB ligands during an Early period of postnatal development. Cereb. Cortex N. Y. N 1991 *14*, 342–351.

van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., and Gage, F.H. (2002). Functional neurogenesis in the adult hippocampus. Nature *415*, 1030–1034.

Preskorn, S., Macaluso, M., Mehra, D.O.V., Zammit, G., Moskal, J.R., Burch, R.M., and GLYX-13 Clinical Study Group (2015). Randomized Proof of Concept Trial of GLYX-13, an N-Methyl-D-Aspartate Receptor Glycine Site Partial Agonist, in Major Depressive Disorder Nonresponsive to a Previous Antidepressant Agent. J. Psychiatr. Pract. *21*, 140–149.

Price, J.L., and Drevets, W.C. (2009). Neurocircuitry of Mood Disorders. Neuropsychopharmacology *35*, 192–216.

Puehringer, D., Orel, N., Lüningschrör, P., Subramanian, N., Herrmann, T., Chao, M.V., and Sendtner, M. (2013). EGF transactivation of Trk receptors regulates the migration of newborn cortical neurons. Nat. Neurosci. *16*, 407–415.

Rajagopal, R., and Chao, M.V. (2006). A role for Fyn in Trk receptor transactivation by G-protein-coupled receptor signaling. Mol. Cell. Neurosci. *33*, 36–46.

Rajagopal, R., Chen, Z.-Y., Lee, F.S., and Chao, M.V. (2004). Transactivation of Trk Neurotrophin Receptors by G-Protein-Coupled Receptor Ligands Occurs on Intracellular Membranes. J. Neurosci. *24*, 6650–6658.

Rantamäki, T., Hendolin, P., Kankaanpää, A., Mijatovic, J., Piepponen, P., Domenici, E., Chao, M.V., Männistö, P.T., and Castrén, E. (2007). Pharmacologically Diverse Antidepressants Rapidly Activate Brain-Derived Neurotrophic Factor Receptor TrkB and Induce Phospholipase-Cγ Signaling Pathways in Mouse Brain. Neuropsychopharmacology *32*, 2152–2162.

Rauskolb, S., Zagrebelsky, M., Dreznjak, A., Deogracias, R., Matsumoto, T., Wiese, S., Erne, B., Sendtner, M., Schaeren-Wiemers, N., Korte, M., et al. (2010). Global deprivation of brain-derived neurotrophic factor in the CNS reveals an area-specific requirement for dendritic growth. J. Neurosci. *30*, 1739–1749.

Reichardt, L.F. (2006). Neurotrophin-regulated signalling pathways. Philos. Trans. R. Soc. Lond. B Biol. Sci. *361*, 1545–1564.

Rex, C.S., Lin, C.-Y., Kramár, E.A., Chen, L.Y., Gall, C.M., and Lynch, G. (2007). Brain-Derived Neurotrophic Factor Promotes Long-Term Potentiation-Related Cytoskeletal Changes in Adult Hippocampus. J. Neurosci. *27*, 3017–3029.

Rico, B., Xu, B., and Reichardt, L.F. (2002). TrkB receptor signaling is required for establishment of GABAergic synapses in the cerebellum. Nat. Neurosci. *5*, 225–233.

Rodriguez-Tébar, A., Dechant, G., and Barde, Y.A. (1990). Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. Neuron *4*, 487–492.

Rodriguez-Tébar, A., Dechant, G., Götz, R., and Barde, Y.A. (1992). Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. EMBO J. *11*, 917–922.

Roo, M.D., Klauser, P., Briner, A., Nikonenko, I., Mendez, P., Dayer, A., Kiss, J.Z., Muller, D., and Vutskits, L. (2009). Anesthetics Rapidly Promote Synaptogenesis during a Critical Period of Brain Development. PLOS ONE *4*, e7043.

Rose, C.R., Blum, R., Pichler, B., Lepier, A., Kafitz, K.W., and Konnerth, A. (2003). Truncated TrkB-T1 mediates neurotrophin-evoked calcium signalling in glia cells. Nature *426*, 74–78.

Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., Fabbri, M.E., Tessarollo, L., Maffei, L., Berardi, N., et al. (2006). Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur. J. Neurosci. *24*, 1850–1856.

