Role of brain-derived neurotrophic factor (BDNF) variants in amygdala-dependent fear memory and extinction learning in mice

Thesis

for the degree of

doctor rerum naturalium (Dr. rer. nat.)

approved by the faculty of Natural Sciences of Otto von Guericke University Magdeburg by **Harish Vuyyuru M.Sc** born on 24.08.1988 in Pedakakani Andhra Pradesh, India

Examiners: Prof. Dr. rer. nat. Volkmar Leßmann

Prof. Dr. rer. nat. Wulf Haubensak

Submitted on 23.08.2023

Defended on 29.04.2024

Table of contents

I. Abbreviations

7,8-DHF	7,8-Dihydroxyflavone
Aa	Amino acids
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP5	D,L-2-amino-5-phosphonovaleric acid
Akt	Protein kinase B
α-2AP	Alpha2-antiplasmin
BA	Basal nucleus of the amygdala
BDNF	Brain-derived neurotrophic factor
BLA	Basolateral amygdala
CaMKII	Ca^{2+} calmodulin-dependent protein kinase II
Ca	Calcium
cDNA	Complementary Deoxyribonucleic acid
CREB	Cyclic adenosine monophosphate calcium response element binding protein
CS	Conditioned stimulus
CeA	Central nucleus of the amygdala
CeL	Lateral nucleus of the central amygdala
CeM	Medial nucleus of the central amygdala
CPP	3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid
ERK	Extracellular signal-regulated kinases
EGFP	Enhanced green fluorescent protein
EPSP	Excitatory postsynaptic potential
E-LTP	Early phase of Long-term potentiation
dB SPL	Decibels, sound pressure level
DR	Dorsal raphe
GABA	Gamma-Aminobutyric acid
GDP	Guanosine diphosphate
h	Hour
HIV	Human immunodeficiency virus
IL mPFC	Infralimbic medial prefrontal cortex
ITCs	Intercalated cell masses
ITI	Inter trial intervals
kDa	Kilo dalton
IRS2, IRS2	Insulin receptor substrate-1 and -2.
LA	Lateral nucleus of the amygdala
LFS	Low frequency stimulation
LTP	Long-term potentiation
LTM	Long-term memories
L-LTP	Late-phase Long-term potentiation
LTD	Long-term depression
µV	Microvolt
mA	Milliampere
MAPK	Mitogen-activated protein kinase
mPFC	Medial prefrontal cortex
Met	Methionine
mGluRs	Metabotropic glutamate receptors
min	Minutes
mRNA	Messenger ribonucleic acid
MMPs	Matrix metalloproteinases
mpITCs	Medial paracapsular Intercalated cell mass
$m\ s^{-1}$	Meter per second

Na	Sodium
NGF	Nerve growth factor
NMDA	N-Methyl-D-aspartic acid
NR2B	N-methyl D-aspartate receptor subtype 2B
NS	Neutral stimulus
NT's	Neurotrophins
PAG	Periaqueductal gray
p75NTR	P75 neurotrophin receptor
PCs	Protein convertases
Pep	Peptide
PKA	Protein kinase A
PL mPFC	Prelimbic medial prefrontal cortex
PLC γ	Phospholipase C-gamma
PI3 Kinase	Phosphatidylinositol 3 kinase
POCD	postoperative cognitive dysfunction
PTSD	Post-traumatic stress disorders
PVT	Paraventricular thalamic nucleus
Raf	Rapidly accelerated fibrosarcoma
Ras	Rat sarcoma
RhoGDI	Rat sarcoma- homolog Guanosine diphosphate –dissociation inhibitor
RhoA	Rat sarcoma homolog family member A
Shc	Proto-oncogene tyrosine-protein kinase homology domain
Src	Proto-oncogene tyrosine-protein kinase
STM	Short-term memory
SKI-1	Subtilisin/kexin-isozyme 1
TGN	Trans Golgi network
Trk	Tropomyosin-related kinase
TAT	Trans activator of transcription
tPA	Tissue plasminogen activator
US	Unconditioned stimulus
Val	Valine
vlPAG	Ventrolateral Periaqueductal gray
VGCCs	Voltage-gated calcium channels
vHPC	Ventral hippocampus

II. List of figures

III. Abstract

Dysfunctions in fear learning are supposed to be one of the major causes for the development of several anxiety disorders, like post-traumatic stress disorder (PTSD). Thus understanding the underlying neuronal mechanisms and circuitries involved in fear learning might lead to novel treatment strategies for anxiety disorders. Fear memory can be quantitatively investigated by using a fear conditioning paradigm. A wide range of research studies demonstrated that the amygdala plays a vital role in fear memory formation as well as the extinction of fear memories. Neurotrophic factors especially mature brain-derived neurotrophic factor (BDNF) and its precursor proBDNF are important mediators for synaptic plasticity and memory formation, acting via activation of TrkB (tropomyosin-related kinase B) and p75 neurotrophin receptors (p75NTR). Several recent studies demonstrated an important role of BDNF-TrkB signaling in the basolateral amygdala (BLA) in regulating fear learning and memory extinction.

However, no studies investigated the role of proBDNF-p75NTR in BLA in mediating the extinction of cued-fear extinction memory. For the first time, we attempted to block proBDNF-p75NTR signaling in the BLA by using TAT-Pep5, an antagonist of proBDNF-p75NTR signaling, and examined the effects of blocking proBDNF-p75NTR signaling on the extinction of fear memory. Infusion of TAT-Pep5 either 20 min before or immediately after extinction training impaired the acquisition of cued-fear extinction and consolidation of extinction memory. This shows that extinction memory formation requires proBDNF-p75NTR signaling in the BLA. Infusion of TAT-Pep5 20 min before fear acquisition did not affect fear memory, indicating that fear memory formation does not require proBDNF-p75NTR signaling in the BLA. Additionally, blocking the proteolysis of proBDNF by infusion of alpha2-antiplasmin *(*α2AP) 20 min before insufficient extinction training enhanced the acquisition of extinction memory. However, the Infusion of α2AP immediately after such insufficient extinction training did not yield successful extinction memory. To confirm the involvement of BDNF in extinction memory consolidation, we performed further experiments. Infusion of TrkB-Fc, a BDNF scavenger, locally into BLA immediately after the extinction training impaired the consolidation of extinction memory. This indicates that extinction memory consolidation requires BDNF-TrkB signaling. Further, infusion of TrkB-Fc, locally into BLA 30 min after the fear training impaired the early consolidation of fear memory as measured during the memory test. In conclusion, the processing of proBDNF, as well as proBDNF-p75NTR and BDNF-TrkB signaling in BLA play major roles in regulating the extinction of cued fear memories. However, cued-fear memory formation relies on BDNF-TrkB signaling but not on proBDNF-p75NTR signaling.

IV. Zusammenfassung

Störungen des Furchtlernens werden als eine der Hauptursachen für die Entwicklung verschiedener Angststörungen, wie den posttraumatischen Belastungsstörungen (PTBS), angesehen. Das Verständnis der zugrundeliegenden neuronalen Mechanismen und Schaltkreise, die am Furchtlernen beteiligt sind, könnte daher zu neuen Behandlungsstrategien für Angststörungen führen. Das Furchtgedächtnis kann mit Hilfe eines Paradigmas zur Furchtkonditionierung quantitativ untersucht werden. Zahlreiche Forschungsstudien haben gezeigt, dass die Amygdala eine entscheidende Rolle bei der Bildung und Extinktion des Furchtgedächtnisses spielt. Neurotrophe Faktoren, insbesondere matures BDNF (Brain-Derived Neurotrophic Factor) und dessen Vorläufer proBDNF, sind wichtige Vermittler für die synaptische Plastizität sowie die Gedächtnisbildung und wirken über die Aktivierung von TrkB-Rezeptoren (Tropomyosin-Related Kinase B) und p75-Neurotrophinrezeptoren (p75NTR). Mehrere neuere Studien haben auch eine wichtige Rolle von BDNF-TrkB-Signalweg in der basolateralen Amygdala (BLA) bei der Regulierung des Furchtlernens und der Gedächtnislöschung gezeigt.

Es wurden bislang jedoch keine Studien durchgeführt, die die Rolle des proBDNF-p75NTR-Signalwegs in der BLA bei der Extinktion des Furchtgedächtnisses untersuchten. Daher haben wir den proBDNF-p75NTR-Signalweg in der BLA blockiert, indem wir TAT-Pep5, einen Antagonisten für diesen Signalweg, zu verschiedenen Zeitpunkten vor oder nach dem Extinktionslernen lokal in die BLA appliziert haben. Die Infusion von TAT-Pep5 20 Minuten vor, oder unmittelbar nach dem Extinktionstraining beeinträchtigte die Akquisition, bzw. die Konsolidierung des Extinktionsgedächtnisses. Dies zeigt, dass die Bildung des Extinktionsgedächtnisses eine proBDNF-p75NTR-Signalgebung in der BLA erfordert. Darüber hinaus hatte die lokale Infusion von TAT-Pep5 20 Minuten vor dem Auslösen des Furcht-Lernens keine Auswirkungen auf die Ausbildung des Furchtgedächtnises, was darauf hindeutet, dass die Bildung des Furchtgedächtnisses nicht die proBDNF-p75NTR-Signalgebung erfordert. Die Blockierung der Proteolyse von proBDNF in der BLA durch die lokale Infusion von alpha2-Antiplasmin (α2AP) 20 Minuten vor einem partiellen, d.h. unvollständigen, Extinktionstraining konnte den Erwerb des Extinktionsgedächtnisses verbessern. Die Infusion von α2AP unmittelbar nach dem partiellen Extinktionstraining führte jedoch nicht zu einer Verbesserung des Extinktionsgedächtnisses. Um die Beteiligung von BDNF an der Konsolidierung des Extinktionsgedächtnisses zu bestätigen, führten wir weitere Experimente durch. Die Infusion von TrkB-Fc, einem „BDNF-Fänger“, in die BLA unmittelbar nach dem Extinktionstraining beeinträchtigte die Konsolidierung des Extinktionsgedächtnisses. Dies deutet darauf hin, dass die Konsolidierung des Extinktionsgedächtnisses einen intakten BDNF-TrkB-Signalweg erfordert. Des Weiteren beeinträchtigte die lokale Infusion

von TrkB-Fc in die BLA 30 Minuten nach dem Furchttraining die frühe Konsolidierung des Furchtgedächtnisses. Zusammenfassend lässt sich sagen, dass die proBDNF-p75NTR- und BDNF-TrkB-Signalgebung in der BLA wichtige Rollen bei der Ausbildung des Extinktionsgedächtnisses spielen. Die Bildung des Furchtgedächtnisses hängt jedoch vom BDNF-TrkB-, nicht aber vom proBDNF-p75NTR-Signalweg ab.

1. Introduction

One of the main goals in cognitive neuroscience is to identify and understand the neural substrates underlying learning and memory. To survive in an environment, any organism must learn relevant environmental cues, such as the presence of stimuli, their valence, and their relation to other stimuli. Therefore, an organism needs the ability to learn and memorize about these stimuli and their features, to subsequently adapt its behavior to the environment. Fear learning is one of the best-studied forms of memory and one of the most powerful emotional experiences in our lives. Frightening experiences can form an emotional memory that leads to long-lasting behavioral changes, which have been observed in many animal species (Blanchard et al., 1993). Fear is an essential adaptive component of a response to potentially threatening stimuli and performs a crucial function for the survival of both, men and animals (Davis, 1992; LeDoux, 2000). However, abnormal fear expression can trigger serious psychiatric problems (Kent & Rauch, 2003; Uys, Stein, Daniels, & Harvey, 2003; Singewald et al., 2015). Thus, understanding the underlying neural basis of fear learning is of great importance. The brain structures and mechanisms mediating fear learning are very similar in different mammalian species (Pape & Pare, 2010). For example, the fear system will trigger similar responses in humans and rodents by employing many defensive response strategies (Kishioka *et al.,* 2009). The mechanisms of fear learning can be easily investigated in the laboratory by using a Pavlovian fear conditioning paradigm. Before going into detail about fear learning, I would like to briefly describe the general processes of memory formation.

Learning and memory are complex processes about which we still do not have a complete consensual picture of its physical nature. In 1900, Müller and Pilzecker proposed the theory of memory consolidation, a fundamental paradigm in the neurobiology and psychobiology of memory (Müller & Pilzecker, 1900; Dudai, 2004). According to this paradigm, memory formation is not a unitary process; it consists of multiple phases such as acquisition (encoding), consolidation (formation), and retrieval. In classical conditioning, acquisition occurs when an individual is subjected to the presentation of a neutral stimulus together with an unconditioned stimulus (US). By this pairing, the neutral stimulus becomes a conditioned stimulus (CS), initially stored in a labile short-term memory (STM). Consolidation occurs when the labile STM is converted into stable long-term memory (LTM), activating various neuronal signaling cascades that involve gene transcription and protein translation. Memory consolidation takes several hours to days or weeks and requires more than one wave of protein synthesis (Dudai, 2004; Quillfeldt, 2016). Memory retrieval is the process where subjects are re-exposed to the CS. Retrieval is a prominent aspect of memory processing, as it is the only way to measure memory (Eisenberg et al., 2003). Memory

retrieval triggers a time interval in which the initially well-stored memory is transferred into an unstable form, allowing the brain to update the stored memory with actual information. To convert this activated, unstable form into a stabilized LTM again, a reconsolidation process is required. Reconsolidation occurs when a consolidated memory is activated by a brief presentation of the CS without the US. Extinction is evoked by re-exposure of the subject to repeated CS presentations in the absence of the US which results in a gradual reduction of the conditioned response. This is due to the fact that during this acquisition of extinction memory, animals learn that the presentation of the CS no longer predicts the occurrence of the US. However, the original memory trace is not erased during the extinction process but is transiently inhibited by newly learned information. (Quillfeldt, 2016; Myers & Davis, 2007).

1.1 Brain-Derived Neurotrophic Factor

Neurotrophins (NT's) are a small family of secretory proteins that consists of the Nerve growth factor (NGF), Brain-derived neurotrophic factor (BDNF), Neurotrophin-3 (NT-3), and Neurotrophin-4/5 (NT-4/5) (Gotz et al., 1994; Nilsson, Fainzilber, Falck, & Ibanez, 1998; Haubensak et al., 1998). Other neurotrophins such as NT-6 and NT-7 genes were identified only in fish and do not have mammalian orthologues (Gotz et al., 1994; Nilsson, Fainzilber, Falck, & Ibanez, 1998). NT's play a prominent role in developing the vertebrate's nervous system, such as promoting survival and differentiation of neurons during development and maintaining neurons viability in adulthood (Korsching, 1993; Segal & Greenberg, 1996; Lewin & Barde, 1996). NT's, regulate their physiological responses through a distinct class of transmembrane receptors, such as the Pan-neurotrophic receptor (p75NTR) and tropomyosin-related kinase (Trk) family of receptor tyrosine kinases (TrkA, TrkB, and TrkC) (Lessmann et al., 2003; Brigadski, et al., 2005; Lessmann & Brigadski, 2009; von Bohlen und Halbach et al., 2003; Calella et al., 2007; Krause et al., 2008; von Bohlen und Halbach, 2010; von Bohlen und Halbach & von Bohlen und Halbach, 2018; Bekinschtein & von Bohlen und Halbach, 2020; Rodriguez-Tebar et al., 1990; Frade & Barde, 1998). As our research work is mainly related to BDNF and its isoforms, I will focus in further sections only on BDNF variants and their role in fear learning and memory.

1.1.1 BDNF structure

All NT's have similar structural and chemical properties and have more than 50% sequence homologies with approximately identical molecular weight (Skaper, 2012). Among NT's, BDNF is a highly conserved protein with a high sequence similarity in all vertebrates, from fish to humans. (Binder & Scharfman, 2004). BDNF is generated as an uncleaved precursor pre-proBDNF protein. This pre-proBDNF form is first cleaved into proBDNF and later on into mature BDNF (will be referred from now on as 'BDNF') (see **Figure 1.1A** for the structure of

proBDNF and BDNF). These two isoforms exert opposing functions by activating distinct receptors in neurons (Lessmann & Brigadski, 2009). BDNF gene structure was described first by Timmusk et al in rats (Timmusk et al, 1993). In rats, the BDNF gene is located on chromosome 11, and the gene consists of eight 5' exons (exons I-VIII) and one 3′ exon (exon IX) in rats and mice. In addition, exon I contains an ATG initiate codon that adds eight amino acids to the pre-proBDNF N-terminus during the translation of exon-I containing BDNF transcript (Timmusk et al., 1995, 1993; Aid, et al., 2007).

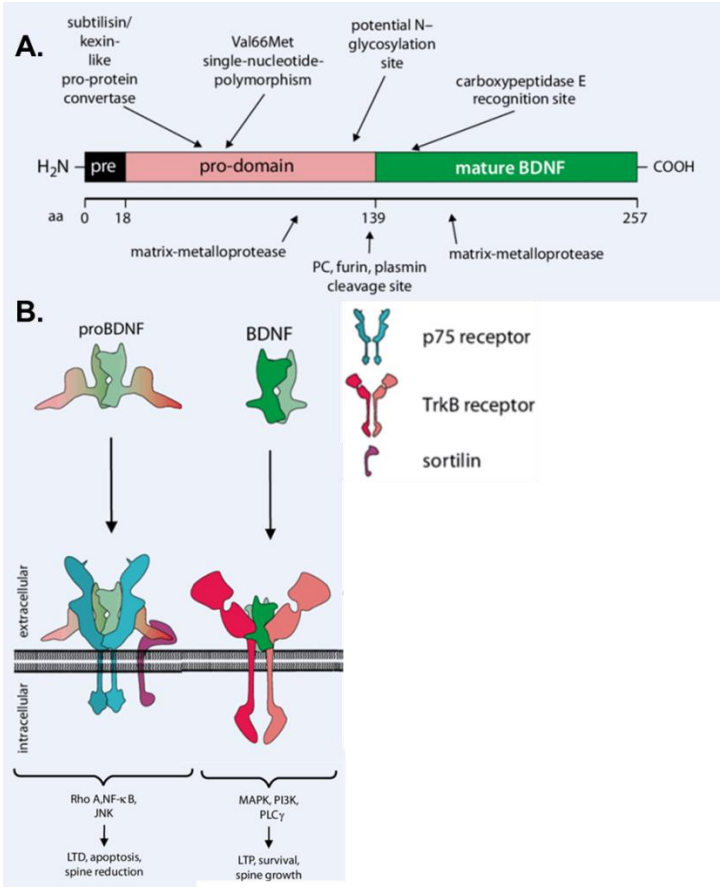

Figure 1.1: Schematic representation of BDNF Variants and its receptors. (Adapted and modified from Brigadski & Leßmann, 2014). **(A) Structure of pre-proBDNF and mature BDNF**: Arrows indicate the different processing sites. The different domains of BDNF along with the length of its amino acid (aa) sequence (Total 249 aa; pre-domain: 18 aa; pro-domain: 112 aa; mature BDNF: 119 aa). Different types of intracellular protein convertases (PCs), plasmin and furin, can cleave at position 130. The position of the val66met single nucleotide polymorphism in the prodomain, the carboxypeptidase recognition site, two putative matrix-metalloproteinase cleavage sites the subtilisin/kexin cleavage site, and a putative n-glycosylation site are indicated.
(B) Simplified model of proBDNF and BDNF binding to its different receptors: BDNF binds to the TrkB receptor and proBDNF binds to p75/sortilin-complex, which activates distinct signaling systems.

1.1.2 Proteolysis of proBDNF into mature BDNF

Synthesis and maturation of BDNF involve various stages and form several precursor isoforms during this process. In the endoplasmic reticulum, BDNF is synthesized and folded as pre-proBDNF. In the Golgi apparatus, the signal sequence of the pre-domain is cleaved off, yielding the 32–35 kDa precursor protein proBDNF (Mowla et al., 1999). This proBDNF protein comprises the N-terminal pro-domain (129 amino acids) and the C-terminal mature domain (118 amino acids) **(Figure 1.1A)**

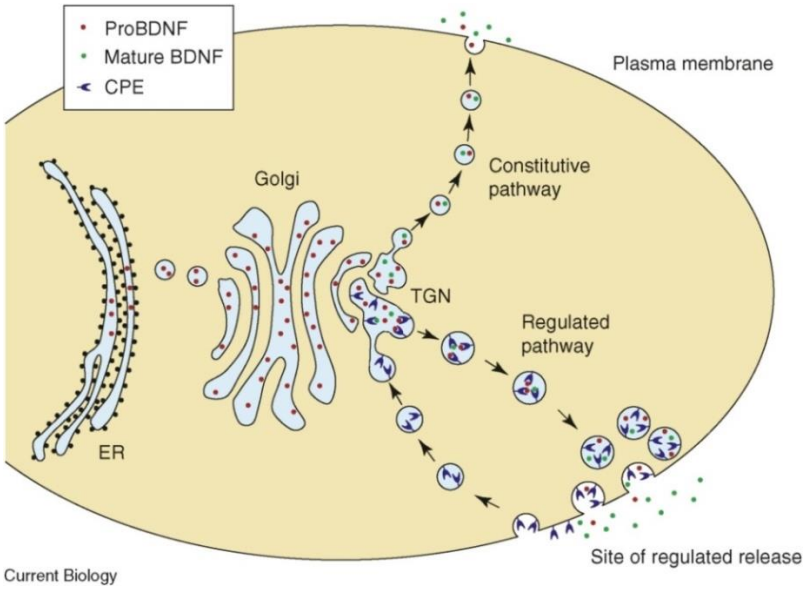

Figure 1.2: BDNF processing, packaging, and secretion in neurons (Lessmann et al., 2003; Thomas & Davies, 2005). BDNF is synthesized in the endoplasmic reticulum (ER) as a 32 kDa precursor protein (proBDNF) that moves though the Golgi apparatus to the trans-Golgi network (TGN), From TGN, BDNF secretion can occur via two processes: vesicles of the constitutive pathway and regulated pathway (Mowla et al., 2001). For instance, in TGN, BDNF binding to the lipid-raft-associated sorting receptor carboxypeptidase E is a prerequisite for sorting into secretory vesicles of the regulated pathway. These secretory vesicles are subsequently transported to appropriate sites and the contents of these secretory vesicles are eventually released upon triggering signals for regulated secretion

Intracellular proBDNF undergoes proteolytic cleavage either by Golgi resident subtilisin-kexin family of endoproteases or secretary granule resident convertases (furin and protein-Convertases 1-7) to yield 13 kDa mature BDNF (BDNF) (Mowla et al., 2001; Seidah et al., 1996; Lessmann & Brigadski, 2009; Brigadski & Lessmann, 2020; Bathina & Das, 2015). proBDNF can also be secreted and cleaved extracellularly by serine protease plasmin (Pang et al., 2004) or by selective matrix metalloproteinases (MMPs). Tissue plasminogen activator

(tPA), a secreted protein, converts the plasminogen to plasmin, and this plasmin cleaves proBDNF into BDNF (Gray and Ellis 2008; Lee et al., 2001; Pang et al., 2004; von Bohlen und Halbach & von Bohlen und Halbach, 2018; Gottmann, et al., 2009, Cunha et al., 2010; Lessmann & Brigadski, 2009; Brigadski & Lessmann, 2020). BDNF secretion can occur via two processes: vesicles of the (i) constitutive pathway and (ii) regulated pathway (Mowla et al., 2001) **(Figure 1.2).** Trafficking of BDNF is a highly regulated process. Two sorting molecules have been identified that play a significant role in sorting newly synthesized BDNF, driving towards a regulated secretory pathway. Sortilin interacts with the BDNF pro-domain, and carboxypeptidase E interacts with the mature domain of BDNF (Song et al., 2017). After being released into the synaptic cleft, proBDNF binds specifically to the p75NTR, and BDNF binds preferentially to TrkB receptors **(Figure 1.1B)** and activates distinct intracellular secondary messengers, which elicit different cellular responses (Brigadski & Lessmann, 2020; Lessmann et al., 2003; Lessmann & Brigadski, 2009; Cunha et al., 2010). BDNF binds to the TrkB receptor with high affinity and triggers the dimerization of TrkB receptors and autophosphorylation of specific receptor tyrosine kinases in the cytoplasmic kinase domain, which act as a docking site for effector molecules. This process further triggers the activation of one of three major signaling cascades: phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinases (ERK), phospholipase Cγ (PLCγ), and. For instance, phosphorylation of TrkB at phosphorylation site Y785 recruits PLCγ, and this event leads to the release of Ca^{2+} from intracellular stores and activation of Ca^{2+} calmodulin-dependent kinase (CaMKII). This CaMKII activates the cyclic adenosine monophosphate -calcium response element binding protein (CREB) via phosphorylation. The transphosphorylation of TrkB at phosphorylation site Y490 triggers the association of proto-oncogene tyrosine-protein kinase (Src)-homology-type 2 linker proteins such as src homology domain (Shc) containing insulin receptor substrate-1 and -2 (IRS1, IRS2). Src sequentially recruits the growth factor receptor-bound protein-2 and the guanine nucleotide exchange factor son of sevenless, initiating the Guanosine-5'-triphosphate loading and rat sarcoma(Ras) activation, which leads to the rapidly accelerated fibrosarcoma (Raf) and ERK kinase cascade. This activated ERK translocates to the nucleus to activate CREB to regulate BDNF gene expression. Growth factor receptor-bound protein-2 also recruits growth factor receptor-bound protein 2-associated protein 1, to activate PI3K and Akt (protein kinase B) kinase cascade. Eventually, the activated CREB either from one of these three pathways triggers the BDNF gene transcription (Cunha et al., 2010). ProBDNF binds to p75NTR, which triggers pro-apoptotic Jun kinase signaling cascades (Woo et al., 2005, Cunha et al., 2010). However, the exact signaling cascades activated by p75NTR signaling are not yet fully resolved. Both BDNF and proBDNF have distinct effects on cellular physiology. BDNF-TrkB signaling stimulates synapse strengthening, neuronal survival

(Reichardt, 2006; Hennigan et al., 2007), functional synaptic plasticity in the form of long-term potentiation (LTP) (von Bohlen und Halbach & von Bohlen und Halbach, 2018; (Bekinschtein & von Bohlen und Halbach, 2020; Korte et al., 1995; Figurov et al., 1996) and has a prominent role in learning and memory (Heldt et al., 2007). Instead, proBDNF-p75NTR signaling promotes apoptosis, presynaptic terminal retraction and decreases synaptic transmission (Yang et al., 2014), which is linked to N-Methyl-D-aspartic acid receptor (NMDAR)-dependent long term depression (LTD) (Woo et al. 2005; Yang et al., 2009), Thus, proBDNF and mature BDNF interact with different receptors and activate distinct signaling systems which play a major role on synaptic plasticity and learning and memory (von Bohlen und Halbach & von Bohlen und Halbach) **(Figure 1.1B)**.