Rowland, L.M., Bustillo, J.R., Mullins, P.G., Jung, R.E., Lenroot, R., Landgraf, E., Barrow, R., Yeo, R., Lauriello, J., and Brooks, W.M. (2005). Effects of ketamine on anterior cingulate glutamate metabolism in healthy humans: a 4-T proton MRS study. Am. J. Psychiatry *162*, 394–396.

Ruhé, H.G., Mason, N.S., and Schene, A.H. (2007). Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol. Psychiatry *12*, 331–359.

Russo-Neustadt, A., Beard, R.C., and Cotman, C.W. (1999). Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. *21*, 679–682.

Russo-Neustadt, A., Ha, T., Ramirez, R., and Kesslak, J.P. (2001). Physical activity–antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav. Brain Res. *120*, 87–95.

Saarelainen, T., Lukkarinen, J.A., Koponen, S., Gröhn, O.H.J., Jolkkonen, J., Koponen, E., Haapasalo, A., Alhonen, L., Wong, G., Koistinaho, J., et al. (2000). Transgenic Mice Overexpressing Truncated trkB Neurotrophin Receptors in Neurons Show Increased Susceptibility to Cortical Injury after Focal Cerebral Ischemia. Mol. Cell. Neurosci. *16*, 87–96.

Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., Agerman, K., Haapasalo, A., Nawa, H., Aloyz, R., et al. (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. J. Neurosci. *23*, 349–357.

Sairanen, M., Lucas, G., Ernfors, P., Castrén, M., and 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. J. Neurosci. *25*, 1089–1094.

Sanacora, G., and Schatzberg, A.F. (2015). Ketamine: Promising Path or False Prophecy in the Development of Novel Therapeutics for Mood Disorders? Neuropsychopharmacology *40*, 259–267.

Savitz, J., and Drevets, W.C. (2009). Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. Neurosci. Biobehav. Rev. *33*, 699–771.

Scharfman, H., Goodman, J., Macleod, A., Phani, S., Antonelli, C., and Croll, S. (2005). Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. Exp. Neurol. *192*, 348–356.

Schecterson, L.C., and Bothwell, M. (2010). Neurotrophin receptors: Old friends with new partners. Dev. Neurobiol. *70*, 332–338.

Schecterson, L.C., Hudson, M.P., Ko, M., Philippidou, P., Akmentin, W., Wiley, J., Rosenblum, E., Chao, M.V., Halegoua, S., and Bothwell, M. (2010). Trk activation in the secretory pathway promotes Golgi fragmentation. Mol. Cell. Neurosci. *43*, 403–413.

Schildkraut, J.J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiatry *122*, 509–522.

Schneider, R., and Schweiger, M. (1991). A novel modular mosaic of cell adhesion motifs in the extracellular domains of the neurogenic trk and trkB tyrosine kinase receptors. Oncogene *6*, 1807–1811.

Segal, R.A., Bhattacharyya, A., Rua, L.A., Alberta, J.A., Stephens, R.M., Kaplan, D.R., and Stiles, C.D. (1996). Differential Utilization of Trk Autophosphorylation Sites. J. Biol. Chem. *271*, 20175–20181.

Seidah, N.G., Benjannet, S., Pareek, S., Chrétien, M., and Murphy, R.A. (1996). Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. FEBS Lett. *379*, 247–250.

Sen, S., Duman, R., and Sanacora, G. (2008). Serum Brain-Derived Neurotrophic Factor, Depression, and Antidepressant Medications: Meta-Analyses and Implications. Biol. Psychiatry *64*, 527–532.

Shen, J., and Maruyama, I.N. (2012). Brain-derived neurotrophic factor receptor TrkB exists as a preformed dimer in living cells. J. Mol. Signal. *7*, 2.

Shi, Y., Mantuano, E., Inoue, G., Campana, W.M., and Gonias, S.L. (2009). Ligand binding to LRP1 Transactivates Trk Receptors by a Src Family Kinase-dependent Pathway. Sci. Signal. *2*, ra18.

Shimizu, E., Hashimoto, K., Okamura, N., Koike, K., Komatsu, N., Kumakiri, C., Nakazato, M., Watanabe, H., Shinoda, N., Okada, S., et al. (2003). Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol. Psychiatry *54*, 70–75.