1.2 Fear conditioning and fear extinction.

Fear conditioning is one of the most recognized experimental paradigms for investigating the underlying neurobiological mechanisms of learning and memory in the mammalian brain (Kim & Jung, 2006; Rescorla, 1968). Fear conditioning is a type of associative learning that enables animals to predict adverse events based on environmental cues accompanying those outcomes. Therefore, fear learning is highly beneficial and adaptive for survival, because it helps animals to avoid and escape potentially dangerous situations (LeDoux, 2000). However, despite being an adaptive ability, malfunctions in fear learning are often thought to contribute to the development of fear and anxiety-related disorders in humans (Mineka & Zinbarg, 2006; Singewald et al., 2015). Excessive and overwhelming fears result in suffering and avoidance of circumstances essential to the quality of life. Thus, increased knowledge of the dynamics of fear learning will further enhance the efficacy of therapies to minimize the impact of these disorders and reduce human suffering (Craske et al., 2014; Vervliet et al., 2013).

In my thesis, I used the auditory cued-fear conditioning paradigm, also often termed delayed fear conditioning, and therefore, I would like to describe this procedure in more detail. During auditory cued-fear conditioning in rodents, a tone is used as a neutral stimulus, which is paired one or several times with a mild aversive electrical stimulus as the fear-inducing US. During the conditioning, the animal forms an association between the two stimuli, by which the tone stimulus becomes a CS. This form of fear learning has been extensively studied in both, humans and rodents for several decades (Fanselow & Poulos, 2005; Johansen et al., 2011; Kargl et al., 2020; Pliota et al., 2018).

Before the main phase of the experiment, the animals are allowed to explore and adapt to the experimental chamber where the conditioning will occur, which is termed as habituation phase. Then, during the fear acquisition (training) phase, a sine tone (neutral stimulus, NS) is paired repeatedly with the co-terminating US, i.e. a mild electrical foot shock.

After 1-5 paired presentations of NS-US, subjects learn to associate both stimuli with each other, and the NS becomes a conditioned stimulus (CS) **(Figure 1.3A)** (LeDoux, 2000). Twenty-four hours after the fear conditioning training, the subject is exposed to CS alone in the absence of US, which in case of successful learning will elicit fear behavior, often shown in increased freezing behavior. Freezing is a complete cessation of body movements, except for respiratory-related movements (Fanselow, 1990). This freezing response acts as an essential defensive behavioral response and increases the attention to aversive stimuli (Davis, 1992; Fendt & Fanselow, 1999).

During extinction training **(Figure 1.3B),** animals are exposed repeatedly to multiple CS presentations in the absence of the US. So animals learn that the previously predicted aversive outcome of the US will no longer occur, thus reducing the conditioned fear (Davis, 1992; Fendt & Fanselow, 1999; Phelps, 2006; Phelps & LeDoux, 2005; Singewald et al., 2015). Extinction is a new inhibitory learning and does not erase the original CS-US association. This can be seen by the fact that successfully extinguished fear is not permanent: fear will return with the passage of time, known as spontaneous recovery. Fear may also return when fear-conditioned subjects are exposed to the US alone without the CS; this process is called reinstatement. Extinguished fear memories can also be recovered by exposing the animals to CS presentations in a novel context, termed as a renewal. From a neural perspective, the phenomenon mentioned above clearly shows that extinction training does not erase fear memories. Hence, extinction itself is a new separate learning process that inhibits the CS-US associated fear memories (Yuan et al., 2018; Bouton, 2002; Dunsmoor et al., 2015; Vervliet et al., 2013). However, some research studies evidenced that fear memories can be erased by extinction training (Lin, Lee, & Gean, 2003; Kim, Lee, Park, Song, et al., 2007; Dalton et al., 2008; Mao et al., 2013). Recently, one research study proposed a possible explanation for this ambiguity: inhibition and erasure mechanisms are active at different phases of extinction (early or late). The inhibitory mechanism is intact with single-session extinction training. However, the erasure mechanism becomes prevalent when single-session extinction training is repeated multiple times. Thus, the inhibitory mechanism may operate mainly during the early stages of extinction training, while the erasure mechanism becomes active after that (An et al., 2017). As discussed earlier, memory retrieval is the only possibility to measure the acquired memory from the previous learning process. In this respect, memory retrieval of the fear memory is performed one day after the fear acquisition or at the beginning of the extinction training **(Figure 1.3B, Day 2 early extinction)** to measure the fear memory. Memory retrieval performed after the extinction training **(Figure 1.3C, Day 3)** measures the extinction memory. Besides cued or delayed fear conditioning, there are also other types of fear conditioning paradigms such as contextual or trace fear conditioning. In contextual fear conditioning, there is no specific cue

serving as CS; instead, the whole environment is associated with foot shock (US). Upon re-exposure to the same environment, animals recall contextual fear memory, which elicits fear responses, as e.g. freezing (Kim & Fanselow, 1992). In contrast to cued or delayed fear conditioning, trace conditioning introduces a time gap between the end of the CS and the start of the US. So there is a stimulus-free interval between the CS and the US for a few seconds resulting in a "trace" period (Lugo et al., 2014). However, since we used only auditory cued-fear conditioning in the present thesis, most of the following chapters focus on auditory cued-fear conditioning, while contextual fear conditioning is only briefly discussed when required.

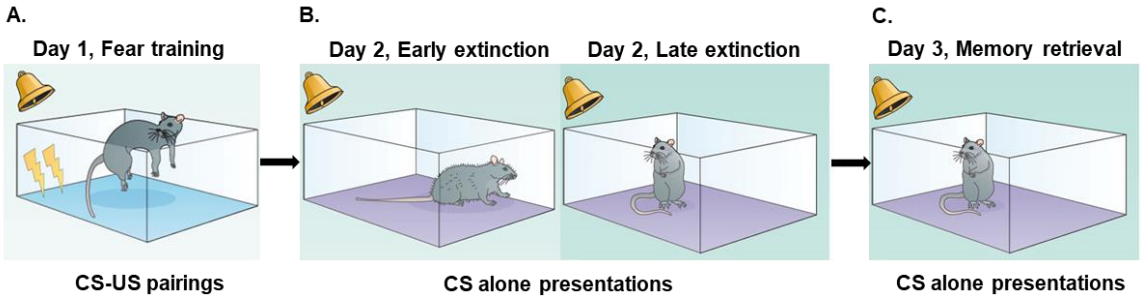

Figure 1.3: Schematic representation of cued-Fear conditioning and fear extinction (Modified from Maren et al., 2013). **(A) Fear training**: On day 1, by the end of fear training animals show unconditional response. **(B) Early and Late extinction training**: On day 2, at the beginning of the extinction training, animals show high freezing levels when exposed to the CS (i.e. fear memory retrieval). When the CS is repeatedly presented many times, the animals show a decline in freezing levels indicating extinction of conditioned fear**. (C) Memory retrieval**: On day 3, animals show reduced freezing levels when exposed to the CS indicating the successful retrieval of fear extinction memory. (CS)-Conditional Stimulus; (US)-Unconditional Stimulus

1.3 Neural circuits involved in cued-fear learning.

In recent decades, significant progress has been made in revealing the fundamental neural circuitry involved in fear learning. A wide range of research studies demonstrated that the amygdala plays a vital role in the acquisition, consolidation, storage, and expression of conditioned fear memory (Johansen et al., 2011). The amygdala, also known as corpus amygdaloideum, is an almond-shaped bilateral structure with a cluster of nuclei located deep in the anterior section of the medial temporal lobe in humans and primates and is one of the main structures of the limbic system (Isaacson, 1974). The amygdala can be divided into several sub-nuclei. For fear learning are especially the lateral amygdala (LA), basal amygdala (BA), and central amygdala (CeA) of importance. The CeA is further divided into the lateral nucleus of central amygdala (CeL) and the medial nucleus of central amygdala (CeM). These sub-nuclei consist of various sub-regions with distinct cellular

compositions and connectivity (Wank et al., 2021). The LA and BA sub-regions form together the so-called basolateral amygdala (BLA) (Krause et al., 2008). The BLA consists of approximately 80% glutamatergic (principal or pyramidal neurons) and 20% GABAergic inhibitory interneurons (Washburn & Moises, 1992; Faber et al., 2001. The nuclei within the BLA are widely interconnected (Pitkanen et al., 1997), and incoming CS/US information is processed first in the LA, which contributes to encoding the conditioned fear memory. Then the CS/US input signals are relayed to CeA which then triggers the physiological responses of fear through divergent projections to the hypothalamus and brainstem areas **(see Figure 1.4)** (Maren & Quirk, 2004; Pliota et al., 2018).

During auditory fear conditioning, the LA receives CS information (tone) from the auditory thalamus and cortex (LeDoux et al., 1984; Luchkina & Bolshakov, 2019; Phelps & LeDoux, 2005; Melzer & Monyer, 2020; Melzer et al., 2020). In addition, the LA also receives US information (such as shock) from the somatosensory cortex and thalamus (Luchkina & Bolshakov, 2019; Phelps & LeDoux, 2005). In naive animals without fear training, the US evokes strong activation on LA pyramidal neurons, while the neutral stimulus (e.g. tone) elicits only weak activation on LA pyramidal neurons. During fear training, when CS is paired with the US, the strong activation caused by US input will enhance the CS input on LA neurons due to their simultaneous temporal activation. After fear conditioning, CS alone could produce robust activation of LA pyramidal neurons during memory retrieval (Rogan et al., 1997). This process triggers the activation of output pathways in the amygdala to elicit defensive responses (Johansen et al., 2011; LeDoux, 2000). GABAergic neurons play a major role in the temporal coordination of neuronal activity in various distant brain circuits (Caputi et al., 2013). For instance, CeM sends inhibitory GABAergic projections to downstream structures, such as ventrolateral periaqueductal gray (vlPAG), which regulates freezing behavior (Haubensak et al., 2010; Herry & Johansen, 2014). These GABAergic projections from the CeM to the vlPAG inhibit a specific sub-set of neurons, leading to activation (disinhibition) of another subset of neurons in vlPAG, which leads to increased freezing behavior. Recently, a research study identified a circuit reciprocally connecting the CeA and vlPAG/ dorsal raphe (DR) region that gates fear learning (Groessl et al., 2018). The medial prefrontal cortex (mPFC) also plays a significant role in regulating fear memory. The mPFC mainly consists of two sub-regions: the prelimbic medial prefrontal cortex (PL mPFC) and the infralimbic medial prefrontal cortex (IL mPFC). Activation of PL mPFC enhances fear expression and memory consolidation, while IL mPFC activation reduces fear expression. The mPFC and BLA demonstrate robust reciprocal connectivity. For instance, strong communication between BLA and PL mPFC triggers the increased activation of CeM neurons **(see Figure 1.4)**, which leads to the disinhibition of vlPAG neurons, resulting in increased expression of fear (Hübner et al., 2014; McGarry & Carter, 2017).

The hippocampus is another important central region of the limbic system that plays a significant role in contextual fear learning. The ventral hippocampus (vHPC) projections towards BLA play a significant role in encoding the context-dependency of fear-related behavior (Orsini & Maren, 2012; Herry et al., 2010). Primarily, ventral CA1 of hippocampal neurons encodes and transmits contextual representations through monosynaptic projections to the amygdala, which elicit defensive behavior (Tovote et al., 2016; Kim & Cho, 2017). Thus, the reciprocal amygdala, hippocampus, and other cortical structures plays a major role in fear learning and memory (discussed in Halbach & Albrecht, 2002). In the present thesis, we have focused only on the importance of the amygdala region (specifically the basolateral amygdala) in regulating cued-fear memory. Therefore, further sections are mainly focused on the amygdala, while the other brain regions are only briefly described and discussed when required.

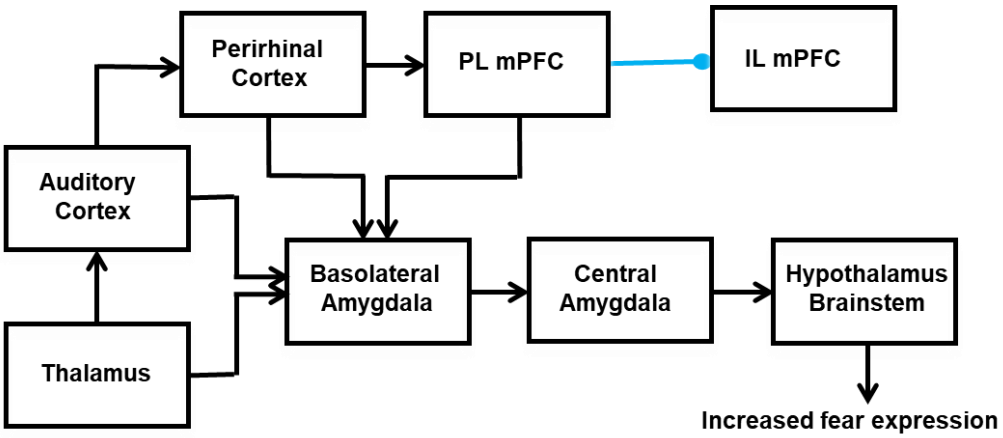

Figure 1.4: Schematic representation of neural circuits involved in cued-fear learning (designed based on Maren, 2011). During auditory cued-fear conditioning, conditional stimulus (CS) and unconditional stimulus (US) inputs from the Auditory cortex and thalamus converge on to the basolateral lateral amygdala (BLA), resulting in the potentiation of auditory responses of BLA neurons. The BLA projects to the medial nucleus of central amygdala (CeM) followed by descending outputs of the CeM to the brainstem and hypothalamic structures triggering fear responses. Additionally, strong communication between BLA and PL mPFC as well as BLA and perirhinal cortex triggers the increased activation of CeM neurons, resulting in increased expression of fear.PL mPFC = prelimbic medial prefrontal cortex, IL mPFC = infralimbic medial prefrontal cortex, **↑=Excitatory inputs, ⚲ =Inhibitory inputs**

1.3.1 Cellular and molecular mechanisms of cued-fear learning

In 1894, Spanish neuroanatomist Santiago Ramon y Cajal proposed the concept of synaptic plasticity for the first time. He hypothesized that memories could be formed by strengthening

existing neuronal connections (Ramón y Cajal, S, 1894). Later in 1949, Donald Hebb, a psychologist, proposed that neurons which “fire together, wire together”, meaning that if two neurons are constantly firing simultaneously, the connection between the two neurons strengthens further (Hebb, D. O, 1949). On the cellular level, the Hebbian plasticity form of associative fear learning has been linked to the concept of LTP. Various research studies demonstrated that both auditory fear conditioning and LTP share similar mechanisms of induction and expression (McKernan & Shinnick-Gallagher, 1997; Rogan et al., 1997). During fear training, an extracellular auditory evoked potential was measured in the LA of control (unpaired) and conditioned (paired) rat groups in response to the CS tones. During fear conditioning and fear memory retrieval, the animals of the paired training group elicited high freezing levels, and an increase in the slope and amplitude of the auditory-evoked potentials were observed. Further, presentations of CS tone alone extinguished the behavioral response, and the auditory-evoked potentials returned to baseline. This data postulates that the enhancement of the auditory evoked LA response shows similar changes as observed during electrical induction of LTP in the thalamo-LA path (Rogan et al., 1997). Therefore, the cellular mechanisms of fear learning can be investigated by combining the *in-vivo, ex-vivo,* and *in-vitro* recordings in LA using LTP as an appropriate model. In this line, several research studies have explored the underlying molecular and biochemical events of LTP in the LA (Huang et al., 2000; Schafe et al., 2000; Brambilla et al., 1997)

During fear training, CS-US convergence triggers associative plasticity in LA projection neurons, resulting in increased cellular activity when subjected later to CS alone during memory retrieval (Sigurdsson et al., 2007; Sah et al., 2008; Johansen et al., 2012; Sears et al., 2014). After fear training, auditory evoked potentials to the CS alone are increased in the LA *in-vitro* and *in-vivo* (Schafe et al., 2001; Sigurdsson et al., 2007; Sah et al., 2008; Johansen et al., 2012). It has been postulated that the CS-US inputs to LA of trigger glutamate release into the synaptic cleft, which binds to glutamate receptors. The binding of glutamate to post-synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors leads to the opening of the AMPA receptors, allowing the influx of positively charged ions, causing an excitatory post-synaptic potential (EPSP). On the other hand, another type of glutamate receptors, the NMDA receptors (NMDARs) are initially blocked by magnesium ions and cannot trigger the influx of ions to the post-synaptic cell. The increased influx of Na^+ ions after stronger/more intense AMPA receptor stimulation induces a higher positive charge (depolarization) inside the post-synaptic neuron. This increased positive charge repels the magnesium ions from the channel pores of the NMDARs, allowing the influx of positively charged calcium ions into post-synaptic neurons. Increased intracellular Ca^{2+} concentration also triggers the further release of Ca^{2+} from intracellular stores in post-synaptic neurons, which leads to the activation of various downstream signaling molecules

like Ca^{2+}calmodulin-dependent protein kinase II (CaMKII) and other protein kinases (Dityatev & Bolshakov, 2005). During fear conditioning, elevated levels of an active auto-phosphorylated form of CaMKII are observed in the dendritic spines of LA (Rodrigues, Farb, Bauer, LeDoux, & Schafe, 2004). These activated CaMKII translocate from the cytosol to synapses and modulate the properties of various synaptic proteins and their interaction via phosphorylation (Shen & Meyer, 1999; Li et al., 2013; Nonaka et al., 2014). This entire process lasts around 1-4 h, which is referred to as early LTP, mainly involved in forming short-term memory. Thus, NMDARs, particularly the NR2B subunit and CaMKII, appear to be involved in the acquisition and initial formation of STM during fear conditioning. For instance, infusion of NMDA antagonist 2-amino-5-phosphonopentanoate (AP5) into the lateral amygdala (LA) blocked the LTP in *in-vitro* electrophysiology experiments and disrupted fear learning when infusions were performed before fear conditioning (Campeau et al., 1992; Miserendino, Sananes, Melia, & Davis, 1990). In another study, the Infusion of Ifenprodil, an antagonist for NR2B subunits of the NMDA receptor, into LA brain slices impaired the LTP in *in-vitro* electrophysiology experiments (Bauer et al., 2002) and disrupted the fear learning when infusions were performed before fear conditioning (Rodrigues et al., 2001).

As discussed above, the increase in intracellular Ca^{2+} during fear training (CS-US pairing) activates protein kinase second messenger pathways (such as the Ras/mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3K) pathway), which are required to form LTM. Upon activation, MAPK and protein kinase A (PKA) translocate to the cell nucleus and trigger gene transcription factor CAMP response element binding protein (CREB) via phosphorylation. This phosphorylated CREB binds to DNA and initiates the gene transcription and protein translation required for temporary cellular changes to be turned into persistent modifications and thus the formation of LTM. Intracellular Ca^{2+} signaling activates the gene transcription, protein translation, and structural changes in cells referred to as late LTP. Late LTP is assumed as one underlying neural process for the formation of LTM. Early LTP is a swift process that causes immediate changes to synapses. In contrast, late LTP is slower, as it relies on gene transcription, and thus, synaptic changes last longer or are often permanent. Thus, LTP might probably be one of the underlying synaptic plasticity mechanisms for fear memory formation (Malenka &Nicoll, 1999; Haubrich & Nader, 2018).

1.3.2 Role of BDNF-TrkB signaling in cued-fear learning

BDNF-TrkB signaling is a key regulator of adult fear circuitry and significant progress has been made in understating the role of BDNF in the fear circuitry over the last decade. Research studies performed in transgenic animal models explored and evidenced the involvement of BDNF-TrkB signaling in mediating fear acquisition. For instance, mice

carrying a point mutation in the phosphorylation sites of TrkB receptors such as Y816 (PLCγ) or Y515 (Shc) showed impaired fear learning. While PLCγ is mainly involved in fear acquisition, the Shc site is responsible for fear memory consolidation (Musumeci et al. 2009). Moreover, heterozygous BDNF knock-out ($BDNF^{+/-}$) rats showed diminished conditioned fear responses (Harris et al., 2016). In another research study, adult $BDNF^{+/-}$ mice exhibited an age-dependent fear learning impairment starting from 3 months of age, which could be attributable to a memory consolidation deficiency (Endres & Lessmann, 2012; Meis et al., 2018). $BDNF^{+/-}$ mice are heterozygous knock-out mice with about half the normal levels of BDNF protein (Bartoletti et al., 2002). Another research study demonstrated that both $BDNF^{Val66Met}$ and $BDNF^{+/-}$ transgenic mice exhibited deficits in contextual fear learning (Chen et al., 2006). $BDNF^{Val66Met}$ is a common single nucleotide polymorphism of rs6265 in the BDNF gene, which causes a substitution of valine (Val) to methionine (Met) at codon 66 in the prodomain (Val66Met), which regulates the activity-dependent release of the BDNF protein (Lessmann & Brigadski, 2009; Kuczewski et al., 2010; Psotta et al., 2013; Brigadski & Lessmann, 2020).

Behavioral research studies evidenced the involvement of BDNF-TrkB signaling in various brain areas mediating fear learning. For instance, the medial prefrontal cortex, specifically the PL, is an emerging modulator of fear behavior. Kerry J Ressler and his colleagues demonstrated that regional knock-out of BDNF in the PL mPFC of mice resulted in impaired fear memory consolidation. Following fear training, local PL mPFC-BDNF knockout mice exhibited robust deficits in freezing at both 1 h and 24 h after fear conditioning. Further, systemic administration of TrkB agonist, 7,8-Dihydroxyflavone (7,8-DHF) into these PL-BDNF knockout animals rescued these impairments (Choi et al., 2010). BDNF-TrkB signaling in the perirhinal cortex also plays a major role in fear memory formation. Interfering with Trk-signaling with local infusion of k252a into the perirhinal cortex 120 min after fear conditioning training also impaired fear memory consolation (Schulz-Klaus, et al., 2013). BDNF-TrkB signaling also plays a vital role in mediating contextual fear memory in the hippocampus. For instance, Barry J. Everitt and colleagues demonstrated that after contextual fear conditioning, BDNF mRNA levels were increased in the hippocampus of rats (Hall, et al., 2000). In another research study, blocking the translation of endogenous BDNF by the infusion of antisense complementary deoxyribonucleic acid (cDNA) into the hippocampus had blocked the contextual fear learning, and this effect was reversed by the co-administration of antisense cDNA with BDNF (Lee et al., 2004). Mutant mice with disrupted PLCγ binding site of TrkB exhibited impaired hippocampal LTP as well as associative learning indicating the importance of TrkB signaling in hippocampal plasticity mechanisms (Minichiello et al., 2002; Gartner et al., 2006; Gruart et al., 2007).