Shinawi, M., Sahoo, T., Maranda, B., Skinner, S.A., Skinner, C., Chinault, C., Zascavage, R., Peters, S.U., Patel, A., Stevenson, R.E., et al. (2011). 11p14.1 microdeletions associated with ADHD, autism, developmental delay, and obesity. Am. J. Med. Genet. A. *155A*, 1272–1280.

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

Silos-Santiago, I., Fagan, A.M., Garber, M., Fritzsch, B., and Barbacid, M. (1997). Severe Sensory Deficits but Normal CNS Development in Newborn Mice Lacking TrkB and TrkC Tyrosine Protein Kinase Receptors. Eur. J. Neurosci. *9*, 2045–2056.

Sirianni, R.W., Olausson, P., Chiu, A.S., Taylor, J.R., and Saltzman, W.M. (2010). The behavioral and biochemical effects of BDNF containing polymers implanted in the hippocampus of rats. Brain Res. *1321*, 40–50.

Sisti, D., Segal, A.G., and Thase, M.E. (2014). Proceed with Caution: Off-label Ketamine Treatment for Major Depressive Disorder. Curr. Psychiatry Rep. *16*, 1–5.

Siuciak, J.A., Boylan, C., Fritsche, M., Altar, C.A., and Lindsay, R.M. (1996). BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. Brain Res. *710*, 11–20.

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

Skolnick, P., Layer, R.T., Popik, P., Nowak, G., Paul, I.A., and Trullas, R. (1996). Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. Pharmacopsychiatry *29*, 23–26.

Smith, K. (2014). Mental health: A world of depression. Nature *515*, 180–181.

Smith, E.G., Deligiannidis, K.M., Ulbricht, C.M., Landolin, C.S., Patel, J.K., and Rothschild, A.J. (2013). Antidepressant augmentation using the N-methyl-D-aspartate antagonist memantine: a randomized, double-blind, placebo-controlled trial. J. Clin. Psychiatry *74*, 966–973.

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

Smith, M.A., Makino, S., Kim, S.Y., and Kvetnansky, R. (1995b). Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology *136*, 3743–3750.

Soliman, F., Glatt, C.E., Bath, K.G., Levita, L., Jones, R.M., Pattwell, S.S., Jing, D., Tottenham, N., Amso, D., Somerville, L.H., et al. (2010). A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. Science *327*, 863–866.

Sommerfeld, M.T., Schweigreiter, R., Barde, Y.A., and Hoppe, E. (2000). Down-regulation of the neurotrophin receptor TrkB following ligand binding. Evidence for an involvement of the proteasome and differential regulation of TrkA and TrkB. J. Biol. Chem. *275*, 8982–8990.

Song, M., Giza, J., Proenca, C.C., Jing, D., Elliott, M., Dincheva, I., Shmelkov, S.V., Kim, J., Schreiner, R., Huang, S.-H., et al. (2015). Slitrk5 Mediates BDNF-Dependent TrkB Receptor Trafficking and Signaling. Dev. Cell *33*, 690–702.

Stephens, R.M., Loeb, D.M., Copeland, T.D., Pawson, T., Greene, L.A., and Kaplan, D.R. (1994). Trk receptors use redundant signal transduction pathways involving SHC and PLC-γ1 to mediate NGF responses. Neuron *12*, 691–705.

Stoilov, P., Castren, E., and Stamm, S. (2002). Analysis of the Human TrkB Gene Genomic Organization Reveals Novel TrkB Isoforms, Unusual Gene Length, and Splicing Mechanism. Biochem. Biophys. Res. Commun. *290*, 1054–1065.

Stone, J.M., Dietrich, C., Edden, R., Mehta, M.A., De Simoni, S., Reed, L.J., Krystal, J.H., Nutt, D., and Barker, G.J. (2012). Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. Mol. Psychiatry *17*, 664–665.

Takei, N., Inamura, N., Kawamura, M., Namba, H., Hara, K., Yonezawa, K., and Nawa, H. (2004). Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. J. Neurosci. *24*, 9760–9769.

Taliaz, D., Loya, A., Gersner, R., Haramati, S., Chen, A., and Zangen, A. (2011). Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. J. Neurosci. *31*, 4475–4483.

Taliaz, D., Nagaraj, V., Haramati, S., Chen, A., and Zangen, A. (2013). Altered brain-derived neurotrophic factor expression in the ventral tegmental area, but not in the hippocampus, is essential for antidepressant-like effects of electroconvulsive therapy. Biol. Psychiatry *74*, 305–312.

Tanaka, J.-I., Horiike, Y., Matsuzaki, M., Miyazaki, T., Ellis-Davies, G.C.R., and Kasai, H. (2008). Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. Science *319*, 1683–1687.

Tao, X., Finkbeiner, S., Arnold, D.B., Shaywitz, A.J., and Greenberg, M.E. (1998). Ca2+ Influx Regulates BDNF Transcription by a CREB Family Transcription Factor-Dependent Mechanism. Neuron *20*, 709–726.

Tao, X., West, A.E., Chen, W.G., Corfas, G., and Greenberg, M.E. (2002). A Calcium-Responsive Transcription Factor, CaRF, that Regulates Neuronal Activity-Dependent Expression of BDNF. Neuron *33*, 383–395.

Taylor, M.J., Tiangga, E.R., Mhuircheartaigh, R.N., and Cowen, P.J. (2012). Lack of effect of ketamine on cortical glutamate and glutamine in healthy volunteers: a proton magnetic resonance spectroscopy study. J. Psychopharmacol. (Oxf.) *26*, 733–737.

Teng, H.K., Teng, K.K., Lee, R., Wright, S., Tevar, S., Almeida, R.D., Kermani, P., Torkin, R., Chen, Z.-Y., Lee, F.S., et al. (2005). ProBDNF Induces Neuronal Apoptosis via Activation of a Receptor Complex of p75NTR and Sortilin. J. Neurosci. *25*, 5455–5463.

Thoenen, H. (1995). Neurotrophins and neuronal plasticity. Science *270*, 593–598.

Tongiorgi, E., Armellin, M., Giulianini, P.G., Bregola, G., Zucchini, S., Paradiso, B., Steward, O., Cattaneo, A., and Simonato, M. (2004). Brain-Derived Neurotrophic Factor mRNA and Protein Are Targeted to Discrete Dendritic Laminas by Events That Trigger Epileptogenesis. J. Neurosci. *24*, 6842–6852.

Trullas, R., and Skolnick, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. Eur. J. Pharmacol. *185*, 1–10.

Tsankova, N.M., Berton, O., Renthal, W., Kumar, A., Neve, R.L., and Nestler, E.J. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat. Neurosci. *9*, 519–525.

Urfer, R., Tsoulfas, P., O'Connell, L., Shelton, D.L., Parada, L.F., and Presta, L.G. (1995). An immunoglobulin-like domain determines the specificity of neurotrophin receptors. EMBO J. *14*, 2795–2805.

Valentine, G.W., Mason, G.F., Gomez, R., Fasula, M., Watzl, J., Pittman, B., Krystal, J.H., and Sanacora, G. (2011). The antidepressant effect of ketamine is not associated with changes in occipital amino acid neurotransmitter content as measured by [1H]-MRS. Psychiatry Res. Neuroimaging *191*, 122–127.

Verhagen, M., van der Meij, A., van Deurzen, P. a. M., Janzing, J.G.E., Arias-Vásquez, A., Buitelaar, J.K., and Franke, B. (2010). Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. Mol. Psychiatry *15*, 260–271.

Vicario-Abejón, C., Collin, C., McKay, R.D., and Segal, M. (1998). Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. J. Neurosci. *18*, 7256–7271.

Wang, J.-W., David, D.J., Monckton, J.E., Battaglia, F., and Hen, R. (2008). Chronic Fluoxetine Stimulates Maturation and Synaptic Plasticity of Adult-Born Hippocampal Granule Cells. J. Neurosci. *28*, 1374–1384.

Watson, F.L., Porcionatto, M.A., Bhattacharyya, A., Stiles, C.D., and Segal, R.A. (1999a). TrkA glycosylation regulates receptor localization and activity. J. Neurobiol. *39*, 323–336.

Watson, F.L., Heerssen, H.M., Moheban, D.B., Lin, M.Z., Sauvageot, C.M., Bhattacharyya, A., Pomeroy, S.L., and Segal, R.A. (1999b). Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex. J. Neurosci. *19*, 7889–7900.

Watson, F.L., Heerssen, H.M., Bhattacharyya, A., Klesse, L., Lin, M.Z., and Segal, R.A. (2001). Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat. Neurosci. *4*, 981–988.