As mentioned above, the amygdala is another important neural structure that plays a significant role in mediating auditory cued-fear memory. BDNF and TrkB receptor mRNA and protein levels were detected at higher levels in various sub-regions of the amygdala. Sensory inputs such as those encoding CS and US information enter LA from cortical and thalamic regions. BDNF is highly expressed in soma and fibers of thalamic neurons (Kawamoto et al., 1996; Conner et al., 1997). Moreover, the temporal association cortex, which also has significant BDNF expression, transmits auditory information about the CS to the LA (Romanski & LeDoux, 1993; Shi & Cassell, 1997; Conner et al., 1997). Thus, the BLA contains high levels of BDNF and its receptor TrkB, and TrkB activation in the BLA is needed to acquire fear memories (Rattiner et al., 2004; Rattiner et al., 2005; Cowansage et al., 2010; Musumeci & Minichiello, 2011; Ehrlich & Josselyn, 2016). TrkB-Fc is a TrkB-specific 'receptor body' composed of the ligand-binding domain of the TrkB receptor coupled to the Fc fragment of human immunoglobulin. This TrkB-Fc acts as a false receptor and scavenges unbound TrkB ligands such as BDNF (Shelton et al., 1995; Patterson et al., 2001). Infusion of either TrkB-Fc or a lentiviral vector expressing a non-functional dominant-negative TrkB isoform (TrkB.T1) or into BLA before fear training had disrupted fear memory formation (Rattiner et al., 2004; Ou & Gean, 2006). Additionally, activation of TrkB receptors by infusion of exogenous BDNF into the BLA or by systemic administration of the TrkB receptor agonist 7,8-dihydroxyflavone (7,8-DHF) facilitated fear learning (Ou & Gean, 2006; Andero et al., 2011). After cued-fear conditioning, increased BDNF mRNA levels were observed in the rodent BLA (Rattiner et al., 2004; Jones, Stanek-Rattiner et al., 2007). Specifically, an increase in BDNF transcripts containing exons I and III were observed (Rattiner et al., 2004; Ou & Gean, 2007). Additionally, Ou and colleagues demonstrated that BDNF protein levels and TrkB phosphorylation were temporarily increased in the BLA after fear training. Upregulation of BDNF expression levels mainly requires calcium influx and phosphorylation of various kinases such as (PI-3) kinase and mitogen-activated protein kinase (MAPK), and PKA (Calella et al., 2007; Ou & Gean, 2007). The same research group postulated that the proteolytic cleavage of proBDNF into mature BDNF was required for fear learning which further supports the crucial role of mature BDNF for fear learning. Besides BLA, the central amygdala (CeA) is another sub-region that plays a significant role in fear learning (Ehrlich & Josselyn, 2016; Fadok et al., 2018). Specifically, the Paraventricular thalamic nucleus (PVT)/CeA circuitry plays a major role in fear learning. Selective deletion of TrkB receptors in lateral nucleus of central amygdala (CeL) or BDNF expression in the PVT impaired the fear learning, and infusion of BDNF into the CeL facilitated fear learning (Penzo et al., 2015).

Considerable research studies indicate the involvement of BDNF in BLA in mediating fear memory consolidation. For example, Ou and colleagues reported increased BDNF

protein expression in the amygdala of rats at 1 h and 12 h after fear training. Their study further revealed that intra-amygdala infusion of either a TrkB ligand scavenger or K252a 9 h after fear training had impaired the long-term fear memory consolidation when tested seven days but not one day after the conditioning (Ou et al., 2010). This indicates that late consolidation of fear memory is required to form stable LTM (i.e. 7 days), which relies on BDNF-TrkB signaling in the amygdala. However, no research was performed to test the involvement of elevated BDNF levels at 1 h after fear conditioning. As mentioned earlier, Gean and colleagues infused TrkB-Fc only before the fear training, which does not allow to differentiate the time resolution of BDNF-TrkB-signaling between fear acquisition and memory consolidation. Thus, in one experiment of this thesis, we further investigated the role of BDNF-TrkB signaling one hour after conditioning for its impact on cued-fear memory consolidation.

1.4 Neural circuits involved in the extinction of cued-fear memory.

Fear extinction is a new inhibitory learning that enables the adaptive regulation of conditioned fear responses. For more than a decade, understanding the neural basis of fear extinction has gained much attention because of its clinical significance in the context of post-traumatic stress disorders in humans. It is well known that the amygdala is one of the main sites for fear learning and fear extinction learning (Bruchey et al., 2007; Corcoran & Quirk, 2007; Hefner et al., 2008; Chang et al., 2009). Within the amygdala, sub-regions such as BLA, intercalated cells (γ-aminobutyric acid (GABA)-releasing densely packed groups of cells) (Melzer & Monyer, 2020) located around the BLA, play significant roles in the acquisition, consolidation, and retrieval of extinction memory (Likhtik et al., 2008; Amano et al., 2010; Maren & Quirk, 2004; Santini et al., 2004; Quirk et al., 2006; Sierra-Mercado et al., 2011; Bloodgood et al., 2018). During extinction training, BLA encodes the CS information and sends this information to IL mPFC (Davis et al., 2017; Klavir et al., 2017). Although the specifics of the IL mPFC -amygdala interactions are still up for debate, it is believed that the IL mPFC regulates the expression of fear primarily through its projections to the amygdala **(see Figure 1.5)** (Bukalo et al., 2015). Research findings demonstrated that activation of the IL mPFC inhibits the CeM neurons (Quirk et al., 2003). CeM is a subdivision of the central nucleus of the amygdala and has direct neuronal projections to the Periaqueductal gray (PAG), ventromedial and lateral hypothalamus that regulate the fear-related responses (Hopkins & Holstege, 1978; Cassell et al., 1986). Though IL mPFC stimulation inhibits the CeM, CeM only receives very few projections from the IL mPFC, indicating that the inhibitory effect is indirect (McDonald et al., 1996). Research studies revealed that Intercalated cell (ITCs) mass surrounding the BLA are key components of this inhibitory circuit (Millhouse, 1986) and also play a key role in the acquisition of extinction memory and its retention (Pare

et al., 2004; Likhtik et al., 2008; Amano et al., 2010). The IL mPFC sends strong projections to medial paracapsular ITCs (mpITCs) located between BLA and CeA. Stimulation of IL mPFC triggers the neuronal activity in the ITCs, and this activation of ITC reduces the activation of CeA neurons, potentially weakening fear responses **(see Figure 1.5)** (Quirk et al., 2003; Paré & Smith, 1993; Royer et al., 1999). Thus, ITCs may incorporate inputs from BLA and IL mPFC to evoke the inhibition of CeM during the acquisition, consolidation, and retrieval of extinction memory (Duvarci & Pare, 2014; Orsini & Maren, 2012; Tovote et al., 2015).

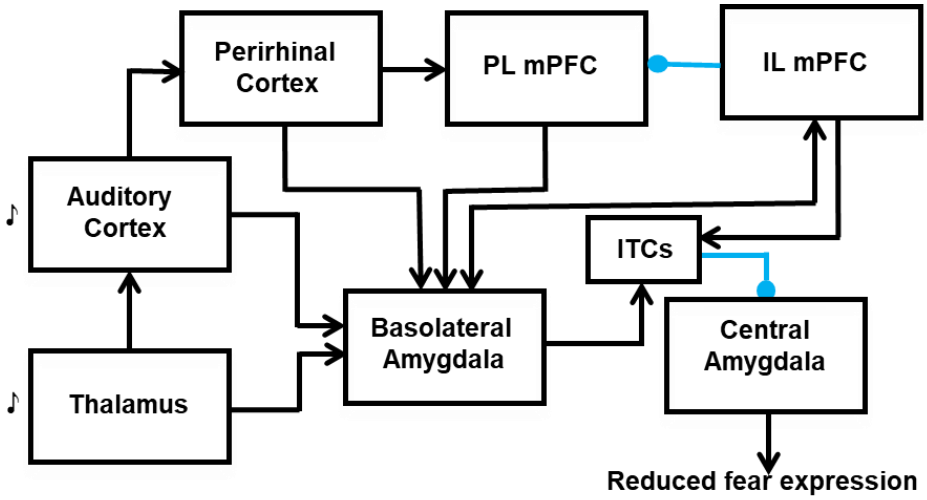

Figure 1.5: Schematic representation of neural circuits involved in the extinction of cued-fear memory (designed based on Maren, 2011). Following extinction, the basolateral amygdala (BLA) encodes the conditional stimulus (CS) information and sends this information to IL mPFC. The activated IL mPFC reciprocally activates the glutamatergic neurons in BLA, which in turn stimulate inhibitory ITCs, thus resulting in the inhibition of the medial nucleus of central amygdala (CeM) output neurons and reduced fear responses. Another possible pathway is the stimulation of IL mPFC triggers the neuronal activity in the ITCs, and this activation of inhibitory ITCs reduces the activation of central amygdala (CeA) neurons, potentially weakening fear responses. However, the specifics of the IL mPFC and amygdala interactions are still up for debate. Further, extinction is expressed only in the same context in which it occurred (mediated by the hippocampus, not shown in the figure). PL mPFC = prelimbic medial prefrontal cortex, IL mPFC = infralimbic medial prefrontal cortex, ITCs= Intercalated cells, ↕= Reciprocal excitatory projections, ↑=Excitatory inputs, ⊥ =Inhibitory inputs

Even though IL mPFC inhibits the conditioned fear responses through neuronal projections either to the BLA or ITCs, there might also be alternative routes with direct projections to CeA (McDonald, 1998) or the hypothalamus that regulate the conditioned fear responses (Fisk & Wyss, 2000). The specific pathway(s) by which the BLA and IL mPFC inhibit

neuronal activity within the medial CeA are still not well understood

After the extinction of conditioned fear, memory for the fear extinction experience becomes context-dependent. Fear is suppressed in the extinction context but can be renewed in other contexts. This 'renewal of extinguished fear memories' concept has become a significant clinical relevance since context-dependent relapses of pathological fear and anxiety are often observed during therapy of various anxiety disorders (Bouton, 1988; Hermans et al., 2006; Craske et al., 2008). The connection between the hippocampus, mPFC, and BLA plays a significant role in mediating the renewal of extinguished contextual fear memories (Maren & Quirk, 2004; Maren, 2005). When animals are subjected to an extinguished CS in the extinction context, the hippocampus induces the IL mPFC inhibition of the BLA to suppress the fear (Maren & Quirk, 2004; Maren, 2005). However, when animals experience an extinguished CS outside of the extinction context, the hippocampus inhibits the IL mPFC activity thereby enhancing the BLA activity to renew fear (Knapska & Maren, 2009). Research studies show that the hippocampus also triggers fear renewal through its direct projections to the basolateral amygdala (Herry et al., 2008). Thus, the hippocampal inputs onto subpopulations of BLA neurons either directly or indirectly, via mPFC, contributes to the context-dependent renewal of conditioned fear (Reviewed in Pape and Pare, 2010). In the present thesis, we have focused only on the importance of the amygdala region in mediating the extinction of cued-fear memory. Therefore, further sections are mainly focused on the amygdala region, while other brain areas are only briefly described when required.

1.4.1 Cellular and molecular mechanisms of extinction of cued-fear memory

There are different potential mechanisms by which fear responses could be diminished following extinction training. There has been a long-lasting debate whether original fear memory is erased completely or transiently inhibited after extinction training. As outlined in the previous chapter, several previous research studies evidenced and supported the inhibitory mechanism of fear extinction by which extinction training forms a new memory, and this new memory inhibits the original fear memory stored in BLA (Maren &Quirk, 2004; LeDoux, 2014). The underlying neural circuits of the inhibition mechanism of fear extinction have been identified in BLA, ITC, and PFC (Likhtik et al., 2008; Amano et al., 2010; Holmes & Singewald, 2013). Conversely, there is also compelling research evidence supporting erasing mechanisms, by which a fear memory that has been stored in the BLA gets removed (Lin, Lee, & Gean, 2003; Kim, Lee, Park, Hong, et al., 2007; Dalton et al., 2008; Mao et al., 2013). Research studies proposed that extinction might reverse or update the prior fear learning if it occurs within a definite time window following memory retrieval, where fear memories become unstable and otherwise susceptible to disruption. During initial fear training, memories are labile but consolidate into long-term memory through protein

synthesis-dependent mechanisms. However, upon retrieval, these memories become labile again and must be reconsolidated through a second round of protein synthesis (Nader & Einarsson, 2010; Monfils et al., 2009). For instance, Monfils et al., 2009, evidenced that this reconsolidation window may allow erasing of fear memories through extinction training. After fear training, retrieving the fear memory in rats with a single isolated CS just before extinction training led to a re-evaluation of the CS as nonthreatening in rats. Then, rats that underwent extinction training shortly after retrieval did not exhibit spontaneous recovery, reinstatement, or renewal of fear (Monfils et al., 2009), indicating that fear memories could be erased through extinction training. Thus, identifying the molecular mechanisms behind reconsolidation is of apparent importance given the significant therapeutic potential of erasing fear memories. Increasing research evidence indicates that extinction may update fear memories by reversing the conditioning-induced processes within the amygdala. Reducing synaptic strength at cortico-amygdala or thalamo-amygdala synapses after fear conditioning by long-term depression (LTD) or depotentiation mechanisms might be another underlying neuronal mechanism of fear extinction learning (Kim, Lee, Park, Hong, et al., 2007; Hong et al., 2009). Precisely calcineurin signaling cascade is involved in the reverse of LTP, resulting in the extinction of fear memory (Lin, Lee, & Gean, 2003; Lin, Yeh, Leu, et al., 2003). Extinction training triggers the upregulation of calcineurin (a protein phosphatase), and this upregulated calcineurin inactivates various protein kinases such as MAPK and Akt via dephosphorylation. Dephosphorylation of protein kinases induces synaptic depotentiation, a physiological process of reversing LTP (Lin, Lee, & Gean, 2003; Lin, Yeh, Leu, et al., 2003; Myers & Davis, 2007). Consistent with the reversal of conditioning-induced changes, extinction training led to depotentiation of CS inputs to the LA and triggers AMPA receptor endocytosis (Lin, Lee, & Gean, 2003; Kim, Lee, Park, Hong, et al., 2007). Indeed, one research study demonstrated that metabotropic glutamate receptors (mGluR1) might enhance the susceptibility of fear memories to disruption by extinction (Clem & Huganir, 2010). Mice that underwent fear conditioning showed a considerable increase in AMPA receptor-mediated synaptic transmission in the LA *in-vitro.* Low-frequency electrical stimulation of LA induced the mGluR1-dependent LTD, which was mediated by a reduction in AMPA receptor-mediated current. Similarly, subjecting mice to extinction trials 30 min after retrieval of the fear memory also reduced the AMPA receptor-mediated current in the LA *in-vitro* and inhibited the recovery of fear memory that generally occurs after extinction. When mice are treated with an mGluR1 antagonist, mice exhibited a recurrence of fear during spontaneous recovery and fear renewal. This indicates that mGluR1-mediated synaptic depression mediates the erasure of fear memory (Clem & Huganir, 2010). Still, it is in debate whether the original fear memory is inhibited or completely erased after extinction (Quirk et al., 2010, An et al., 2017). Besides the just described mechanisms, also other general

cellular and molecular events are involved in fear extinction learning and memory formation, which are described in the next paragraph.

Various research studies evidenced the involvement of multiple receptors, intracellular molecules, and other intra-cellular processes in regulating extinction memory formation. Michael Davis group reported for the first time that NMDARs play a significant role in mediating fear extinction learning. For instance, infusion of NMDAR antagonist D,L-2-amino-5-phosphonovaleric acid (AP5) directly into BLA before extinction training impaired the extinction of conditioned fear (Falls et al., 1992). Similarly, systemic infusion of NMDA-R antagonist, 3-(2-Carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP) either before or after extinction training impaired the retention of extinction memory (Santini et al., 2001). Similarly, Burgos-Robles and colleagues showed that the infusion of CPP into the ventromedial prefrontal cortex (vmPFC) before or immediately after extinction training impaired extinction recall. Multichannel recordings performed by the same groups showed that infusion of CPP reduced burst firing in vmPFC neurons. They have also observed a high degree of bursting in IL mPFC neurons after extinction training, suggesting that NMDAR-dependent bursting in the IL mPFC is required for recall of extinction memory (Burgos-Robles et al., 2007). Another research study shows that the specific NR2B-containing NMDARs are involved in meditating extinction memory. For instance, either systemic or intra-amygdala infusion of Ifenprodil, an antagonist for NR2B-containing NMDARs, before extinction training impaired the initial acquisition and retrieval of fear extinction (Sotres-Bayon et al., 2007). Furthermore, the infusion of Ifenprodil locally into vmPFC immediately after extinction training impaired the extinction consolidation (Sotres-Bayon, Diaz-Mataix, Bush, & LeDoux, 2009). In addition, infusion of NMDAR agonist D-cycloserine into the hippocampus either before or after extinction training enhanced the acquisition and retention of extinction memory and increased the expression level of NR2B protein in the CA1, CA3, and dentate gyrus of the hippocampus (Ren et al., 2013; reviewed in Holmes & Singewald, 2013). These results show that NMDARs in multiple brain areas are involved in fear extinction, and specifically, NR2B-containing NMDARs are required.

It is no surprise that GABA neurotransmission is involved in fear extinction because it is regarded as inhibitory learning. For example, Justin A Harris and colleagues found that systemic injections of GABA antagonists hindered the acquisition and context-dependent expression of extinction in one of the first studies addressing GABA's function in extinction (Harris & Westbrook, 1998). Infusions of GABA agonists into the IL mPFC or BLA, on the other hand, aid oblivion (Akirav et al., 2006). In another study, after fear training, mRNA levels of the $GABA_A$ receptor subtypes α1, α5, and the GABA-synthetizing enzyme GAD were reduced. However, after extinction training, mRNA levels of α2, β2, gephyrin, GAD, and GABA transporter GAT1 were elevated (Heldt & Ressler, 2007). In addition, GAD65-deficient

mice showed impaired cued-fear extinction, both within extinction sessions and during retention (Sangha et al., 2009). Various research studies showed that the extinction of fear memory is dependent on different receptors and intracellular molecules, such as the activation of metabotropic glutamate receptors (mGluRs) (Kim, Lee, Park, Hong, et al., 2007; Fontanez-Nuin et al., 2011;), $GABA_A$ receptors (Chhatwal et al., 2005; Akirav et al., 2006; Sierra-Mercado et al., 2011) L-type VGCCs (Cain et al., 2005), mitogen-activated protein kinase (Lu et al., 2001), phosphatidyl inositol 3 (PI-3) kinase (Lin, Yeh, Lu, & Gean, 2003; Mao et al., 2006), calcineurin (Lin et al., 2003; Almeida-Correa et al., 2015), and new protein synthesis (Lin, Yeh, Lu & Gean, 2003).

1.4.2 Role of BDNF-TrkB signaling in the extinction of cued-fear memory

Several behavioral studies using pharmacological or transgenic approaches, demonstrated the role of BDNF signaling in regulating the extinction of cued-fear memory. For instance, our group (Psotta et al., 2013) demonstrated that $BDNF^{+/-}$ mice exhibit age-dependent impairments in fear extinction learning. In another research study, human BDNF Met allele carriers showed impaired extinction of conditioned fear, which was associated with abnormal fronto-amygdala activity (Soliman et al., 2010). $BDNF^{Val/Met}$ mice also exhibited impaired extinction of conditioned fear responses (Soliman et al., 2010). Research studies also demonstrated the crucial role of BDNF signaling in the amygdala, hippocampus, and IL mPFC in the establishment of fear extinction memories (Heldt et al., 2007; Peters et al., 2010; Rosas-Vidal et al., 2014; Singewald et al., 2015). For instance, the infusion of BDNF into IL mPFC reduced conditioned fear even in the absence of extinction training, indicating that exogenously applied BDNF levels in IL mPFC substituted for extinction training (Peters et al., 2010). Infusion of anti-BDNF antibodies into IL mPFC prior to extinction training impaired the acquisition and retention of extinction memories (Rosas-Vidal et al., 2018). Similarly, infusion of BDNF into vHPC induces fear extinction and increases the firing of IL mPFC neurons. Immunohistochemistry experiments showed that extinction training increases BDNF expression in vHPC neurons projecting to IL mPFC and PL mPFC. Further, disrupting the BDNF production within the vHPC blocked the extinction of avoidance memory (Peters et al., 2010). These research findings support the notion that extinction depends on the release of BDNF in the IL mPFC from projections originating in the hippocampus (Rosas-Vidal et al., 2014; Peters et al., 2010). Further, after extinction training, BDNF protein levels in the ventral hippocampus are elevated at first and then followed by extinction-induced BDNF expression in the basal amygdala (Rosas-Vidal et al., 2014). This indicates that BDNF-TrkB signaling in BLA also plays a role in mediating the formation of extinction memories. One research study showed that the infusion of exogenous BDNF into BLA enhanced fear extinction and elevated the TrkB phosphorylation in the IL mPFC, indicating

the importance of BLA-IL mPFC projection in regulating the extinction memory formation (Xin et al., 2014). In addition, neuronal expression of CC1-EGFP in the basolateral amygdala (BLA) disrupted the extinction of fear memory and blocked the BLA-induced enhancement of TrkB phosphorylation in the IL mPFC. CC1-EGFP (construct of fluorescent CC1 and enhanced green fluorescent protein (EGFP) fused protein) was shown to interrupt the TrkB anterograde axonal transport and its localization at the pre-synaptic site. These research findings indicate that pre-synaptic TrkB in BLA neurons are essential for memory extinction and facilitates the BDNF signaling transduction from the BLA to IL mPFC (Li et al., 2017). Furthermore, Kerry J Ressler and colleagues showed that BDNF mRNA levels were increased in BLA after extinction training. Moreover, infusion of lentiviral vectors expressing TrkB.T1 into BLA in rats before extinction training had impaired the retrieval of extinction but not within-session extinction (Chhatwal et al., 2006). Thus, this research evidence suggests that BDNF-TrkB signaling in BLA is essential in regulating fear memory. However, Kerry J Ressler and colleagues induced a chronic local overexpression of the non-functional TrkB.T1 receptor expressing lentivirus in the BLA of rats, resulting in diminished BDNF/TrkB signaling. The chronic overexpression of TrkB-T1 does not allow a time resolution of BDNF-TrkB-signaling between extinction acquisition and memory consolidation. Thus, in the present thesis, we attempted to disrupt the BDNF-TrkB signaling immediately after extinction training by the infusion of TrkB Fc, which is a BDNF scavenger and to observe its effects on memory consolidation.

1.4.3 Role of proBDNF-p75NTR signaling in the extinction of cued-fear memory.

ProBDNF and p75NTR expression in rodents is developmentally controlled, with the highest levels in the first and second postnatal weeks synchronizing well with synapse formation (Yang et al., 2009). While P75NTR are widely expressed in the central nervous system during developmental stages, their abundance is substantially reduced in adulthood (Underwood & Coulson, 2008; Foltran & Diaz, 2016). ProBDNF is produced in various brain regions such as the amygdala, thalamus cortex, hippocampus, nucleus basalis, and cerebellum (Michalski & Fahnestock, 2003). Immunoblotting experiments revealed the p75NTR expression in the adult murine amygdala (Algamal et al., 2018; Barnes & Thomas, 2008; Colyn et al., 2019). Due to its widespread distribution, proBDNF-p75NTR signaling plays a major role in various physiological functions in adult animals. Research studies demonstrated that the uncleaved proBDNF plays an essential role in regulating synaptic plasticity in the brain and spinal cord (Fahnestock et al., 2002; Zhou et al., 2004). Cleavage of proBDNF into BDNF is also an important mechanism underlying memory processes as it directly affects the proBDNF/BDNF availability. So far, research studies have been

performed in investigating the role of proteolysis of proBDNF in mediating hippocampus-dependent extinction of contextual fear memory. Barnes and colleagues show that proBDNF signaling is necessary for hippocampus-dependent extinction of contextual-fear memory. Blocking the proteolysis of proBDNF to the mature form of BDNF by applying tpa-stop into the CA1 of the hippocampus improved contextual fear extinction. Additionally, the infusion of antisense oligonucleotides targeting BDNF in CA1 of the hippocampus before extinction training enhanced the extinction learning and increased the proBDNF protein expression (Barnes & Thomas, 2008). Additionally, infusion of anisomycin, which is a protein translation inhibitor into CA1 of the hippocampus immediately after the extinction training, impaired the extinction memory formation. Thus, this research study indicates that proteolysis of proBDNF in the hippocampus is a key regulator in the protein synthesis-dependent extinction of contextual fear memory. Interestingly, the infusion of recombinant BDNF into CA1 of the hippocampus before extinction training impaired the contextual fear extinction (Kirtley & Thomas, 2010). However, as mentioned in sub-section 1.5.2, mature BDNF (BDNF) is also required for extinction memory consolidation (Chhatwal et al., 2006). Similarly, the infusion of BDNF into IL mPFC and hippocampal inputs to the IL mPFC also facilitated the cued-fear extinction (peters et al., 2010). Moreover, the extinction of contextual fear memory had elevated both pro- and mature BDNF in the CA1 of the hippocampus (Barnes & Thomas, 2008). These results suggest that a specific ratio of both proBDNF/BDNF expression levels is required for extinction memory consolidation. Additionally, the proteolytic cleavage of proBDNF might influence extinction acquisition and memory consolidation. ProBDNF signaling in IL mPFC also plays a significant role in mediating cued-fear extinction. For instance, Sun, Li, & An, 2018, demonstrated that blocking proBDNF signaling in the I mPFC L delayed cued-fear extinction, and applying exogenous proBDNF to the IL mPFC improved cued-fear extinction (Sun, Li, & An, 2018).

As discussed in section 1.5.1, extinction learning is linked to a reduction in synaptic strength in the LA (Lin, Lee, & Gean, 2003; Kim, Lee, Park, Hong, et al., 2007; Dalton et al., 2008; Mao et al., 2013). The calcineurin cascade is a prominent cellular mechanism that plays a major role in the reversal of LTP and the extinction of fear memory (Lin, Lee, & Gean, 2003; Lin, Yeh, Leu, et al., 2003). Previous research studies showed that the low-frequency stimulation (LFS) of rat LA slices that underwent fear conditioning triggers the LTD at thalamic and cortical afferents to the rat LA (Kim, Lee, Park, Hong, et al., 2007; Hong et al., 2009). However, this depotentiation was occluded by extinction learning, supporting the notion that LTD expression and extinction learning depend on similar cellular mechanisms (Kim, Lee, Park, Hong, et al., 2007; Hong et al., 2009). Supporting this, the electrophysiology team in our lab also observed occlusion of LTD in *ex-vivo* LA slices collected from mice that experienced fear conditioning followed by extinction training (Ma et

al., 2021). Electrophysiological studies in our lab also demonstrated that proBDNF binding to p75NTR is essential to induce LTD in the LA of mice. In particular, activation of p75NTR is necessary for LTD at thalamic and cortical afferents to the LA of mice. Inhibition of proBDNF-p75NTR signaling by applying TAT-Pep5 blocked the LTD at both, the thalamic and cortical afferents to the LA of mice (Ma et al., 2021). TAT-Pep5 is a p75NTR inhibitor, where Pep5 is made cell-permeable by fusing it with the N-terminal protein transduction domain sequence from HIV protein TAT. TAT-Pep5 binds p75NTR intracellularly and blocks-guanosine diphosphate (GDP) (inactive form) from binding to the p75NTR (Pearn et al., 2012). Since LTD is regulated by proBDNF signaling (Lu et al., 2005; Gibon & Barker, 2017), proBDNF is a promising candidate that may regulate the control of LTD in the LA and fear extinction learning. Thus, it indicates that proBDNF-p75NTR signaling in BLA might play a significant role in regulating the extinction memory, and LTD is one of the underlying plasticity-related mechanisms. So, in my thesis, we attempted to block the proBDNF-p75NTR signaling in the amygdala of mice by infusing the TAT-Pep5 in order to see its effect on fear extinction learning. As mentioned earlier, the processing and availability of proBDNF is the crucial step that modulates extinction acquisition and memory consolidation. Thus, we also attempted to block the proteolysis of proBDNF by the infusion of alpha2-antiplasmin (α2AP) in the absence of proper extinction training and to see its effects on it.

2. Aims and objectives

Cued-fear learning and extinction of cued-fear memories rely on neural circuitries containing the amygdala as a core region. The amygdala, especially the basolateral amygdala, is one of the main regions of interest to further determine the local mechanism mediating cued-fear learning and memory extinction. Since proBDNF and BDNF are important mediators of synaptic plasticity, we want to further elucidate their local role in the BLA in mediating cued-fear learning and memory extinction in mice.

Aim (I): The role of proBDNF-p75NTR signaling in the BLA in learning and extinction of cued-fear memory

Several recent studies demonstrated that extinction learning of cued-fear memories resulted in depotentiation at cortico-amygdala and thalamo-amygdala synapses (Kim, Lee, Park, Hong, et al., 2007; Hong et al., 2009; Lin, Lee, & Gean, 2003). Interestingly, depotentiation is mechanistically very similar to de novo LTD in the lateral amygdala, which is well investigated in various research studies (Kim, Lee, Park, Hong, et al. 2007, Hong et al. 2009; Mirante et al. 2014; Collingridge et al., 2010). Furthermore, proBDNF-p75NTR signaling is a well-described mediator for LTD in the Hippocampus (Woo et al. 2005), and very recent findings of our lab showed that LTD at both cortical and thalamic input synapses to the amygdala relies on proBDNF-p75NTR signaling. Thus, we hypothesize that proBDNF-p75NTR signaling could be an essential mediator in the BLA for cued-fear extinction learning. To test this, we locally applied TAT-Pep5, a blocker of p75NTR receptors, which binds to the p75NTR receptor intracellularly and blocks guanosine-diphosphate (GDP) (inactive form) from binding to the p75NTR (Pearn et al., 2012). We applied TAT-Pep5 into BLA of mice either 20 min before extinction training, to investigate their role in the acquisition of fear extinction memories, or immediately after extinction training, to interfere with memory consolidation. In addition, we also tested whether proBDNF-p75NTR signaling in BLA of mice is involved in mediating cued-fear learning. Therefore, we attempted to block proBDNF-p75NTR signaling in BLA by infusing the TAT-Pep5 20 min before the fear learning.

Aim (II): The role of BDNF-TrkB signaling in the BLA on fear extinction memory consolidation

A previous study suggested that the BDNF-TrkB signaling in BLA is more likely involved in the consolidation than in the acquisition of fear extinction memory (Chhatwal et al., 2006). They induced a chronic local overexpression of the non-functional TrkB.T1 receptor in the BLA of rats, resulting in diminished BDNF/TrkB signaling. However, chronic overexpression of TrkB-T1 does not allow a time resolution of BDNF-TrkB-signaling, and hence their

observations are rather indirect. To draw more clear conclusions about the involvement of BDNF in the BLA for extinction memory consolidation, we performed only a temporal interference with BDNF/TrkB signaling by infusing TrkB-Fc into BLA immediately after the extinction training. TrkB-Fc acts as a false receptor and scavenges unbound BDNF (Shelton et al., 1995; Patterson et al., 2001).

Aim (III): Improvement of cued-fear extinction learning by modulating proBDNF processing

As mentioned in section 1.5.3, cleavage of proBDNF into BDNF is also an important mechanism underlying memory processes as it directly affects the proBDNF/BDNF availability. Furthermore, in our first set of TAT-Pep5 experiments (Aim (I)), we found that proBDNF-p75NTR signaling in the BLA plays an essential role in the acquisition and consolidation of fear extinction memory. Thus, we hypothesize that elevating proBDNF levels in the BLA could improve fear extinction learning. Therefore, we developed a partial fear extinction paradigm that did not yield successful fear extinction learning. Next, we attempted to inhibit the proteolysis of proBDNF in the amygdala of mice and assess the impact of locally elevated proBDNF levels in mediating the extinction of cued-fear memory. To this aim, we infused alpha2-antiplasmin (α2AP) either 20 min before partial extinction training or immediately after partial extinction training. The α2AP is a physiological plasmin inhibitor and belongs to the serine protease inhibitor (serpin) family. This α2AP has been studied in various *in-vivo* studies and has proven to efficiently inhibit the proteolysis of proBDNF into mature BDNF (Dulka et al., 2016).

Aim (IV): The role of BDNF- TrkB signaling in the BLA on cued-fear learning

Infusion of either a lentiviral vector expressing a TrkB.T1 or TrkB-Fc into BLA before fear training impaired fear memory formation (Ou & Gean, 2006), pointing towards a crucial role of BDNF-TrkB-signaling for fear memory acquisition. Moreover, Po-Wu Gean and colleagues reported elevated amygdala BDNF protein expression levels at 1 h after fear training, suggesting an additional role in the early consolidation of fear memories (Ou et al., 2010). To test this hypothesis, we locally injected the specific BDNF scavenger TrkB-Fc into BLA after the fear training to hinder the BDNF-TrkB signaling exclusively during fear memory consolidation. With our approach, we can directly differentiate the involvement of BDNF-TrkB signaling between fear acquisition and consolidation.

3. Materials and Methods

3.1 Animals

C57BL/6J (Charles River, Sulzfeld, Germany) male mice between 2-4 months of age were used for all experiments. The animals were maintained at a 12–12 h light-dark-cycle (lights on at 7:00 a.m.) with a continuous supply of food and water *ad libitum*. All behavioral experiments were performed during the light phase of the animals and were in accordance with the ethical guidelines for using laboratory animals in experiments (EU directive 63/10) and approved by the local animal care committee (Landesverwaltungsamt Sachsen-Anhalt, IPHY/G/01-1383).

3.1.1 Stereotactic surgery procedure

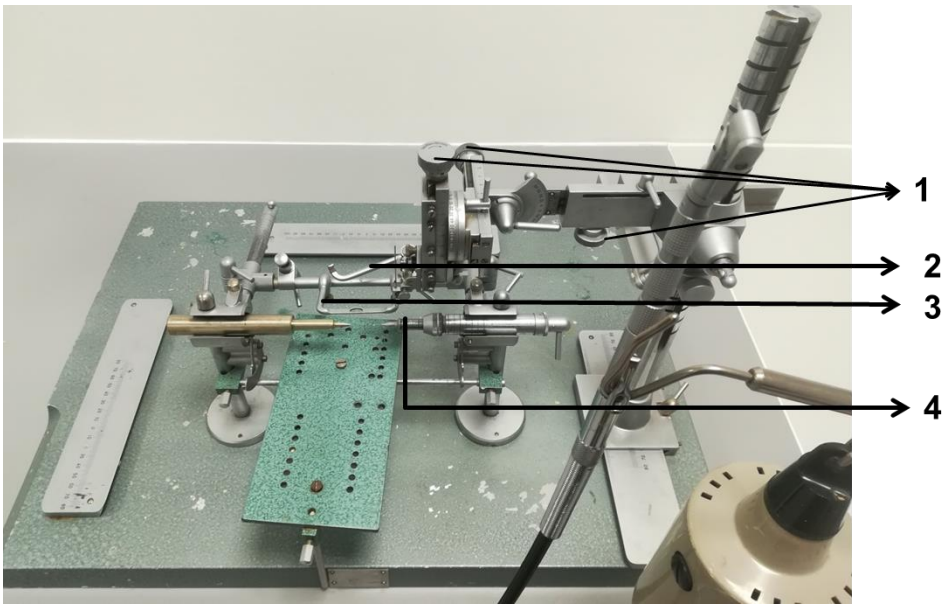

Figure 3.1: Stereotactic frame. Mice head is stabilized using ear bars **(4)** and head holder **(3).** The stereotactic manipulator **(2)** is equipped with injection cannulas that serve as a holder for the guide cannulas during surgery. The manipulator is operated by three navigation screws **(1).**

Mice were deeply anesthetized with Ketamine (200 mg/kg body weight)–Xylazine (10 mg/kg body weight) mixture and the depth of anesthesia were checked by a lack of the toe retracting reflex. Before surgery, mice received a subcutaneous injection of Carprofen (5 mg/kg body weight; Rimadyl®, Zoetis Deutschland GmbH, Berlin, Germany) to reduce pain. Then, mice were restrained onto a stereotactic apparatus, and a 1 cm long incision was made on the frontal part of the animal skull. Hydrogen peroxide (3%) was applied to the skull to remove and clean the soft tissue and debris from the skull. Then two holes were drilled,

and stainless steel guide cannulas (22 gauge) were implanted bilaterally, aiming to BLA (relative to bregma: anterior-posterior, +1.4 mm; medio-lateral, ± 3.2 mm; dorso-ventral, 3.9 mm, coordinates according to Paxinos & Franklin 2013). The cannulas were fixed to the skull by connecting it with dental cement (Hoffmann Dental Manufaktur GmbH, Berlin, Germany) to a screw (Bilany consultant GmbH, Düsseldorf, Germany) that acts as an anchor to the skull. After cannulas were fixed, Hoffmann's phosphate cement was further covered with water-insoluble acrylic cement ("Speiko", Dr. Speier GmbH, Münster, Germany) to protect Hoffmann's phosphate cement throughout the experimental period. After surgery, the animals were constantly monitored until they were awake from anesthesia. To reduce post-surgery pain, mice received directly after the surgery a subcutaneous injection of Metamizole (200 mg/kg bodyweight; Wirtschaftsgenossenschaft Deutscher Tierärzte, Garbsen, Germany) after the surgery. Animals were constantly monitored until they were fully awake and were then put back in their home cages to recover for at least one week before performing behavioral experiments.

3.1.2 Instrumentation for Pavlovian cued-fear conditioning

For the cued-fear conditioning experiments, a fear conditioning system (TSE Systems, Bad Homburg, Germany) was used. The FCS system consisted of a test box, a housing chamber, and a control unit (PCI interface). Each test box had a quadratic (23 × 23 cm^2) shape and was equipped with a loudspeaker on the ceiling to present auditory stimuli, serving as conditioned stimuli (CS), and a grid floor to provide electrical foot shocks, serving as unconditional stimuli (US). The test box was equipped with a sensor frame-mounted with infrared light beams to detect animal movements. In this frame, the test box could be inserted that consisted either of black or transparent Perspex®. The whole system was located in a sound-attenuating housing chamber.

We have used the same contextual settings described below for all the experiments in the present thesis. For fear acquisition, a transparent box, a light intensity of 400, and 70 % ethanol as a cleaning agent was used **(Figure 3.3A)**. For fear extinction training and memory retrieval, a dark box, a light intensity of 90, and Decosept AF (Dr. Schumacher GmbH, Malsfeld Germany) as the cleaning agent were used to provide different contextual settings **(Figure 3.3B & C)**. Additionally, during extinction training and memory retrieval, the grid floor was covered by a dark plate. A tone (8-kHz) with an intensity of 70 dB SPL lasting 30 seconds was used as CS paired with a co-terminating scrambled foot shock (0.4 mA, 1 sec), which served as the US. As an indicator of fear behavior, we used mice freezing time as an index. The freezing behavior of the mice was automatically assessed by an array of infrared light beams surrounding the test box and the automatic freezing detection threshold was 4 seconds.

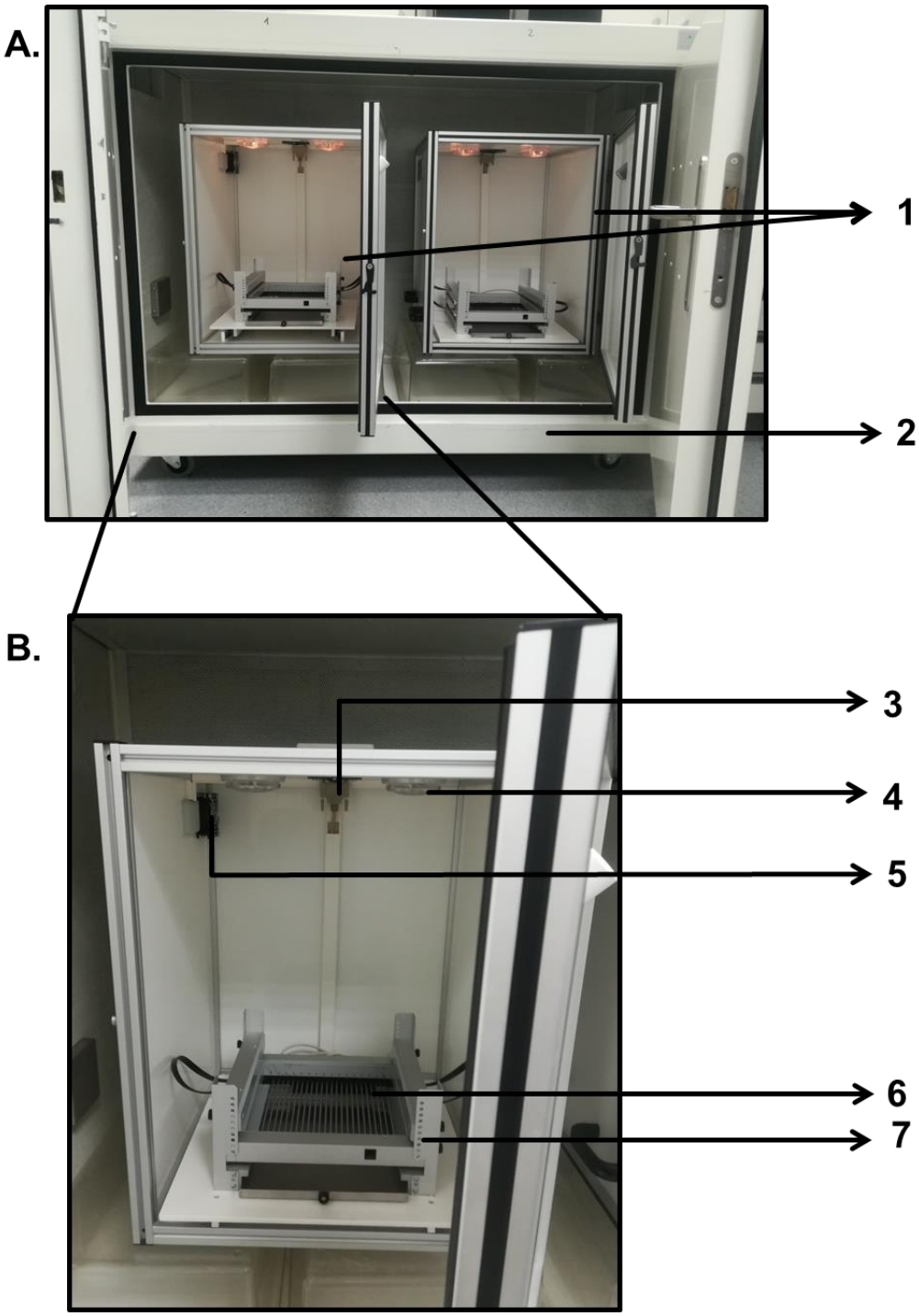

Figure 3.2: (A) TSE Fear conditioning system. (B) Enlarged representation of one experimental chamber (1). During fear training, mice were placed inside the transparent box on the foot shock grid **(6).** During memory retrieval and extinction training, the grid floor was covered by a plate. Animal movements were registered with a sensor frame equipped with infrared light beams **(7).** The tone was presented via the loudspeaker **(3).** Illumination **(4)** and ventilation fan **(5)** were used as variable contextual elements. The whole test box was placed in a sound-attenuating chamber **(2).**

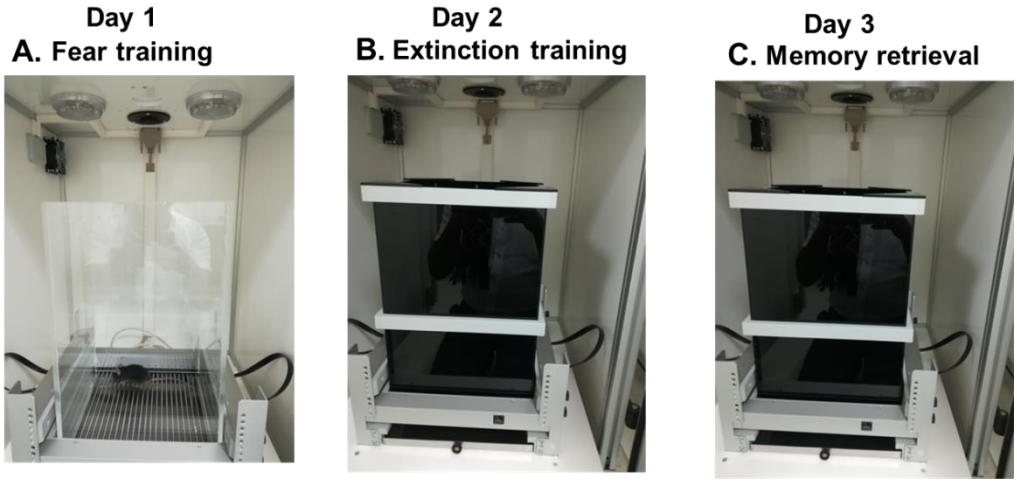

Figure 3.3: Schematic representation of fear conditioning and extinction paradigm. (A) Fear training: On day 1, animals are placed on a grid floor inside a transparent box and are subjected three times to 30 sec of tone (CS) co-terminating with 1 sec shock (US). **(B) Extinction training**: On day 2, animals are placed inside the dark box, and grid floors are covered by a dark plate. Then animals are subjected to 4 times CS with 120 sec inter-trial interval followed by 21 times CS with 30 sec inter-trial interval. **(C) Memory retrieval**: On day 3, animals are subjected 5 times only to the CS**.**

3.1.3 Drug preparation:

Muscimol working solution (1.6 µg/µl) was prepared by dissolving 10 mg of Muscimol (Merck KGaA, Darmstadt, Germany) in 5,975 ml of 0.9% NaCl. TAT-Pep5 working solution (10 ng/µl) was prepared by dissolving 100 µg of TAT-Pep5 (Merck KGaA, Darmstadt, Germany) in 50 ml of 0.9% NaCl. TrkB-Fc (R&D systems, Minneapolis, United States) working solution (6.6 µg/µl) was prepared by dissolving 100 µg of TrkB-Fc in 15 µl of PBS. Alpha2-antiplasmin (α2AP) working solution (0.66µg/µl) was prepared by dissolving 100 µg of α2AP (Merck KGaA, Darmstadt, Germany) in 150 µl of 0.9% NaCl. All working solutions were stored in a freezer at -20^{o}C

3.2 To investigate the role of proBDNF-p75NTR signaling in BLA on cued-fear learning and extinction memories.

In our first set of experiments, we aimed to investigate the role of proBDNF-p75NTR signaling in BLA of mice in regulating the cued- cued-fear learning and extinction memories.

3.2.1 Experiment Ia: To investigate the role of proBDNF-p75NTR signaling in BLA on the acquisition and consolidation of fear extinction memories.

For fear training on day 1, mice were exposed three times to a tone (CS, 8 kHz, 720 dB SPL) that was paired with a co-terminating scrambled foot shock (US, 0.4 mA), with variable inter-

trial intervals of 60 to 120 sec. On day 2, the extinction training was performed in a novel context **(see section 3.1.2 & Figure 3.3B)**. After a 2 min habituation period, animals were exposed to 4 CS with inter-trial intervals of 120 sec first to assess the fear memory retrieval, followed by 21 CS presentations with inter-trial intervals of 5 sec to foster fear extinction learning. Either 20 minutes before or immediately after the extinction training, mice received bilateral microinjections of 0.4 µl TAT-Pep5 (4 ng per side of BLA) from the working solution or 0.9% NaCl alone as the vehicle. An infusion pump (World Precision Instruments Germany GmbH, Friedberg, Germany) equipped with a 2 µl micro syringe (Hamilton, Nevada, Unites states) was used to infuse the drug into BLA at a constant rate of 0.1 µl/min over a period of 3 min. Cannulas were left in place for an additional 2 min to allow complete diffusion of the drug into the BLA tissue. To assess the fear extinction memory, a memory retention test was performed 24 h after the extinction training. Therefore, mice were subjected to 5 times the CS with inter-trial intervals of 120 sec in the same context as the extinction training took place **(Figure 3.3C)**.