Webster, M.J., Weickert, C.S., Herman, M.M., and Kleinman, J.E. (2002). BDNF mRNA expression during postnatal development, maturation and aging of the human prefrontal cortex. Brain Res. Dev. Brain Res. *139*, 139–150.

Webster, M.J., Herman, M.M., Kleinman, J.E., and Shannon Weickert, C. (2006). BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan. Gene Expr. Patterns GEP *6*, 941–951.

Weeks, H.R., III, Tadler, S.C., Smith, K.W., Iacob, E., Saccoman, M., White, A.T., Landvatter, J.D., Chelune, G.J., Suchy, Y., Clark, E., et al. (2013). Antidepressant and Neurocognitive Effects of Isoflurane Anesthesia versus Electroconvulsive Therapy in Refractory Depression. PLoS ONE *8*, e69809.

West, A.E., Chen, W.G., Dalva, M.B., Dolmetsch, R.E., Kornhauser, J.M., Shaywitz, A.J., Takasu, M.A., Tao, X., and Greenberg, M.E. (2001). Calcium regulation of neuronal gene expression. Proc. Natl. Acad. Sci. *98*, 11024–11031.

West, A.E., Pruunsild, P., and Timmusk, T. (2014). Neurotrophins: transcription and translation. Handb. Exp. Pharmacol. *220*, 67–100.

Wetsel, W.C., Rodriguiz, R.M., Guillemot, J., Rousselet, E., Essalmani, R., Kim, I.H., Bryant, J.C., Marcinkiewicz, J., Desjardins, R., Day, R., et al. (2013). Disruption of the expression of the proprotein convertase PC7 reduces BDNF production and affects learning and memory in mice. Proc. Natl. Acad. Sci. *110*, 17362–17367.

Wiese, S., Jablonka, S., Holtmann, B., Orel, N., Rajagopal, R., Chao, M.V., and Sendtner, M. (2007). Adenosine receptor A2A-R contributes to motoneuron survival by transactivating the tyrosine kinase receptor TrkB. Proc. Natl. Acad. Sci. U. S. A. *104*, 17210–17215.

Windisch, J.M., Auer, B., Marksteiner, R., Lang, M.E., and Schneider, R. (1995). Specific neurotrophin binding to leucine-rich motif peptides of TrkA and TrkB. FEBS Lett. *374*, 125–129.

Winnubst, J., Cheyne, J.E., Niculescu, D., and Lohmann, C. (2015). Spontaneous Activity Drives Local Synaptic Plasticity In Vivo. Neuron *87*, 399–410.

Woo, N.H., Teng, H.K., Siao, C.-J., Chiaruttini, C., Pang, P.T., Milner, T.A., Hempstead, B.L., and Lu, B. (2005). Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat. Neurosci. *8*, 1069–1077.

Wu, K., Xu, J., Suen, P., Levine, E., Huang, Y., Mount, H.T.J., Lin, S., and Black, I.B. (1996). Functional trkB neurotrophin receptors are intrinsic components of the adult brain postsynaptic density. Mol. Brain Res. *43*, 286–290.

Wu, M.V., Shamy, J.L., Bedi, G., Choi, C.-W.J., Wall, M.M., Arango, V., Boldrini, M., Foltin, R.W., and Hen, R. (2014). Impact of Social Status and Antidepressant Treatment on Neurogenesis in the Baboon Hippocampus. Neuropsychopharmacology *39*, 1861–1871.

Xu, B., Zang, K., Ruff, N.L., Zhang, Y.A., McConnell, S.K., Stryker, M.P., and Reichardt, L.F. (2000a). Cortical degeneration in the absence of neurotrophin signaling: dendritic retraction and neuronal loss after removal of the receptor TrkB. Neuron *26*, 233–245.

Xu, B., Gottschalk, W., Chow, A., Wilson, R.I., Schnell, E., Zang, K., Wang, D., Nicoll, R.A., Lu, B., and Reichardt, L.F. (2000b). The Role of Brain-Derived Neurotrophic Factor Receptors in the Mature Hippocampus: Modulation of Long-Term Potentiation through a Presynaptic Mechanism involving TrkB. J. Neurosci. *20*, 6888–6897.

Yamashita, T., and Tohyama, M. (2003). The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. Nat. Neurosci. *6*, 461–467.