3.2.2 Experiment Ib: To investigate the role of proBDNF-p75NTR signaling on cued-fear learning in BLA

For fear training on day 1, the tone (CS) was paired three times with co-terminating scrambled foot shock (US) with variable inter-trial intervals of 60 to 120 sec **(see section 3.2)**. Twenty minutes before the fear training, mice received bilateral microinjections of 0.4 µl TAT-Pep5 (4 ng per side of BLA) from the working solution or 0.9% NaCl as vehicle control into BLA. An infusion pump equipped with a 2 µl micro syringe was used to infuse drugs into BLA at a constant rate of 0.1 µl/min over 4 min. Cannulas were left in place for an additional 1 min to allow complete drug diffusion into the BLA tissue. A memory retrieval test, identical to that used to test for fear extinction memory described in **section 3.2**, was performed 24 h after the fear training to assess the cued-fear memory.

3.3 Experiment II: To investigate the role of BDNF-TrkB signaling in BLA on consolidation of fear extinction memories

We used the same fear training and extinction protocol as described in **section 3.2**. However, on day 2, immediately after the extinction training, mice received bilateral microinjections of 0.3 µl TrkB-Fc (2 µg) from working solution or PBS alone as the vehicle. An infusion pump equipped with a 2 µl micro syringe was used to infuse drugs into BLA at a constant rate of 0.1 µl/min for 3 minutes. Cannulas were left in place for an additional 1 min to allow complete diffusion of the drug into the BLA tissue. To assess the fear extinction memory, a memory retention test was performed 24 h after the extinction training.

3.4 Experiment III: To investigate the role of elevated proBDNF levels on improving the acquisition and consolidation of extinction memories

To analyze the potential memory-improving effect of elevated proBDNF levels on fear extinction learning, we first had to establish a partial extinction protocol, i.e. an extinction paradigm that did not induce a successful extinction memory. Our partial extinction protocol includes only 8 CS presentations, which are not sufficient to induce a successful extinction of cued-fear memory. This was a prerequisite for the planned series of experiments in which we wanted to test whether elevating proBDNF levels in the BLA could improve extinction learning. For fear training on day 1, the CS was paired three times with co-terminating scrambled foot shock (US) with variable inter-trial intervals of 60 to 120 sec **(see section 3.2)**. On day 2, the extinction training was performed in a novel context. First, animals were exposed to 4 CS with 120 sec of time intervals to assess the fear memory retrieval, followed by either 21 CS (normal extinction) or 4 CS presentations (partial extinction) with only 5 sec of time intervals to foster fear extinction learning. To assess the fear extinction memory, a memory retention test was performed 24 h after the extinction training. Therefore, mice were subjected to 5 times the CS with inter-trial intervals of 120 sec in the same context as the extinction training took place. After we verified that the partial extinction paradigm failed to induce successful fear extinction learning, we used this paradigm to test whether elevated levels of proBDNF in the BLA might yield successful fear extinction learning in this paradigm. Therefore, we blocked the proteolysis of proBDNF in the BLA by performing bilateral infusions of α2AP and performed the partial extinction protocol. To this aim, on day 2, either 20 min before or immediately after the partial extinction training, mice received bilateral microinjections of 0.3 µl α2AP (200 ng per side of BLA) from the working solution or 0.9% NaCl as the vehicle. An infusion pump equipped with a 2 µl micro syringe was used to infuse drugs into BLA at a constant rate of 0.1 µl/min for 4 min. Cannulas were left in place for an additional 1 min to allow complete diffusion of the drug into the BLA tissue. To assess the fear extinction memory, a memory retention test was performed 24 h after the partial extinction training.

3.5 Experiment IV: To investigate the role of BDNF-TrkB signaling on cued-fear memory consolidation

For fear training on day 1, the tone (CS) was paired five times with co-terminating scrambled foot shock (US) with variable inter-trial intervals of 60 to 120 sec. Thirty minutes after fear training, mice received bilateral microinjections of either TrkB-Fc human chimeric antibody dissolved in PBS or PBS alone as a vehicle through implanted cannulas directly into BLA. Considering that TrkB-Fc requires at least 30 min to distribute in the tissue (Ou, Yeh, & Gean, 2010), drugs were infused 30 minutes after the fear training to scavenge BDNF 60 min

after conditioning, as BDNF levels in BLA are high at this time point. An infusion pump equipped with a 2 µl micro syringe was used to infuse either the 0.3 µl TrkB-Fc (2 µg) from the working solution or PBS at a constant rate of 0.1 µl/min for 3min.. A memory retrieval test, identical to that used to test for fear extinction memory described in **section 3.2**, was performed 24 h after the fear training to assess the cued-fear memory.

3.6 Histology

Upon completing the behavioral experiments, mice were sacrificed with an overdose of isoflurane, and brains were rapidly collected and placed in a 4% formalin (Merck KGaA, Darmstadt, Germany) solution prepared in PBS. After fixation of brains at 4°C for two days, formalin solution was replaced by 30% saccharose (AppliChem GmbH, Darmstadt, Germany) in PBS solution. Saccharose acts as a cryoprotection for the brain tissues during the following cryosectioning process. Brains were stored in that saccharose solution until they were entirely submerged, which indicates sufficient saccharose diffusion into the brain tissue. Later, each brain was fixed to a round metal platform using TissueTec solution (Sakura Finetek, Alphen aan den Rijn, Netherlands) and placed inside the cryotome (Leica CM 3050, Leica Microsystems, Wetzlar, Germany). A series of 50 µm slices were made, aiming for the area where the cannula traces could be visible. Slices were placed on chrome alum gelatin-coated specimen slides (Gerhard Menzel GmbH, Braunschweig, Germany), which provide good adhesion of brain tissue to the glass and prevent damage or loss of slices during the staining procedure. After cryosectioning, glass slides with brain slices were placed on a warm plate at 42°C for 40 minutes for drying. Further, Nissl staining was performed to visualize the cell bodies and the lesions made by the injections. The Nissl staining protocol comprised of the following steps where glass slides with brain slices were immersed accordingly

- Immerse the glass slides with brain slices two times in 100% ethanol (3 min for each step)
- One time Isopropanol (3 min)
- Three times in xylene (3 min for each step)
- One time in Isopropanol (3 min)
- Two times in 100% Ethanol (3 min for each step)
- One time 70% ethanol (3 min)
- One time in distilled water (3 min)
- One time in cresyl violet (slices were periodically checked until sufficient color intensity was observed)
- One time in distilled water (3 min)
- One time in 70% ethanol (2 min)
- Two times in 96% ethanol (1 min each step)

- One time in Isopropanol (1 min)
- Two times in xylene (3 min for each step)

*Xylene (Carl Roth GmbH, Karlsruhe, Germany), Isopropanol (Th. Geyer GmbH & Co. KG, Renningen, Germany), Ethanol (Otto Fischer GmbH & Co. KG, Saarbrücken, Germany), Cresyl violet (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany)

Steps from 100% ethanol to distilled water, preceding the cresyl violet staining agent, were applied to rehydrate the dried slices and remove extra salts. Protocol from 70% ethanol to Isopropanol was aimed to dehydrate the slices and remove the extra staining agent. Xylene was used to prevent extra background staining and clear tissue residuals. After the last step, glass slides with coronal brain sections were mounted with DePeX (SERVA Electrophoresis, Heidelberg, Germany) and left under the hood overnight.

Brain sections were analyzed under a light microscope to observe the accurate placement of cannulas. Mice with the excessive size of the lesions (>1 mm), lesions interrupting the BLA integrity, and any other cannula misplacement (distance from cannula tip to the BLA>1mm) were eliminated from the final analysis.

3.7 Statistical analysis

For behavioral experiments data, Graph Pad Prism 8.0 was used for statistical analysis. Data obtained from fear conditioning and fear extinction training were analyzed using Two-way Repeated Measures (RM) ANOVA and post-hoc Sidak test. Data obtained from the memory retention test were analyzed by unpaired t-test. Data from all experiments are represented as mean and standard error of the mean (±SEM). A p-value < 0.05 was regarded as a significant difference.

4. Results

4.1 Fear extinction learning relies on proBDNF-p75NTR signaling in the BLA.

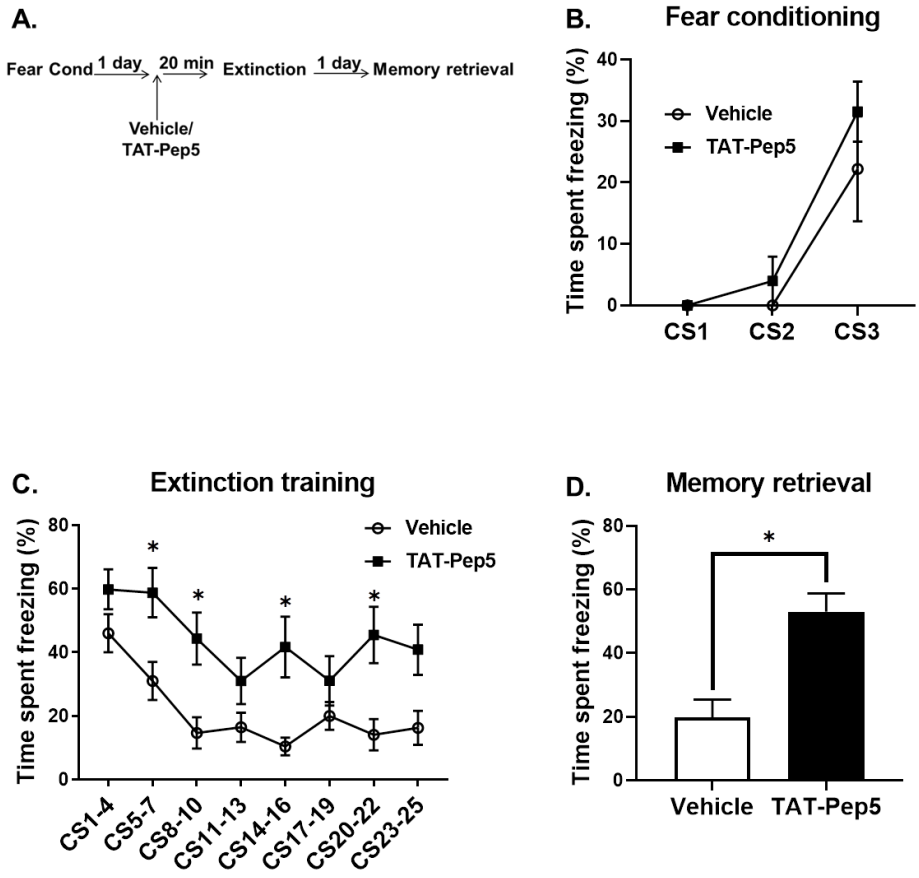

Figure 4.1: Infusion of TAT-Pep5 into BLA 20 min before extinction training impaired cued-fear extinction learning. **(A)** Schematic representation of the experiment to assess the involvement of p75NTR signaling in the BLA for the acquisition of fear extinction memory. **(B)** During fear training, both perspective treatment groups showed similar freezing levels. **(C)** Application of TAT-Pep5 20 min before the extinction training hampered the reduction of freezing during the fear extinction training, suggesting an impaired acquisition of fear extinction memories. **(D)** When tested 24 h later, the TAT-Pep5 (n = 13) treated animals exhibited no memory for the previous fear extinction training, indicated by high levels of freezing to the CS, compared to the vehicle group (n = 12). * indicates $p < 0.05$ (Ma et al., 2021).

So far, no research studies investigated the role of proBNDF-p75NTR signaling in BLA in regulating the extinction of cued fear memory. To test whether fear extinction learning relies on p75NTR signaling in the BLA of mice, we locally applied the p75NTR-Antagonist TAT-Pep5 before the extinction training. TAT-Pep5 is a functional antagonist of p75NTR receptors

and it blocks the Ras homolog family member A (RhoA) activation by preventing the p75NTR-activated displacement of RhoA from Rho GDP dissociation inhibitors (Buhusi et al., 2017; Yamashita & Tohyama, 2003). During fear training, both perspective treatment groups showed a similar level of freezing **(Figure 4.1B)**. A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{2,46}$ = 24.18, p < 0.001), indicating a general increase in freezing behavior with ongoing CS-US pairings. However, there was neither a significant main effect of the factor treatment ($F_{1,23}$ = 1.63, p = 0.21) nor a significant interaction of these factors ($F_{2,46}$ = 0.59 p = 0.56) were observed. On day 2, we bilaterally applied TAT-Pep5 or vehicle 20 min before the extinction training and observed that the local application of TAT-Pep5 impaired within-session extinction **(Figure 4.1C)**. A repeated measure ANOVA revealed significant main effects for the factor drug treatment (TAT-Pep5 vs. vehicle, $F_{1,23}$ = 9.64, p = 0.005) as well as for the number of CS presentations ($F_{7,161}$ = 10.37, p < 0.001). However, there was no significant interaction of these two factors ($F_{7,161}$ = 1.71, p = 0.11). Due to the significant main effects, we have performed post-hoc Holm-Sidak multiple comparisons, which revealed significant differences between the two treatment groups for 5 binned CS presentation periods **(CS5-7, CS8-10, CS14-16, and CS20-22)**. Notably, there was no difference during the first 4 spaced CS presentations, indicating that the local *in-vivo* TAT-Pep5 application did not alter the fear memory expression. Twenty-four hours later, we tested for the extinction memory of these animals and observed significantly more freezing in the TAT-Pep5 than in the vehicle-treated animals (unpaired, t-test comparison p < 0.001, **Figure 4.1D)**. These results indicated that the local application of TAT-Pep5 in the BLA impaired the within-session extinction and resulted in a lack of long-term extinction memory, as seen in the retention test 24 h later (Ma et al., 2021).

4.2 Extinction memory consolidation relies on proBDNF-p75NTR signaling in the BLA

In the second set of experiments, we locally applied TAT-Pep5 immediately after the extinction training to test whether p75NTR signaling is involved in the consolidation of fear extinction memories. During fear training, both treatment groups showed the same level of freezing **(Figure 4.2B)**. A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{2,48}$ = 53.02, p < 0.001), indicating a general increase in freezing behavior with ongoing CS-US pairings. However, there was neither a significant main effect of the factor treatment ($F_{1,24}$ = 1.33, p = 0.26), nor an interaction of these two factors were observed ($F_{2,48}$ = 0.63, p = 0.53). On day 2, we bilaterally applied just either TAT-Pep5 or vehicle into the BLA of mice immediately after the extinction training. Therefore, as expected, we observed no differences during the extinction training between the two treatment groups **(Figure 4.2C)**. A repeated measure ANOVA revealed only a significant

main effect for the number of CS presentations ($F_{7,168} = 17.60$, $p < 0.001$), indicating a general extinction of fear. Importantly, there was neither a significant effect of treatment ($F_{1,24} = 0.73$, $p = 0.40$) nor an interaction of both factors ($F_{7,168} = 0.59$, $p = 0.77$) indicating that both groups show similar performances before the drug treatment. When we tested the animals for their fear extinction memories 24 hr later, we observed significantly more freezing behavior (unpaired t-test comparison: $p = 0.004$) in those mice that had been treated with TAT-Pep5 after the extinction training **(Figure 4.2D)**. In conclusion, our results indicated that p75NTR signaling in the amygdala is required to consolidate fear-extinction memories (Ma et al., 2021).

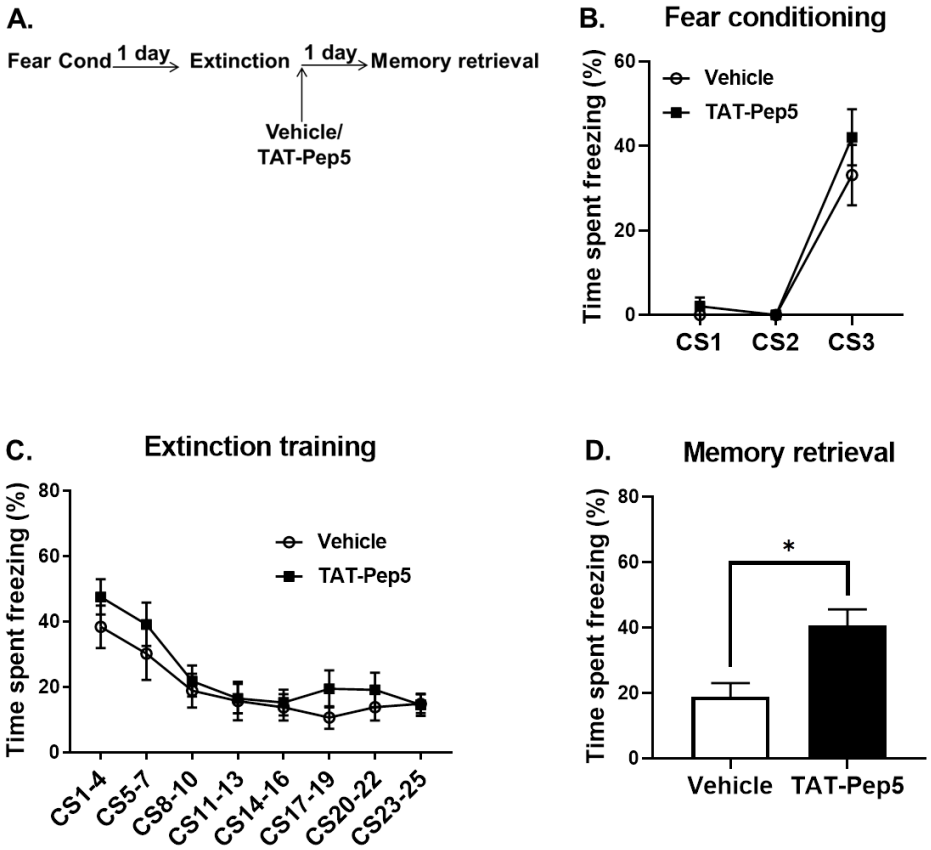

Figure 4.2: Infusion of TAT-PEP5 into BLA of mice immediately after the extinction training impaired the extinction memory consolidation. (A) Schematic representation of the experiment to investigate the involvement of p75NTR signaling in the BLA of mice on the consolidation of fear extinction memory. **(B)** During fear training, both perspective treatment groups showed similar freezing levels. **(C)** Before the application of TAT-Pep5, both groups showed a similar extinction of conditioned fear. **(D)** When tested 24 h later, those animals that received TAT-Pep5 (n = 14) directly after the extinction training, exhibited no memory for the extinction training, indicated by high levels of freezing to the CS, compared to the vehicle-treated group (n = 12). * indicates $p < 0.05$ (Ma et al., 2021).

4.3 Extinction memory consolidation depends on BDNF-TrkB signaling in the BLA

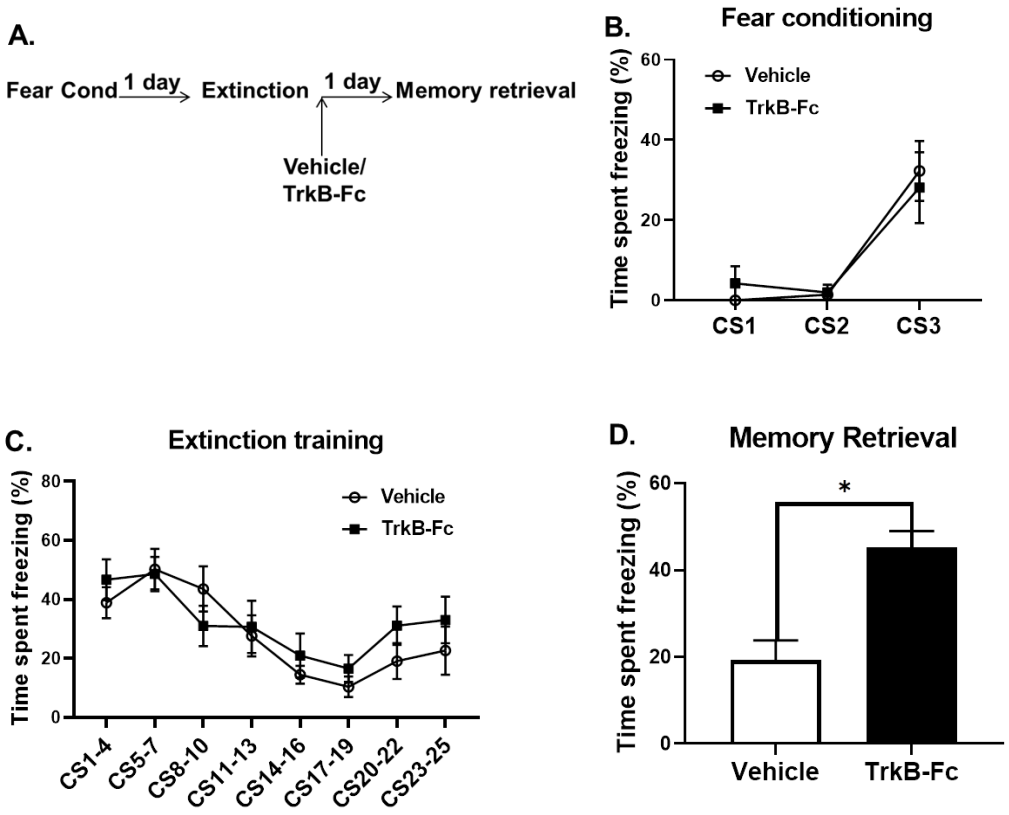

Figure 4.3: Infusion of TrkB-Fc into BLA immediately after the extinction training impaired the extinction memory consolidation. **(A)** Schematic representation of the experiment to assess the effect of TrkB-Fc in the BLA for the consolidation of extinction memory. **(B)** During fear training, both perspective TrkB-Fc (n = 10) and vehicle (n = 10) groups showed similar levels of freezing. **(C)** Before the application of TrkB-Fc, both treatment groups showed a similar extinction of conditioned fear. **(D)** When tested 24 h later, those animals that received TrkB-Fc directly after the extinction training exhibited no memory for the fear extinction training, indicated by high levels of freezing to the CS. * indicates $p < 0.05$.

In our next experiment, we would like to investigate the role of BDNF-TrkB signaling in regulating extinction memory consolidation. To test this, we locally applied TrkB-Fc directly after the fear extinction training to test whether BDNF-TrkB signaling is involved in the consolidation of fear extinction memories. TrkB-Fc is BDNF scavenger, which has been shown to scavenge BDNF efficiently in several previous research studies (Ou et al., 2010; Schildt et al., 2013; Heldt et al., 2014). On day 1, during fear training, both treatment groups show the same level of freezing **(Figure 4.3B).** A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{2,36} = 18.29$, $p < 0.001$),

indicating a general increase in freezing behavior with ongoing CS-US pairings. However, neither a significant main effect of the treatment ($F_{1,18}$ = 0.003, p = 0.96) nor the interaction of these factors ($F_{2,36}$ = 0.30, p = 0.74). On day 2, we first performed extinction training, after which we bilaterally applied either TrkB-Fc or vehicle. As expected, we observed no differences during the extinction training between the two treatment groups **(Figure 4.3C)**. A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{7,126}$ = 10.34, p < 0.001), indicating a general fear extinction. Importantly, neither a significant effect of treatment ($F_{1,18}$ = 0.42, p = 0.52) nor an interaction of both factors ($F_{7,126}$ = 1.08, p = 0.37) were observed, indicating that both groups showed similar performances before the drug treatment. When we tested the animals for their fear extinction memories 24 h later, we observed significantly more freezing behavior (unpaired, t-test comparison: p < 0.001) in those mice that had been treated with TrkB-Fc after the extinction training **(Figure 4.3D)**. In conclusion, our data demonstrated that BDNF-TrkB signaling in the amygdala is required to consolidate fear extinction memories.

4.4 Establishing a partial extinction protocol

Further, we would like to investigate the role of elevated levels of proBDNF alone in regulating the extinction of cued-fear memory formation. For this purpose, we attempt to develop a partial extinction procedure, i.e. an extinction-training paradigm that does not result in successful fear extinction learning. During fear training, both partial and normal extinction groups showed the same level of freezing **(Figure 4.4B)**. A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{2,32}$ = 18.24, p < 0.001), indicating a general increase in freezing behavior with ongoing CS-US pairings. This indicated that both treatment groups show equal manner of fear learning. However, neither a significant main effect between both partial and normal extinction groups ($F_{1,16}$ = 0.12, p = 0.72) nor in the interaction of these factors ($F_{2,32}$ = 0.23, p = 0.79). On day 2, animals that underwent the normal extinction procedure showed reduced freezing levels by the end of training (**See Figure 4.4C, CS22-25)**. A repeated measure ANOVA revealed that there is a significant difference in freezing levels between CS1-4 (start of training) and CS22-24 (end of training) ($F_{1,14}$ = 8.11, p = 0.01). Animals that underwent the partial extinction procedure showed high freezing levels by the end of the training, similar to freezing levels at the start of training **(Figure 4.4D)**. A repeated measure ANOVA revealed no significant differences in freezing levels between CS1-2 (start of training) and CS7-8 (end of training) ($F_{1,18}$ = 0.86, p = 0.36). When tested 24 h later, mice that underwent a partial extinction procedure showed significantly higher freezing levels than the animals that underwent a normal extinction procedure **(Figure 4.4E)** (t-test comparison; P = 0.04). In conclusion, our results showed that the partial extinction protocol with 8 CS presentations does not

extinguish the previously learned fear memory. Further, we used this partial extinction protocol to investigate the role of elevated levels of proBDNF in regulating the extinction of cued-fear memory formation.

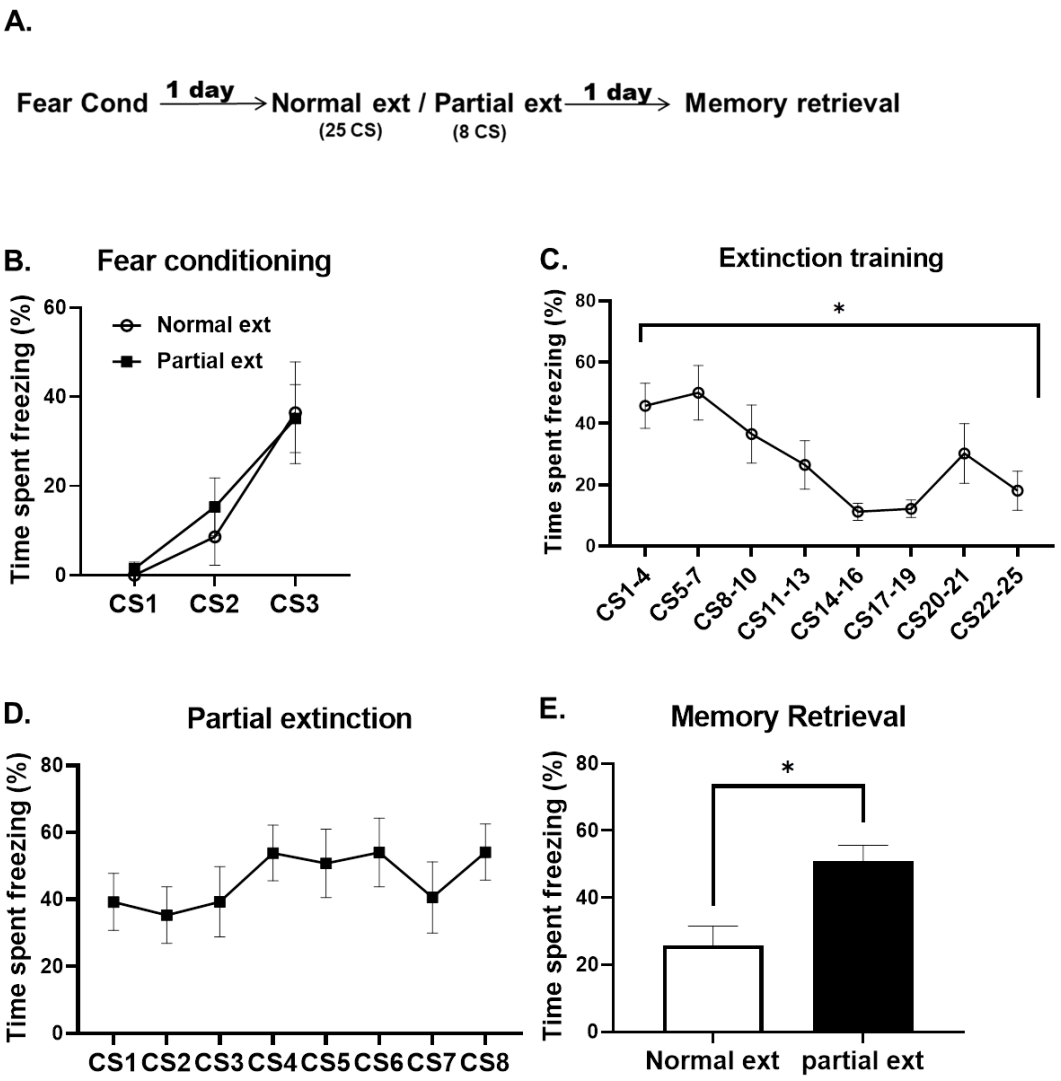

Figure 4.4: Partial extinction (8 CS) procedure does not induce fear extinction learning. (A) Schematic representation of experiment to identify the suitable/successful partial extinction protocol (8 CS). **(B)** During fear training, both partial (8 CS, n=8) and normal extinction (25 CS, n=10) groups showed similar levels of freezing. **(C)** For analysis, 25 CS presentations were averaged to 8 CS presentations for the normal extinction group. Animals showed reduced freezing levels by the end of extinction training **(CS22-25). (D)** Animals showed high levels of freezing to CS presentation by the end of partial extinction training. **(E)** When tested 24 h later, animals underwent partial extinction animals exhibited no memory for the previous fear extinction training, indicated by high levels of freezing to the CS. * indicates $p < 0.05$.

4.5 Inhibition of proteolysis of proBDNF in BLA enhanced fear extinction learning.

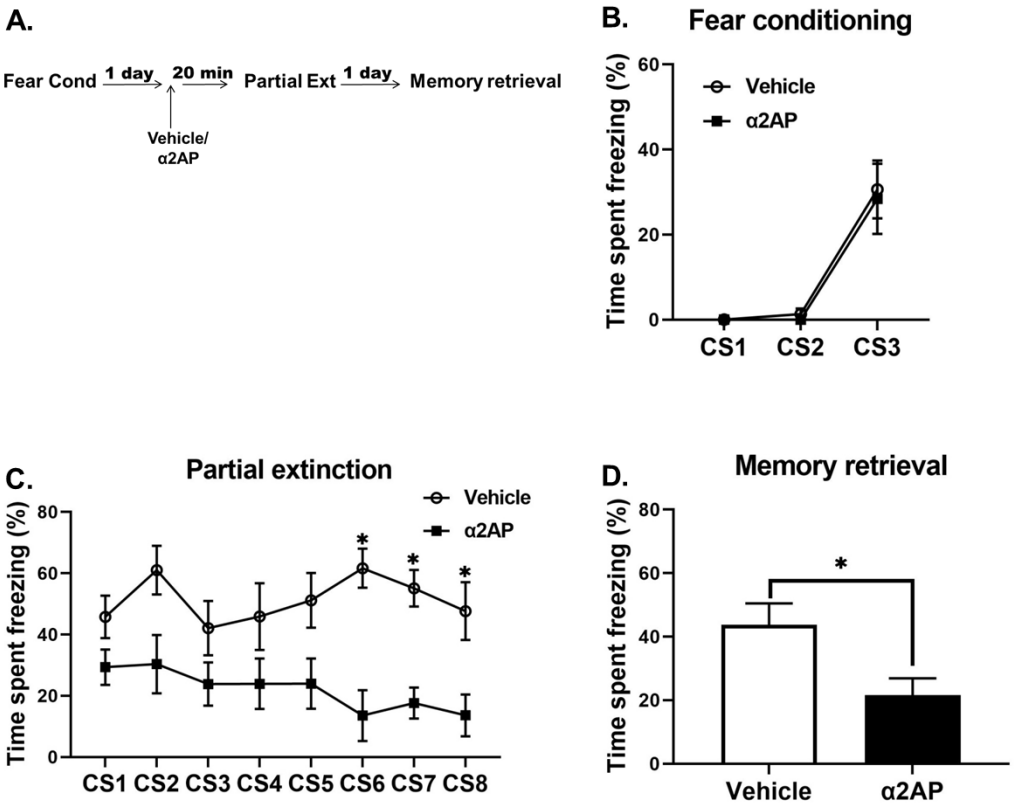

Figure 4.5: Alpha2-antiplasmin (α2AP) infused 20 min before partial extinction enhanced fear extinction memory. (A) Schematic representation of the experiment to assess the influence of inhibition of proteolysis of proBDNF in the BLA on partial extinction learning (8 CS). **(B)** During fear training, both perspective vehicle (n=11) and α2-antiplasmin (n=11) groups showed similar levels of freezing. **(C)** Application of α2AP 20 min before the partial fear extinction training has reduced the freezing levels during the partial extinction training, suggesting an enhanced fear extinction memory. **(D)** When tested 24 h later, the α2AP treated animals exhibited enhanced extinction, indicated by reduced freezing levels to the CS when compared to the vehicle. * indicates $p < 0.05$.

In the previous set of experiments, we optimized a partial extinction protocol and we used the same partial extinction protocol in this set of α2AP experiments. To test whether blocking the proteolysis of pro-BDNF in the amygdala could enhance extinction learning, we locally applied α2AP before the extinction training. During fear training, both vehicle and the supposed α2AP treatment groups show the same level of freezing **(Figure 4.5B)**. A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{2,39} = 29.72$, $p < 0.001$), indicating a general increase in freezing behavior with ongoing CS-US pairings. However, neither a significant main effect of the treatment ($F_{1,20} = 0.096$,

p = 0.75) nor in the interaction of these factors ($F_{2,39}$ = 0.031, p = 0.96) were observed. On day 2, we bilaterally applied either the α2AP or vehicle 20 min before the extinction training and observed that the local application of α2AP improved the within-session extinction **(Figure 4.5C)**. A repeated measure ANOVA revealed significant main effects for the drug treatment (α2AP vs. vehicle, $F_{1,20}$ = 20.95, p < 0.001). However, there was neither a significant difference for the number of CS presentations ($F_{7,140}$ = 0.81, p = 0.57) nor the interaction of these factors ($F_{7,140}$ = 1.17, p = 0.32) were observed. Due to the significant main effects, we performed post-hoc Holm-Sidak multiple post-hoc comparisons, which revealed significant differences between the two treatment groups for the last three CS presentations. Importantly, there was no difference during the first 4 spaced CS presentations, indicating that the local *in-vivo* α2AP application did not alter the fear memory expression. Twenty-four hours later, we tested for the extinction memory of these animals and observed significantly less freezing in the α2AP treated animals than in the vehicle-treated animals (unpaired, t-test comparison; p = 0.017, **Figure 4.5D**). Hence, our results indicated that the local application of α2AP in the BLA enhanced fear extinction learning.

4.6 Inhibition of proteolysis of proBDNF in BLA has no effect on extinction memory consolidation.

We locally applied α2AP directly after the extinction training to test whether blocking the proteolysis of proBDNF has any effect on the consolidation of fear extinction memories. During fear training, both treatment groups showed a similar level of freezing **(Figure 4.6B)**. A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{2,42}$ = 41.78, p < 0.001), indicating a general increase in freezing behavior with ongoing CS-US pairings. However, there was neither a significant main effect of the treatment ($F_{1,21}$ = 0.63, p = 0.43) nor the interaction of these factors ($F_{2,42}$ = 0.22, p = 0.79) were observed. On day 2, we bilaterally applied either α2AP or vehicle immediately after the extinction training. As expected, before drug application, we observed no differences during the extinction training between the two treatment groups **(Figure 4.6C)**. A repeated measure ANOVA revealed no significant effect of treatment between α2AP and vehicle-treated animals ($F_{1,21}$ = 0.16, p = 0.68). Further, no significant differences were observed in freezing between the ongoing CS presentations in each treated group ($F_{7,147}$ = 1.95, p = 0.06). ANOVA revealed no significant interaction of these factors ($F_{7,147}$ = 0.27, p = 0.96), indicating that both groups showed similar performances before the drug treatment. During memory retrieval on day 3, no significant differences were observed in freezing behavior (unpaired t-test comparison: p = 0.55) between the α2AP and vehicle-treated mice **(Figure 4.6D)**. Hence, our results indicated that the local application of α2AP in the BLA did not affect the consolidation of fear extinction memory.

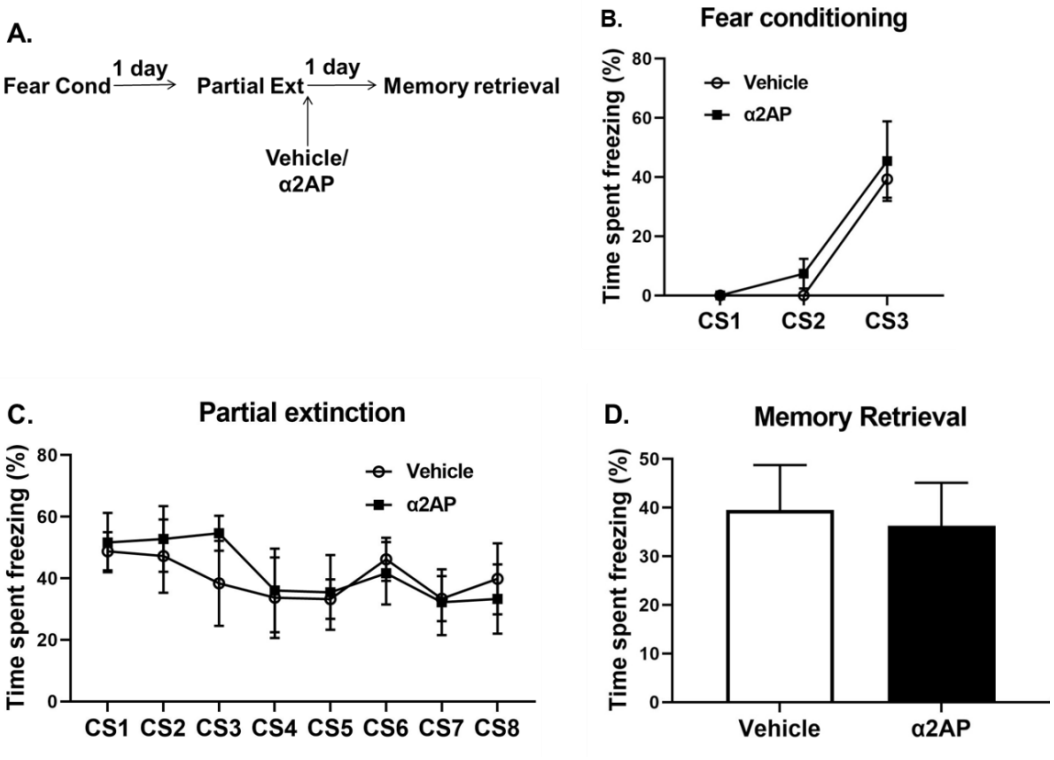

Figure 4.6: Alpha2-antiplasmin (α2AP) infused into BLA immediately after the partial extinction training had no effect on fear extinction memory. (A) Schematic representation of the experiment to assess the influence of inhibition of proteolysis of proBDNF in the amygdala on extinction memory consolidation (8 CS). **(B)** During fear training, both vehicle (n=12) and α2AP (n =11) groups showed similar levels of freezing. **(C)** Before the application of α2AP, both treatment groups showed a similar level of freezing during partial extinction training. **(D)** When tested 24 h later, both α2AP and vehicle-treated animals exhibited similar levels of freezing to the CS.

4.7 Acquisition of cued-fear does not rely on proBDNF-p75NTR signaling in the BLA

To test whether fear learning relies on p75NTR signaling in the amygdala, we locally applied the p75NTR -antagonist TAT-Pep5 20 min before the fear training. During fear training, both treatment groups showed a similar level of freezing **(Figure 4.7B)**. A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{2,32}$ = 19.71, p < 0.001) indicating a general increase in freezing behavior with ongoing CS-US pairings. However, there was neither a significant main effect of the factor treatment ($F_{1,16}$ = 0.01, p = 0.89) nor in interaction of these two factors ($F_{2,32}$ = 0.06, P = 0.94) were observed. Twenty-four hours later, we tested the fear memory of these animals and observed no significant difference in freezing levels between previously TAT-Pep5 and vehicle-treated animals (unpaired t-test comparison; p = 0.751 (**Figure 4.7C**). Hence, our results indicated that the p75NTR signaling was not involved in fear memory formation (Ma et al., 2021).

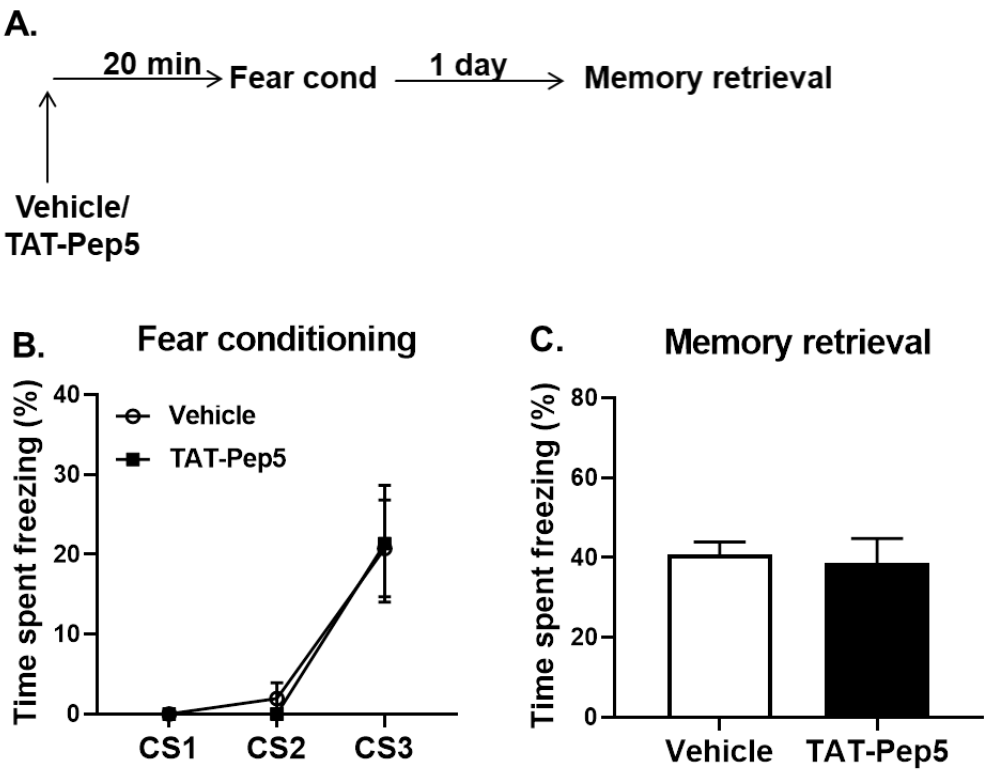

Figure 4.7: Infusion of TAT-Pep5 into BLA 20 min before fear training had no effect on fear learning. (A) Schematic representation of the experiment to assess the involvement of p75NTR signaling in the amygdala for fear learning and memory formation. **(B)** During fear training, both TAT-Pep5 (n = 9) and vehicle (n = 9) treated animals showed similar levels of freezing. **(C)** When tested 24 h later, both TAT-Pep5 and vehicle-treated animals exhibited similar levels of freezing to the CS (Ma et al., 2021).

4.8 Fear memory consolidation depends on BDNF-TrkB signaling in the BLA

To test whether early fear memory consolidation relies on TrkB signaling in the BLA, we locally applied the BDNF scavenger TrkB-Fc 30 min after the fear training. On day 1, during fear training, both treatment groups show the same level of freezing **(Figure 4.8B)**. A repeated measure ANOVA revealed only a significant main effect for the number of CS presentations ($F_{4,84}$ = 25.90, $p < 0.001$), indicating a general increase in freezing behavior with ongoing CS-US pairings. However, there was neither a significant main effect of the factor treatment ($F_{1,21}$ = 0.25, p = 0.62) nor an interaction of these two factors ($F_{4,84}$ = 0.83, p = 0.51). The, we bilaterally applied TrkB-Fc 30 min after the fear training into BLA. Twenty-four hours later, we tested for the fear memory of these animals and observed significantly less freezing in the TrkB-Fc than in the vehicle-treated animals (unpaired t-test comparison $p < 0.003$, **Figure 4.8C**). In conclusion, our results indicated that the local application of TrkB-Fc in the BLA impaired the early fear memory consolidation and resulted in a lack of fear memory, as seen in the retention test 24 h later.

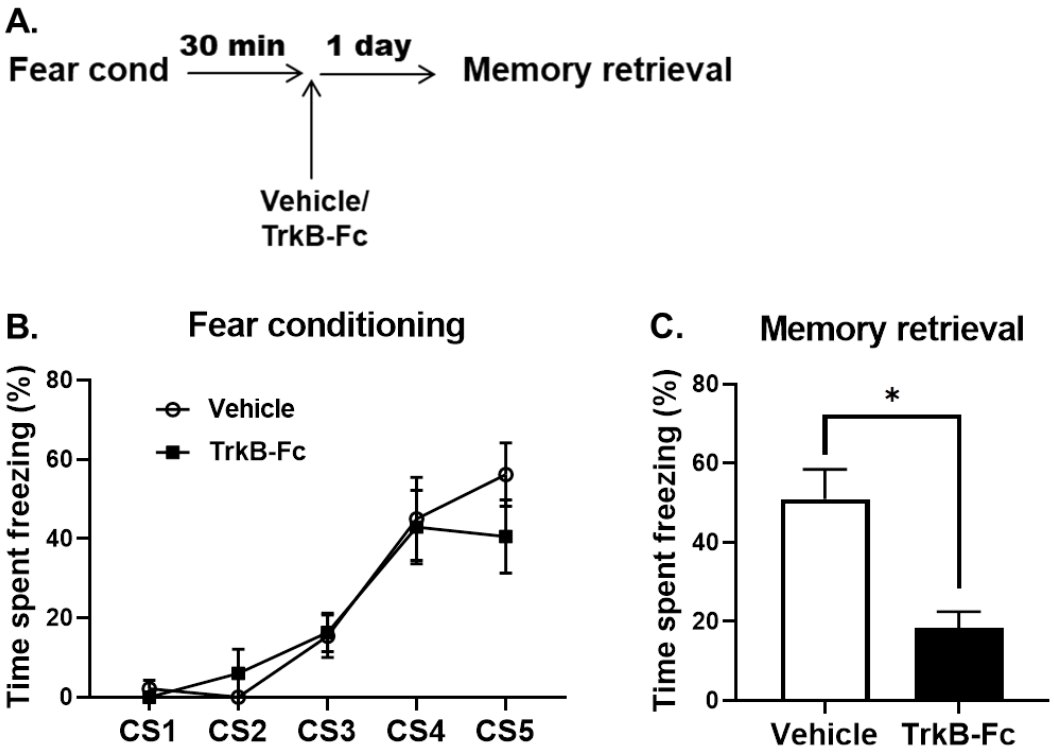

Figure 4.8: Infusion of TrkB-Fc into BLA at 30 min after fear training impaired fear memory consolidation. (A) Schematic representation of the experiment to assess the effect of TrkB-Fc in the BLA for fear memory consolidation. **(B)** During fear training, both perspective vehicle (n=11) and TrkB-Fc (n=12) treatment groups showed similar levels of freezing. **(C)** When tested 24 h later, the animals treated with TrkB-Fc 30 min after fear training exhibited no memory for the previous fear training, indicated by low levels of freezing to the CS. * indicates $p < 0.05$

5. Discussion

The present thesis's main aim was to investigate the role of proBDNF-p75NTR and BDNF-TrkB signaling in the BLA in mediating cued-fear learning and the extinction of cued-fear memory. For the first time, we demonstrated that blocking proBDNF-p75NTR signaling in the BLA impaired both the acquisition and consolidation of cued-fear extinction memory. Furthermore, we showed that elevating proBDNF levels in the BLA by blocking the proteolysis of proBDNF improved fear extinction learning. Thus, our results revealed that the proBDNF-p75NTR signaling in the BLA is an essential additional mechanism contributing to successful fear extinction learning. Besides proBDNF-p75NTR signaling, we demonstrated that in the BLA, BDNF-TrkB signaling is also required for the successful consolidation of fear extinction memories. Thus, both forms of BDNF seem to be important in the BLA for mediating cued-fear extinction memory formation. We also further elucidated the role of proBDNF and BDNF in cued-fear learning and observed that in the BLA BDNF, but not proBDNF is required for successful fear learning. Thus, overall our data revealed new insights into the involvement of BDNF and proBDNF signaling in cued-fear and fear extinction learning.