Yan, Q., Radeke, M.J., Matheson, C.R., Talvenheimo, J., Welcher, A.A., and Felnstein, S.C. (1997). Immunocytochemical localization of TrkB in the central nervous system of the adult rat. J. Comp. Neurol. *378*, 135–157.

Yang, J., Siao, C.-J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z.-Y., Mark, W., Tessarollo, L., Lee, F.S., et al. (2009). Neuronal release of proBDNF. Nat. Neurosci. *12*, 113–115.

Yang, J., Harte-Hargrove, L.C., Siao, C.-J., Marinic, T., Clarke, R., Ma, Q., Jing, D., LaFrancois, J.J., Bath, K.G., Mark, W., et al. (2014). proBDNF Negatively Regulates Neuronal Remodeling, Synaptic Transmission, and Synaptic Plasticity in Hippocampus. Cell Rep. *7*, 796–806.

Yano, H., Lee, F.S., Kong, H., Chuang, J., Arevalo, J., Perez, P., Sung, C., and Chao, M.V. (2001). Association of Trk neurotrophin receptors with components of the cytoplasmic dynein motor. J. Neurosci. *21*, RC125.

Yao, R., and Cooper, G.M. (1995). Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. Science *267*, 2003–2006.

Yeo, G.S.H., Connie Hung, C.-C., Rochford, J., Keogh, J., Gray, J., Sivaramakrishnan, S., O'Rahilly, S., and Farooqi, I.S. (2004). A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. Nat. Neurosci. *7*, 1187–1189.

Ying, S.-W., Futter, M., Rosenblum, K., Webber, M.J., Hunt, S.P., Bliss, T.V.P., and Bramham, C.R. (2002). Brain-Derived Neurotrophic Factor Induces Long-Term Potentiation in Intact Adult Hippocampus: Requirement for ERK Activation Coupled to CREB and Upregulation of Arc Synthesis. J. Neurosci. *22*, 1532–1540.

Zaccaro, M.C., Ivanisevic, L., Perez, P., Meakin, S.O., and Saragovi, H.U. (2001). p75 Co-receptors regulate ligand-dependent and ligand-independent Trk receptor activation, in part by altering Trk docking subdomains. J. Biol. Chem. *276*, 31023–31029.

Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H., and Lindholm, D. (1990). Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. EMBO J. *9*, 3545–3550.

Zafra, F., Castrén, E., Thoenen, H., and Lindholm, D. (1991). Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. Proc. Natl. Acad. Sci. *88*, 10037–10041.

Zakharenko, S.S., Patterson, S.L., Dragatsis, I., Zeitlin, S.O., Siegelbaum, S.A., Kandel, E.R., and Morozov, A. (2003). Presynaptic BDNF required for a presynaptic but not

postsynaptic component of LTP at hippocampal CA1-CA3 synapses. Neuron *39*, 975–990.

Zanos, P., Moaddel, R., Morris, P.J., Georgiou, P., Fischell, J., Elmer, G.I., Alkondon, M., Yuan, P., Pribut, H.J., Singh, N.S., et al. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature.

Zarate CA, Jr, Singh JB, Carlson PJ, and et al (2006). A randomized trial of an n-methyl-d-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatry *63*, 856–864.

Zhao, C., Deng, W., and Gage, F.H. (2008). Mechanisms and Functional Implications of Adult Neurogenesis. Cell *132*, 645–660.

Zhao, L., Sheng, A.-L., Huang, S.-H., Yin, Y.-X., Chen, B., Li, X.-Z., Zhang, Y., and Chen, Z.-Y. (2009). Mechanism underlying activity-dependent insertion of TrkB into the neuronal surface. J. Cell Sci. *122*, 3123–3136.

Zheng, J., Shen, W.-H., Lu, T.-J., Zhou, Y., Chen, Q., Wang, Z., Xiang, T., Zhu, Y.-C., Zhang, C., Duan, S., et al. (2008). Clathrin-dependent endocytosis is required for TrkB-dependent Akt-mediated neuronal protection and dendritic growth. J. Biol. Chem. *283*, 13280–13288.

Zhou, X.F., and Rush, R.A. (1996). Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. Neuroscience *74*, 945–953.

Zigova, T., Pencea, V., Wiegand, S.J., and Luskin, M.B. (1998). Intraventricular Administration of BDNF Increases the Number of Newly Generated Neurons in the Adult Olfactory Bulb. Mol. Cell. Neurosci. *11*, 234–245.