5.1 Role of proBDNF-p75NTR signaling in the extinction of cued-fear memories

In our first set of experiments, we investigated the role of proBDNF-p75NTR signaling in BLA in regulating the extinction of cued-fear memory. Here, we showed for the first time that proBDNF-p75NTR signaling in BLA is required for the extinction of cued-fear memory. To test for its involvement in the acquisition of fear extinction memory, we performed *in-vivo* experiments by infusing TAT-Pep5 directly into the BLA of mice 20 min before the fear extinction training. TAT-Pep5 is a functional antagonist of p75NTR receptors and it blocks the Ras homolog family member A (RhoA) activation by preventing the p75NTR-activated displacement of RhoA from Rho-GDP-dissociation inhibitors (RhoGDIs) (Buhusi et al., 2017; Yamashita & Tohyama, 2003). Since drugs might take some time to diffuse into the BLA and exert their full inhibitory effect, we infused TAT-Pep5 20 minutes before extinction training. At the end of the fear conditioning, mice showed similar freezing levels, indicating the important prerequisite that both groups showed similar fear learning. Importantly, on day 2, mice that received TAT-Pep5 20 min before fear extinction training exhibited a slower and less effective decay in freezing behavior compared to vehicle-treated mice. Similarly, during memory retrieval on day 3, mice treated with TAT-Pep5 before the extinction training exhibited an increased freezing response. These results indicate that blocking proBDNF-p75NTR signaling by the infusion of TAT-Pep5 had impaired the acquisition of fear extinction memories. Thus, proBDNF-p75NTR signaling in BLA is one of the neural signaling pathways

mediating cued-fear extinction learning. Even though research studies showed that BDNF-TrkB signaling in the BLA is required for amygdala-dependent cued-fear extinction memory consolidation (see our results discussed in **section 5.3** and Chhatwal et al., 2006; Andero et al., 2011), one cannot rule out the possible requirement of additional proBDNF-p75NTR signaling in the BLA in mediating cued-fear extinction memory consolidation. Thus, we investigated the role of proBDNF-p75NTR signaling in BLA in mediating extinction memory consolidation. To test this, we infused TAT-Pep5 locally into BLA immediately after the fear extinction training. Mice treated with TAT-Pep5 immediately after extinction training showed an increased freezing response compared to vehicle-treated groups during memory retrieval. This indicates that, in addition to BDNF-TrkB signaling, proBDNF-p75NTR signaling also mediates the cued-fear extinction memory consolidation in the BLA. Previously, Lei An and colleagues observed that, blocking the proBDNF signaling in the infralimbic medial prefrontal cortex (IL mPFC) of rats by applying an anti-proBDNF antibody before extinction training slightly delayed cued-fear extinction. Additionally, applying exogenous proBDNF to the IL mPFC enhanced cued-fear extinction (Sun et al., 2018). However, they investigated the role of proBDNF signaling in IL mPFC of rats, which in their study required different experimental procedures. For the first time, in our study, we demonstrated the function of proBDNF-p75NTR signaling in the BLA of mice contributing to cued-fear extinction learning and memory consolidation. Moreover, these results indicate that both mice and rats share a similar signaling mechanism for the extinction of cued-fear memory

Previous research studies have assessed the role of proBDNF signaling in different brain areas that play a major role in the extinction of different aversive memories such as contextual fear memory (Barnes & Thomas, 2008), conditioned place aversion (Martínez-Laorden et al., 2019), and inhibitory avoidance memory (Radiske et al., 2015), which relay on different neural circuits. For instance, a research study focusing on the extinction of hippocampus-dependent contextual fear memory observed that inhibiting the proteolysis of proBDNF to BDNF in the CA1 of the hippocampus enhanced contextual fear extinction. Furthermore, proBDNF levels were increased in CA1 of the hippocampus 6 h after contextual fear extinction training supporting the significant role of proBDNF in mediating the extinction of contextual fear memory (Barnes & Thomas, 2008). Radiske and her colleagues performed inhibitory avoidance experiments in rats and demonstrated that BDNF and proBDNF levels were increased in dorsal CA1 of the hippocampus after extinction memory reactivation, indicating that both proBDNF and BDNF are required for the retrieved fear extinction engram (Radiske et al., 2015). In another research study, Pilar Almela and colleagues performed conditioned place aversion experiments in mice to assess the role of BDNF and proBDNF on the extinction of conditioned aversive memory. Conditioned place aversion has been a popular paradigm to assess the aversive aspects of withdrawal. In this paradigm, the

aversive memory is associated with drug withdrawal that can induce an emotional or motivational state, which leads to compulsive drug taking. In this study, they have two groups of mice, one treated with saline and another group treated with morphine. After conditioned place aversion extinction, they observed reduced levels of BDNF, but no significant changes in expression levels of proBDNF when compared to saline-treated animals (Martínez-Laorden et al., 2019) indicating that extinction of conditioned place aversion doesn't rely on proBDNF signaling. Thus, besides the extinction of cued fear memories, proBDNF signaling is a general mechanism in different brain regions that plays a major role in mediating the extinction of various types of aversive memories (Barnes & Thomas, 2008; Radiske et al., 2015), but not all of them with some exceptions (Martínez-Laorden et al., 2019).

5.1.1 Cellular mechanisms of LTD and extinction

Extinction learning is linked to the reduction of synaptic strength in the LA (Lin, Lee, & Gean, 2003; Kim, Lee, Park, Hong, et al., 2007; Dalton, Wang, Floresco, & Phillips, 2008; Mao et al., 2013) and reducing synaptic strength at cortico-amygdala or thalamic-amygdala synapses after fear conditioning by long-term depression (LTD) might be another underlying neuronal mechanism of fear extinction learning (Kim, Lee, Park, Hong, et al., 2007; Hong et al., 2009). In the present study, we showed for the first time that p75NTR activation in BLA is essential for cued-fear extinction learning and memory consolidation. Of note, the electrophysiology team in our lab also demonstrated that proBDNF binding to p75NTR is essential to induce LTD in *ex-vivo* LA slices of mice at thalamic and cortical afferents to LA: so the application of TAT-Pep5 resulted in a blockage of LTD at both, thalamic and cortical afferents to LA (Ma et al., 2021). They further observed occlusion of LTD by paired-pulse LFS of cortical and thalamic inputs to the LA in *ex-vivo* slices collected from mice that experienced fear conditioning followed by extinction training. Synaptic responses were significantly reduced by paired-pulse LFS in fear-conditioned mice, but LTD was occluded in fear-extinguished mice (Ma et al., 2021). These research findings provide new insights that LTD and cued-fear extinction mechanisms in mice share similar cellular mechanisms. Interestingly, de novo LTD depends on group 1 metabotropic glutamate receptor, NR2B containing NMDARs, calcineurin, and AMPA receptor endocytosis (Kim, Lee, Park, Hong, et al., 2007, Mirante et al., 2014). Converging research evidence supports the notion that proBDNF-p75NTR signaling is also essential for NR2B-dependent LTD (Woo et al., 2005) which plays a major role in the extinction of conditioned fear (Sotres-Bayon et al., 2007; Dalton et al., 2012; Sun et al., 2018). For instance, p75NTR$^{-/-}$ mice showed reduced expression of NR2B which is exclusively involved in the LTD, and activation of p75NTR by application of proBDNF enhanced the NR2B-dependent LTD in hippocampal slices (Woo et al., 2005). In another research study, the systemic application of Ro25-6981 a

blocker for NR2B containing NMDARs into rats before extinction training or recall impaired the extinction memory formation. Similarly, the application of Ro25-6981 at thalamic inputs to LA also impaired LTD induction (Dalton et al., 2012). Moreover, blockade of NR2B by systemic and intra-amygdala infusion of Ifenprodil before extinction training also impaired the extinction learning, and subsequent retrieval of fear extinction (Sotres-Bayon et al., 2007). Thus, these research findings support the idea that the LTD induced at thalamic/cortical afferents to the LA involves similar intracellular signaling pathways as cued-fear extinction learning in rodents. Further, the proBDNF-p75NTR-dependent, LTD could contribute to the extinction of cued-fear memory.

Surprisingly, one research study proposed that the mGluR1-dependent LTD might facilitate the erasure of fear memories by extinction training (Clem & Huganir, 2010). Mice that underwent fear conditioning showed a considerable increase in AMPA-receptor mediated synaptic transmission in the LA *in-vitro.* Next, low-frequency electrical stimulation of LA induced the mGluR1-dependent LTD, which was mediated by a reduction in AMPA receptor-mediated current. Similarly subjecting mice to extinction trials 30 min after retrieval of the fear memory also reduced the AMPA receptor-mediated current in the LA *in-vitro* and also inhibited the recovery of fear memory that generally occurs after extinction. However, when mice were treated with a mGluR1 antagonist, mice exhibited a recurrence of fear during spontaneous recovery and fear renewal. This indicates that mGluR1-mediated synaptic LTD regulates the erasure of fear memory (Clem & Huganir, 2010). However, LTD may not just act as the counterpart of LTP via depotentiating and erasing the originally learned memory. Rather, research studies evidenced distinct roles of LTD in hippocampus-dependent associative learning and encoding of novel information (Kemp & Manahan-Vaughan, 2004; Stacho & Manahan-Vaughan, 2022). For instance, a research study demonstrated that during a spatial object recognition test in rats, exploration of a novel environment containing novel or unfamiliar objects facilitated LTD, but exploring a novel environment alone in the absence of objects impaired the LTD. Further, exploration of an empty novel environment facilitated LTP but simultaneous object exploration caused depotentiation of LTP (Kemp & Manahan-Vaughan, 2004). These results showed that LTD has been involved in the acquisition of information about novel item configurations and updating spatial information (Kemp & Manahan-Vaughan, 2004; Stacho & Manahan-Vaughan, 2022). Thus, hippocampal LTD uniquely contributes to spatial learning and memory, especially through the novel acquisition and updating the spatial content. As mentioned in the introduction chapter **section 1.5.1**, there is a long-lasting debate whether extinction training forms a new updated inhibitory memory or it completely erases the fear memory. Sukwoo Choi and colleagues proposed that the inhibitory mechanism is in play with single-session extinction training (An et al., 2017). However, the erasure mechanism

becomes prevalent when single-session extinction training is repeated multiple times. In our present study, we performed only a single session of extinction training and blocking the proBDNF signaling in BLA either before or after single-session extinction training impaired the extinction memory formation. Thus, based on our results, we could propose that proBDNF signaling meditated extinction memory might be a newly updated inhibitory memory rather than an erasure of fear memory. However, further investigations are required to uncover the role of LTD in updating the cued-fear extinction memories by acquiring novel associations of CS presentation.

5.2 Infusion of alpha2-antiplasmin enhanced extinction memory acquisition but not consolidation

Our previous experiments showed that the extinction of fear memory relies on proBDNF-p75NTR signaling in BLA. Next, we investigated whether elevating the proBDNF levels by blocking the proteolysis of proBDNF could mimic the fear extinction-related behavior even without proper extinction training. For this purpose, we modified the extinction protocol in a way that it just does not yield fear extinction memory, which we termed as partial extinction protocol. On day 1 during fear training, both partial and normal extinction groups expressed similar freezing levels, which confirms that there is no impaired fear learning, and both groups expressed an equal manner of fear learning. On day 2, the partial extinction group was exposed briefly to 8 CS presentations, and normal extinction groups were exposed to 25 CS. During memory retrieval on day 3, mice that underwent partial extinction showed higher freezing levels than mice that underwent normal extinction. This shows that our partial extinction protocol is just below the threshold to induce full extinction of fear memory. Thus, our partial extinction protocol briefly activates the consolidated fear memory but is not adequate to extinguish the fear memory. Extinction of fear memory requires more trials than fear acquisition. A single or two CS-US pairings are already sufficient to induce substantial conditional fear, while several Research studies demonstrated that very few individual CS presentations are insufficient to produce extinction memory and extinction learning requires numerous CS presentations (Oler & Baum, 1968; Pavlov, 1927; Reynolds, 1945). These previous research studies are in line with our results that are shown in this partial extinction experiment. Thus, our partial extinction protocol is insufficient to produce significant extinction learning but instead activates the consolidated fear memory.

Then, we investigated whether elevating proBDNF levels in the amygdala could improve fear extinction learning in this non-effective fear extinction paradigm. To this aim, we blocked the proteolysis of proBDNF by local infusion of α2-antiplasmin (α2AP) directly into the BLA 20 min before the beginning of the partial extinction training on day 2. This resulted in reduced freezing levels compared to vehicle-treated mice during the extinction training.

Similarly, on day 3, α2AP-treated animals showed reduced freezing levels than vehicle-treated mice during memory retrieval. This confirms that the elevation of proBDNF levels through blocking the proteolysis of proBDNF by the infusion of α2AP enhanced extinction learning when compared with the vehicle. Tissue plasminogen activator (tPA) is expressed in most brain regions and tPA converts the plasminogen into plasmin, and this plasmin converts proBDNF to mature BDNF. Thus, high plasmin levels lead to increased proteolysis of neurotrophins and the formation of the mature form of neurotrophins such as BDNF (von Bohlen und Halbach & von Bohlen und Halbach, 2018). Alpha2-antiplasmin (α2AP) is a physiological plasmin inhibitor and belongs to the serine protease inhibitor (serpin) family. Lysine residues at the C-terminus of α2AP bind to lysine-binding sites located in the kringle domains of plasminogen and plasmin, generating plasmin-antiplasmin complexes (Skrzypiec et al., 2008). Thus, α2AP regulates the proteolysis of neurotrophic factors such as BDNF and NGF. The function of α2AP has been studied in *in-vivo* studies and has proven to inhibit the proteolysis of proBDNF into BDNF efficiently (Mizutani et al., 1996). For instance, treatment of rat hippocampal slices with the α2AP inhibits plasmin activity and reduces the cleavage of proBDNF into BDNF, suppressing the induction of LTP (Mizutani et al., 1996). Thus, with our experiments, we conclude that infusion of α2AP 20 min before the partial extinction successfully inhibited the proteolysis of proBDNF into BDNF, resulting in an elevation of proBDNF levels, which facilitated the encoding of extinction. A previous research study showed that proteolysis of proBDNF in the hippocampus is a key regulator in the protein synthesis-dependent extinction of contextual fear memory. For instance, infusion of tPA-STOP (an inhibitor of the proteolysis of proBDNF) into CA1 of the hippocampus has facilitated the extinction of contextual fear memory and elevated the proBDNF levels in CA1 after extinction training. However, this study assessed the role of proBDNF in hippocampus-dependent contextual fear memory, which relies on different neural circuits **(see introduction chapter, section 1.5)**. In the present study, we focused on the role of proBDNF in BLA in regulating the extinction of auditory cued fear memory. For the first time, we showed that blocking the proteolysis of proBDNF with α2AP in BLA of mice before extinction training facilitated the extinction acquisition, supporting the previously established notion **(see section 5.1)** that proBDNF signaling is required for the extinction acquisition.

Next, we assessed the effect of inhibition of this treatment on extinction memory consolidation. For this purpose, we infused α2AP locally into BLA immediately after the partial extinction training. Surprisingly, during memory retrieval, both vehicle and drug-treated groups showed a similar level of freezing. Thus, elevating proBDNF levels by blocking the proteolysis of proBDNF did not enhance extinction memory consolidation. One possible explanation is that partial extinction (weak paradigm) training does not produce enough proBDNF levels for a successful extinction acquisition. Even though the processing of

proBDNF was blocked immediately after the partial extinction training, there was no initial acquired memory to be further consolidated. Another possible explanation could be that the extinction memory consolidation might require both proBDNF and BDNF (**see section 5.3**, Barnes & Thomas, 2008, Kirtley & Thomas, 2010). However, it could that our partial extinction paradigm did not produce enough proBDNF levels to be processed into BDNF and hence, infusion of α2AP into BLA did not show any enhancing effect on extinction memory formation. Thus, both proBDNF and BDNF could play a major role in extinction memory consolidation (see **section 5.3.1** for more details). In conclusion, our present findings proved that extinction memory learning could be improved by elevating proBDNF levels by inhibiting the processing of proBDNF during extinction training.

5.3 Role of BDNF-TrkB signaling in the extinction memory consolidation

In the first set of experiments, we showed that proBDNF-p75NTR signaling plays a major role in regulating cued-fear extinction learning and memory consolidation. Now, we investigated the role of BDNF-TrkB signaling in mediating extinction memory consolidation. Here, we observed that the Infusion of TrkB-Fc into BLA immediately after the extinction training impaired the consolidation of fear extinction memory. TrkB-Fc has been shown to scavenge BDNF efficiently in several previous research studies (Ou et al., 2010; Schildt et al., 2013; Heldt et al., 2014). A previous research study showed that local overexpression of lentiviral vector expressing non-functional dominant-negative TrkB isoform TrkB-T1 receptors into BLA before extinction training impaired extinction memory retention but not encoding of extinction memory (Chhatwal et al., 2006). However, the chronic overexpression of TrkB-T1 does not allow a clear time resolution of BDNF-TrkB-signaling required for acquisition and consolidation, and hence their observations are rather indirect. To gain more clarity on this, we infused the TrkB-Fc immediately after the extinction training to scavenge BDNF and assess its effect on the consolidation of extinction memory. With this approach, we confirmed that BDNF-TrkB signaling is required for the consolidation of extinction memory consolidation. Previously, Kerry J Ressler and colleagues have reported time-dependent upregulation of BDNF exon V-containing mRNA within the BLA compared to non-extinguished controls (Chhatwal et al., 2006). So probably fear extinction learning leads to an upregulation of BDNF, which in turn is used for consolidation of extinction memory. In another study, TrkB phosphorylation levels in the BLA were increased 2 h after fear extinction training, and blockage of the pre-synaptic TrkB in BLA neurons impaired the extinction of cued-fear memory (Li et al., 2017). In our study, scavenging the unbound BDNF in BLA by infusing TrkB-Fc after extinction training impaired extinction memory consolidation. Thus, our results perfectly complement the two previous research studies demonstrating that after extinction training, BDNF expression and BDNF-TrkB signaling in BLA plays a major

role in cued-fear extinction memory consolidation.

5.3.1 Homeostasis between proBDNF and mature BDNF

Our own and the experiments of other groups clearly show that in the BLA both, proBDNF and BDNF are required for the successful consolidation of fear extinction memories. Thus, the balance between the levels of both, proBDNF and BDNF probably plays a major role in mediating the consolidation of extinction memory. In this respect, Barnes and Thomas (2008) demonstrated that inhibiting the proteolysis of proBDNF into BDNF by the infusion of tPA-STOP into CA1 of the hippocampus prior to extinction training has impaired the extinction of contextual fear memory. Additionally, they showed elevated proBDNF levels in hippocampal CA1 of animals that underwent contextual fear extinction (Barnes & Thomas, 2008). In our results, we proved for the first time that inhibiting the proBDNF-p75NTR signaling in BLA plays a major role in cued-fear extinction memory formation. However, our results of the present study and other previous studies (Chhatwal et al., 2006) showed that mature BDNF (BDNF) is also required for the consolidation of fear extinction memory. One possible explanation is that indeed optimal levels of BDNF are required for extinction memory consolidation and deviation from this optimum level (too high or too low) leads to an impairment in extinction memory formation (Trent, Barnes, Hall, & Thomas, 2017). For instance, the application of exogenous recombinant BDNF into the dorsal hippocampus prior to extinction training impaired the extinction of contextual fear memory (Kirtley & Thomas, 2010; Trent et al., 2017). Hence, the high levels of exogenously administered recombinant BDNF into the dorsal hippocampus prior to extinction training might have disrupted the finely controlled mechanisms of endogenous BDNF to orchestrate the extinction of contextual fear memory (Kirtley & Thomas, 2010; Trent et al., 2017). The homeostasis between proBDNF and BDNF in the hippocampus is also important in contributing to cognitive performance in rodent models of Alzheimer's disease. Some Alzheimer’s disease mouse models show high levels of proBDNF/BNDF ratio in the hippocampal CA1 implying that impaired processing of proBDNF cleavage into BDNF. However, normalizing the proBDNF/BNDF ratio in hippocampal CA1 of Alzheimer’s disease mice models improved the cognitive performance in fear conditioning and water maze experiments (Bie et al., 2022). In another research study, postoperative cognitive dysfunction (POCD) mice exhibited reduced levels of BDNF/proBDNF ratio in the hippocampus and impaired consolidation of contextual fear memory. However, infusion of TAT-Pep5 and exogenous BDNF into the hippocampus of POCD mice facilitated the consolidation of contextual fear memory. This study further confirms that the BDNF/proBDNF ratio plays a major role in regulating cognitive behavior in rodent models (Xue et al., 2022). Although these previous research studies were conducted in the hippocampus to assess various cognitive behaviors in rodent models, it is still plausible

to state that the homeostasis between proBDNF and BDNF in BLA is essential for the consolidation of cued-extinction memory. As a final note, our present study confirmed that fear extinction learning relies on proBDNF-p75NTR, but both BDNF-TrkB signaling and proBDNF-p75NTR signaling are required for cued-fear extinction memory consolidation.

5.4 Role of proBDNF-p75NTR signaling in mediating the fear memory

So far, no research studies have investigated the role of proBDNF-p75NTR signaling in BLA in mediating cued-fear learning. Therefore, we blocked proBDNF-p75NTR signaling in the BLA during fear conditioning. Mice infused with TAT-Pep5 20 min before fear training expressed similar freezing levels as vehicle-treated animals throughout the whole fear training session. Similarly, during memory retention, there were no differences in freezing levels between vehicle and TAT-Pep5 treated mice. Thus, our results showed that the proBDNF-p75NTR signaling in BLA is not required for fear memory formation. In line with our results, a previous research study showed that $p75NTR^{ExIII-/-}$ mice showed no impairment in cued and contextual-dependent fear memory formation. $P75NTR^{ExIII-/-}$ knockout mice models are generated by removing the exon III that encodes the extracellular domain of p75NTR receptors. These mice showed similar freezing levels when compared to control mice during retrieval of cued or contextual fear memory indicating that fear memory is intact in $p75NTR^{ExIII-/-}$ knockout mice models (Busch et al., 2017). Interestingly, few other studies demonstrated distinct functions of proBDNF-p75NTR signaling in mediating various other aversive memories in inhibitory avoidance and chronic social defeat stress tasks. For instance, TrkB heterozygous knockout mice showed impaired acquisition of fear memory, while p75NTR knockout mice showed enhanced fear memory in an inhibitory avoidance experiment (Olsen et al., 2013). This shows that proteolysis of proBDNF into BDNF and BDNF-TrkB signaling play important roles in mediating inhibitory avoidance memory. Further, long-term chronic social defeat stressed mice exposed to a fear stimulus showed elevated levels of proBDNF in the BLA when compared to non-stressed control mice and exhibited a marked social avoidance memory. Thus, the enhanced proBDNF expression in BLA induced by aversive stimuli could be a synaptic marker of major depression (Colyn et al., 2019). Hence, based on our present results and previous studies, proBDNF-P75NTR signaling has diversified roles in mediating various types of aversive memories.

5.5 Cued-fear memory consolidation requires BDNF-TrkB signaling in BLA

We further investigated the role of BDNF-TrkB signaling in BLA in mediating cued-fear memory formation. We showed that the elevated BDNF levels in the BLA of mice 60 min after cued-fear training are involved in consolidating early fear memories. Interfering the BDNF-TrkB signaling by the infusion of TrkB-Fc into BLA 30 min after the fear training has

impaired the consolidation of fear memory. BDNF levels started to increase at 30 min and reached the maximum peak at the 60-min time point after cued-fear learning in the amygdala of rats (Ou et al., 2010). Therefore, we performed a local infusion of TrkB-Fc BLA 30-min after fear training. By scavenging the available BDNF by local application of TrkB-Fc, we observed a complete absence of fear memory as measured during the memory test one day later. Our results provided further evidence that TrkB-signaling in the BLA is involved in the early consolidation of cued-fear memories. Further, Po-Wu Gean and colleagues performed their experiments in rats and we performed our experiments in mice, which suggests that both rats and mice have similar neuronal and temporal mechanisms in meditating cued-fear memory formation. Po-Wu Gean and colleagues also demonstrated that after fear training there is a second increase in BDNF expression levels peaking around 9 h and decline back to baseline around 12 h after fear training. Infusion of either the Trk inhibitor k252a or the BDNF scavenger TrkB-Fc into BLA at 9 h after fear training impaired the late consolidation of fear memory resulting in a lack of fear memory when tested after 7 days, while fear memory was still intact when tested 24 h after of fear conditioning (Ou et al., 2010). In our study, scavenging BDNF around 1 h after the fear conditioning point impaired the fear memory consolidation when tested 24 h after fear conditioning. These results show that BDNF expression in BLA is required at early (1 h) and late (9 -12 h) fear memory consolidation and required at least two stages of protein synthesis. Our results also suggests that disruption of early memory consolidation at 1 h time point disrupted the second memory consolidation and hence mice expressed reduced levels of freezing during memory retrieval performed 24 h after fear training. Thus, the disruption of BDNF-TrkB signaling during early fear memory consolidation in a restricted time window could interfere with memory persistence, thereby revealing BDNF-TrkB signaling as a potential target for the treatment of post-traumatic stress disorders.

Previous research studies also investigated the role of BDNF-TrkB signaling in BLA in mediating fear memory formation. For instance, infusion of a lentiviral vector expressing a non-functional dominant-negative TrkB isoform in the amygdala of rats before fear acquisition has impaired the fear memory formation (Rattiner et al., 2004). In another research study during fear-potentiated startle experiments, mice treated with Cre recombinase expressing lentiviral vectors with bilateral amygdala *BDNF* deletion prior to fear training showed reduced freezing levels when compared to lentiviral vectors expressing green fluorescent protein treated control mice (Heldt et al., 2014). However, in our study, we infused the TrkB-Fc in BLA of mice 30 min after fear training, which is a specific time point where BDNF levels started to increase and this differentiates between acquisition and consolidation of fear memory. Scavenging the BDNF levels 30 min after fear training impaired the cued-fear memory consolidation when tested 24 h after fear training. Thus, with

our present study, we showed that early BDNF-TrkB signaling in BLA regulates exclusively the early and late cued-fear memory consolidation.

5.6 Final conclusion

As a final note, we showed for the first time that proBDNF-p75NTR signaling in BLA plays a major role in mediating cued-fear extinction learning. Interestingly, both proBDNF-p75NTR and BDNF-TrkB signaling in BLA are required for extinction memory consolidation. Previous research studies proposed that homeostasis of proBDNF/BDNF plays a major role in regulating the learning and memory of rodents in various behavioral tasks (Barnes & Thomas, 2008; Kirtley & Thomas, 2010; Bie et al., 2022 Xue et al., 2022). In our study, we have assessed the role of proBDNF-p75NTR and BDNF-TrkB signaling in BLA individually in mediating the extinction memory consolidation. However, further research studies are required to assess the role of homeostasis of proBDNF/BDNF in BLA in mediating the extinction of cued-fear memories. Next, the recurrence of fear memory even after extinction is one of the major problems in extinction-based therapies for several anxiety disorders (Bouton, 2002; Vervliet et al., 2013; Dunsmoor et al., 2015; Yuan et al., 2018). Still, it is a debate whether the extinction training updates the original fear memory by transiently inhibiting CS-US associated fear memory or it erases the fear memory completely. Based on our results and previous research studies (An et al., 2017), we suggest that both, proBDNF and BDNF signaling in BLA are contributing to the formation of extinction memory, which itself is a newly updated memory that transiently inhibits the CS-US associated fear memory rather than a complete erasure of fear memories. However, further research studies are required to draw final conclusions here. Additionally, we showed that disruption of BDNF-TrkB signaling arround 1 h after fear training impaired the cued-fear memory consolidation. Thus, interfering with the early cued-fear memory consolidation could potentially hinder long-term fear memory persistence, thereby revealing BDNF-TrkB signaling as a potential target for the treatment of post-traumatic stress disorders. In conclusion, our present research provides novel insights about the role of proBDNF-p75NTR and BDNF-TrkB signaling in BLA in mediating cued-fear learning and extinction memory consolidation, which might be a useful basis for addressing the above-mentioned still unanswered notions in future research studies.

6. References

Adachi, N., Numakawa, T., Richards, M., Nakajima, S., & Kunugi, H. (2014). New insight in expression, transport, and secretion of brain-derived neurotrophic factor: Implications in brain-related diseases. *World J Biol Chem, 5*(4), 409-428. doi: 10.4331/wjbc.v5.i4.409

Aid, T., Kazantseva, A., Piirsoo, M., Palm, K., & Timmusk, T. (2007). Mouse and rat BDNF gene structure and expression revisited. *J Neurosci Res, 85*(3), 525-535. doi: 10.1002/jnr.21139

Akirav, I., Raizel, H., & Maroun, M. (2006). Enhancement of conditioned fear extinction by infusion of the GABA(A) agonist muscimol into the rat prefrontal cortex and amygdala. *Eur J Neurosci, 23*(3), 758-764. doi: 10.1111/j.1460-9568.2006.04603.x

Algamal, M., Ojo, J. O., Lungmus, C. P., Muza, P., Cammarata, C., Owens, M. J., . . . Crawford, F. (2018). Chronic Hippocampal Abnormalities and Blunted HPA Axis in an Animal Model of Repeated Unpredictable Stress. *Front Behav Neurosci, 12*, 150. doi: 10.3389/fnbeh.2018.00150

Almeida-Correa, S., Moulin, T. C., Carneiro, C. F., Goncalves, M. M., Junqueira, L. S., & Amaral, O. B. (2015). Calcineurin inhibition blocks within-, but not between-session fear extinction in mice. *Learn Mem, 22*(3), 159-169. doi: 10.1101/lm.037770.114

Amano, T., Unal, C. T., & Pare, D. (2010). Synaptic correlates of fear extinction in the amygdala. *Nat Neurosci, 13*(4), 489-494. doi: 10.1038/nn.2499

Amir, A., Amano, T., & Pare, D. (2011). Physiological identification and infralimbic responsiveness of rat intercalated amygdala neurons. *J Neurophysiol, 105*(6), 3054-3066. doi: 10.1152/jn.00136.2011

An, B., Kim, J., Park, K., Lee, S., Song, S., & Choi, S. (2017). Amount of fear extinction changes its underlying mechanisms. *Elife, 6*. doi: 10.7554/eLife.25224

Andero, R., Heldt, S. A., Ye, K., Liu, X., Armario, A., & Ressler, K. J. (2011). Effect of 7,8-dihydroxyflavone, a small-molecule TrkB agonist, on emotional learning. *Am J Psychiatry, 168*(2), 163-172. doi: 10.1176/appi.ajp.2010.10030326

Barde, Y. A., Edgar, D., & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *EMBO J, 1*(5), 549-553.

Barnes, P., & Thomas, K. L. (2008). Proteolysis of proBDNF is a key regulator in the formation of memory. *PLoS One, 3*(9), e3248. doi: 10.1371/journal.pone.0003248

Bartoletti, A., Cancedda, L., Reid, S. W., Tessarollo, L., Porciatti, V., Pizzorusso, T., & 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*(23), 10072-10077.

Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci, 11*(6), 1164-1178. doi: 10.5114/aoms.2015.56342

Bauer, E. P., Schafe, G. E., & LeDoux, J. E. (2002). NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. *J Neurosci, 22*(12), 5239-5249.

Bekinschtein, P., & von Bohlen und Halbach, O. (2020). Editorial: Cellular and molecular mechanisms of neurotrophin function in the nervous system. *Frontiers in Cellular Neuroscience*, *14*. https://doi.org/10.3389/fncel.2020.00101

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived neurotrophic factor. *Growth Factors, 22*(3), 123-131. doi: 10.1080/08977190410001723308

Bie, B., Wu, J., Lin, F., Naguib, M., & Xu, J. (2022). Suppression of hippocampal GABAergic transmission impairs memory in rodent models of alzheimer's disease. *European Journal of Pharmacology*, *917*, 174771. https://doi.org/10.1016/j.ejphar.2022.174771

Blanchard, R. J., Yudko, E. B., Rodgers, R. J., & Blanchard, D. C. (1993). Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. *Behav Brain Res, 58*(1-2), 155-165. doi: 10.1016/0166-4328(93)90100-5.

Bloodgood, D. W., Sugam, J. A., Holmes, A., & Kash, T. L. (2018). Fear extinction requires infralimbic cortex projections to the basolateral amygdala. *Transl Psychiatry, 8*(1), 60.

Bouton, M. E. (1988). Context and ambiguity in the extinction of emotional learning: implications for exposure therapy. *Behav Res Ther, 26*(2), 137-149. doi: 10.1016/0005-7967(88)90113-1

Bouton, M. E. (2002). Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biol Psychiatry, 52*(10), 976-986. doi: 10.1016/s0006-3223(02)01546-9

Brambilla, R., Gnesutta, N., Minichiello, L., White, G., Roylance, A. J., Herron, C. E., . . . Klein, R. (1997). A role for the Ras signalling pathway in synaptic transmission and long-term memory. *Nature, 390*(6657), 281-286. doi: 10.1038/36849

Brigadski, T., Hartmann, M., & Lessmann, V. (2005). Differential vesicular targeting and time course of synaptic secretion of the mammalian neurotrophins. *J Neurosci, 25*(33), 7601-7614. doi: 10.1523/JNEUROSCI.1776-05.2005

Brigadski, T., & Leßmann, V. (2014). BDNF: a regulator of learning and memory processes with clinical potential. *E-Neuroforum*, *20*(1), 1-11. doi: 10.1515/s13295-014-0053-9

Brigadski, T., & Lessmann, V. (2020). The physiology of regulated BDNF release. *Cell Tissue Res, 382*(1), 15-45. doi: 10.1007/s00441-020-03253-2

Brown, T. H., Kairiss, E. W., & Keenan, C. L. (1990). Hebbian synapses: biophysical mechanisms and algorithms. *Annu Rev Neurosci, 13*, 475-511. doi: 10.1146/annurev.ne.13.030190.002355

Bruchey, A. K., Shumake, J., & Gonzalez-Lima, F. (2007). Network model of fear extinction and renewal functional pathways. *Neuroscience, 145*(2), 423-437. doi: 10.1016/j.neuroscience.2006.12.014

Buhusi, M., Etheredge, C., Granholm, A. C., & Buhusi, C. V. (2017). Increased Hippocampal proBDNF Contributes to Memory Impairments in Aged Mice. *Front Aging Neurosci, 9*, 284. doi: 10.3389/fnagi.2017.00284

Busch, R., Baldus, M., Vogt, M. A., Berger, S. M., Bartsch, D., Gass, P., & von Bohlen und Halbach, O. (2017). Effects of p75ntr deficiency on cholinergic innervation of the amygdala and anxiety-like behavior. *Journal of Neurochemistry*, *141*(3), 461–471. https://doi.org/10.1111/jnc.14006

Bukalo, O., Pinard, C. R., Silverstein, S., Brehm, C., Hartley, N. D., Whittle, N., Colacicco, G., Busch, E., Patel, S., Singewald, N., & Holmes, A. (2015). Prefrontal inputs to the amygdala instruct fear extinction memory formation. Science Advances, 1(6). https://doi.org/10.1126/sciadv.1500251.

Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E., & Quirk, G. J. (2007). Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. *Neuron, 53*(6), 871-880. doi: 10.1016/j.neuron.2007.02.021

Cain, C. K., Godsil, B. P., Jami, S., & Barad, M. (2005). The L-type calcium channel blocker nifedipine impairs extinction, but not reduced contingency effects, in mice. *Learn Mem, 12*(3), 277-284. doi: 10.1101/lm.88805

Campeau, S., Miserendino, M. J., & Davis, M. (1992). Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behav Neurosci, 106*(3), 569-574. doi: 10.1037//0735-7044.106.3.569

Cassell, M. D., Gray, T. S., & Kiss, J. Z. (1986). Neuronal architecture in the rat central nucleus of the amygdala: a cytological, hodological, and immunocytochemical study. *J Comp Neurol, 246*(4), 478-499. doi: 10.1002/cne.902460406

Calella, A. M., Nerlov, C., Lopez, R. G., Sciarretta, C., von Bohlen und Halbach, O., Bereshchenko, O., & Minichiello, L. (2007). Neurotrophin/Trk receptor signaling mediates C/EBPA, -β and NeuroD recruitment to immediate-early gene promoters in neuronal cells and requires c/ebps to induce immediate-early gene transcription. *Neural Development*, *2*(1). https://doi.org/10.1186/1749-8104-2-4

Caputi, A., Melzer, S., Michael, M., & Monyer, H. (2013). The long and short of GABAergic neurons. *Current Opinion in Neurobiology*, *23*(2), 179–186. https://doi.org/10.1016/j.conb.2013.01.021

Chang, C. H., Knapska, E., Orsini, C. A., Rabinak, C. A., Zimmerman, J. M., & Maren, S. (2009). Fear extinction in rodents. *Curr Protoc Neurosci, Chapter 8*, Unit8 23. doi: 10.1002/0471142301.ns0823s47

Chen, Z.-Y., Jing, D., Bath, K. G., Ieraci, A., Khan, T., Siao, C.-J., Herrera, D. G., Toth, M., Yang, C., McEwen, B. S., Hempstead, B. L., & Lee, F. S. (2006). Genetic variant BDNF (val66met) polymorphism alters anxiety-related behavior. Science, 314(5796), 140–143. https://doi.org/10.1126/science.1129663

Chhatwal, J. P., Myers, K. M., Ressler, K. J., & Davis, M. (2005). Regulation of gephyrin and GABAA receptor binding within the amygdala after fear acquisition and extinction. *J Neurosci, 25*(2), 502-506. doi: 10.1523/JNEUROSCI.3301-04.2005

Chhatwal, J. P., Stanek-Rattiner, L., Davis, M., & Ressler, K. J. (2006). Amygdala BDNF signaling is required for consolidation but not encoding of extinction. *Nat Neurosci, 9*(7), 870-872. doi: 10.1038/nn1718

Chhatwal, J. P., & Ressler, K. J. (2007). Modulation of fear and anxiety by the endogenous cannabinoid system. *CNS Spectr, 12*(3), 211-220. doi: 10.1017/s1092852900020939

Choi, D. C., Maguschak, K. A., Ye, K., Jang, S. W., Myers, K. M., & Ressler, K. J. (2010). Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proc Natl Acad Sci U S A, 107*(6), 2675-2680. doi: 10.1073/pnas.0909359107

Choi, D. C., Gourley, S. L., & Ressler, K. J. (2012). Prelimbic BDNF and TrkB signaling regulates consolidation of both appetitive and aversive emotional learning. *Transl Psychiatry, 2*, e205. doi: 10.1038/tp.2012.128

Colyn, L., Venzala, E., Marco, S., Perez-Otaño, I., & Tordera, R. M. (2019). Chronic social defeat stress induces sustained synaptic structural changes in the prefrontal cortex and amygdala. Behavioural Brain Research, 373, 112079. https://doi.org/10.1016/j.bbr.2019.112079

Clem, R. L., & Huganir, R. L. (2013). Norepinephrine enhances a discrete form of long-term depression during fear memory storage. *J Neurosci, 33*(29), 11825-11832. doi: 10.1523/JNEUROSCI.3317-12.2013

Clem, R. L., & Huganir, R. L. (2010). Calcium-Permeable AMPA Receptor Dynamics Mediate Fear Memory Erasure. *Science*, *330*(6007), 1108–1112. https://doi.org/10.1126/science.1195298

Collingridge, G., Peineau, S., Howland, J., & Wang, Y. (2010). Long-term depression in the CNS. *Nature Reviews Neuroscience*, *11*(7), 459-473. doi: 10.1038/nrn2867

Colyn, L., Venzala, E., Marco, S., Perez-Otano, I., & Tordera, R. M. (2019). Chronic social defeat stress induces sustained synaptic structural changes in the prefrontal cortex and amygdala. *Behav Brain Res, 373*, 112079. doi: 10.1016/j.bbr.2019.112079

Conner, J. M., Lauterborn, J. C., Yan, Q., Gall, C. M., & 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*(7), 2295-2313.

Corcoran, K. A., & Quirk, G. J. (2007). Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. *J Neurosci, 27*(4), 840-844. doi: 10.1523/JNEUROSCI.5327-06.2007

Cowansage, K. K., LeDoux, J. E., & Monfils, M. H. (2010). Brain-derived neurotrophic factor: a dynamic gatekeeper of neural plasticity. *Curr Mol Pharmacol, 3*(1), 12-29. doi: 10.2174/1874467211003010012

Craske, M. G., Kircanski, K., Zelikowsky, M., Mystkowski, J., Chowdhury, N., & Baker, A. (2008). Optimizing inhibitory learning during exposure therapy. *Behav Res Ther, 46*(1), 5-27. doi: 10.1016/j.brat.2007.10.003

Craske, M. G., Treanor, M., Conway, C. C., Zbozinek, T., & Vervliet, B. (2014). Maximizing exposure therapy: an inhibitory learning approach. *Behav Res Ther, 58*, 10-23. doi: 10.1016/j.brat.2014.04.006

Cunha, C., Brambilla, R., & Thomas, K. L. (2010). A simple role for BDNF in learning and memory? *Front Mol Neurosci, 3*, 1. doi: 10.3389/neuro.02.001.2010

Dalton, G. L., Wu, D. C., Wang, Y. T., Floresco, S. B., & Phillips, A. G. (2012). NMDA GluN2A and GluN2B receptors play separate roles in the induction of LTP and LTD in the amygdala and in the acquisition and extinction of conditioned fear. *Neuropharmacology, 62*(2), 797-806. doi: 10.1016/j.neuropharm.2011.09.001

Dalton, G. L., Wang, Y. T., Floresco, S. B., & Phillips, A. G. (2008). Disruption of AMPA receptor endocytosis impairs the extinction, but not acquisition of learned fear. *Neuropsychopharmacology, 33*(10), 2416-2426. doi: 10.1038/sj.npp.1301642

Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annu Rev Neurosci, 15*, 353-375. doi: 10.1146/annurev.ne.15.030192.002033

Davis, M., Ressler, K., Rothbaum, B. O., & Richardson, R. (2006). Effects of D-cycloserine on extinction: translation from preclinical to clinical work. *Biol Psychiatry, 60*(4), 369-375. doi: 10.1016/j.biopsych.2006.03.084

Davis, P., Zaki, Y., Maguire, J., & Reijmers, L. G. (2017). Cellular and oscillatory substrates of fear extinction learning. *Nat Neurosci, 20*(11), 1624-1633. doi: 10.1038/nn.4651

Dityatev, A. E., & Bolshakov, V. Y. (2005). Amygdala, long-term potentiation, and fear conditioning. *Neuroscientist, 11*(1), 75-88. doi: 10.1177/1073858404270857

Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annu Rev Psychol, 55*, 51-86. doi: 10.1146/annurev.psych.55.090902.142050

Dunsmoor, J. E., Niv, Y., Daw, N., & Phelps, E. A. (2015). Rethinking Extinction. *Neuron, 88*(1), 47-63. doi: 10.1016/j.neuron.2015.09.028

Duvarci, S., & Pare, D. (2014). Amygdala microcircuits controlling learned fear. *Neuron, 82*(5), 966-980. doi: 10.1016/j.neuron.2014.04.042

Dulka, B., Ford, E., Lee, M., Donnell, N., Goode, T., Prosser, R., & Cooper, M. (2016). Proteolytic cleavage of proBDNF into mature BDNF in the basolateral amygdala is necessary for defeat-induced social avoidance. *Learning & Memory*, *23*(4), 156-160. doi: 10.1101/lm.040253.115

Edelmann, E., Lessmann, V., & Brigadski, T. (2014). Pre- and postsynaptic twists in BDNF secretion and action in synaptic plasticity. *Neuropharmacology, 76 Pt C*, 610-627. doi: 10.1016/j.neuropharm.2013.05.043

Ehrlich, D. E., & Josselyn, S. A. (2016). Plasticity-related genes in brain development and amygdala-dependent learning. *Genes Brain Behav, 15*(1), 125-143. doi: 10.1111/gbb.12255

Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., & Luthi, A. (2009). Amygdala inhibitory circuits and the control of fear memory. *Neuron, 62*(6), 757-771. doi: 10.1016/j.neuron.2009.05.026

Eisenberg, M., Kobilo, T., Berman, D. E., & Dudai, Y. (2003). Stability of retrieved memory: inverse correlation with trace dominance. *Science, 301*(5636), 1102-1104. doi: 10.1126/science.1086881

Endres, T., & Lessmann, V. (2012). Age-dependent deficits in fear learning in heterozygous BDNF knock-out mice. *Learn Mem, 19*(12), 561-570. doi: 10.1101/lm.028068.112

Faber, E. S., Callister, R. J., & Sah, P. (2001). Morphological and electrophysiological properties of principal neurons in the rat lateral amygdala in vitro. *J Neurophysiol, 85*(2), 714-723. doi: 10.1152/jn.2001.85.2.714

Fadok, J. P., Markovic, M., Tovote, P., & Luthi, A. (2018). New perspectives on central amygdala function. *Curr Opin Neurobiol, 49*, 141-147. doi: 10.1016/j.conb.2018.02.009

Fahnestock, M., Garzon, D., Holsinger, R. M., & Michalski, B. (2002). Neurotrophic factors and Alzheimer's disease: are we focusing on the wrong molecule? *J Neural Transm Suppl*(62), 241-252. doi: 10.1007/978-3-7091-6139-5_22

Falls, W. A., Miserendino, M. J., & Davis, M. (1992). Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. *J Neurosci, 12*(3), 854-863.

Fanselow, M. S., & Kim, J. J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behav Neurosci, 108*(1), 210-212. doi: 10.1037//0735-7044.108.1.210

Fanselow, M. S., & Poulos, A. M. (2005). The neuroscience of mammalian associative learning. *Annu Rev Psychol, 56*, 207-234. doi: 10.1146/annurev.psych.56.091103.070213

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev, 23*(5), 743-760. doi: 10.1016/s0149-7634(99)00016-0

Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T., & Lu, B. (1996). Regulation of synaptic responses to high-frequency stimulation and LTP by Neurotrophins in the hippocampus. *Nature*, *381*(6584), 706–709. https://doi.org/10.1038/381706a0

Finn, P. J., Ferguson, I. A., Renton, F. J., & Rush, R. A. (1986). Nerve growth factor immunohistochemistry and biological activity in the rat iris. *J Neurocytol, 15*(2), 169-176. doi: 10.1007/BF01611653

Fisk, G. D., & Wyss, J. M. (2000). Descending projections of infralimbic cortex that mediate stimulation-evoked changes in arterial pressure. *Brain Res, 859*(1), 83-95. doi: 10.1016/s0006-8993(00)01935-1

Foltran, R. B., & Diaz, S. L. (2016). BDNF isoforms: a round trip ticket between neurogenesis and serotonin? *J Neurochem, 138*(2), 204-221. doi: 10.1111/jnc.13658

Fontanez-Nuin, D. E., Santini, E., Quirk, G. J., & Porter, J. T. (2011). Memory for fear extinction requires mGluR5-mediated activation of infralimbic neurons. *Cereb Cortex, 21*(3), 727-735. doi: 10.1093/cercor/bhq147

Frade, J. M., & Barde, Y. A. (1998). Nerve growth factor: two receptors, multiple functions. *Bioessays, 20*(2), 137-145. doi: 10.1002/(SICI)1521-1878(199802)20:2<137::AID-BIES6>3.0.CO;2-Q

Furukawa, S., Sugihara, Y., Iwasaki, F., Fukumitsu, H., Nitta, A., Nomoto, H., & Furukawa, Y. (1998). Brain-derived neurotrophic factor-like immunoreactivity in the adult rat central nervous system predominantly distributed in neurons with substantial amounts of brain-derived neurotrophic factor messenger RNA or responsiveness to brain-derived neurotrophic factor. *Neuroscience, 82*(3), 653-670. doi: 10.1016/s0306-4522(97)00315-1

Gartner, A., Polnau, D. G., Staiger, V., Sciarretta, C., Minichiello, L., Thoenen, H., . . . Korte, M. (2006). Hippocampal long-term potentiation is supported by presynaptic and postsynaptic tyrosine receptor kinase B-mediated phospholipase Cgamma signaling. *J Neurosci, 26*(13), 3496-3504. doi: 10.1523/JNEUROSCI.3792-05.2006

Gray, K., & Ellis, V. (2008). Activation of pro-BDNF by the pericellular serine protease plasmin. FEBS Letters, 582(6), 907–910. https://doi.org/10.1016/j.febslet.2008.02.026

Gibon, J., & Barker, P. A. (2017). Neurotrophins and Proneurotrophins: Focus on Synaptic Activity and Plasticity in the Brain. *Neuroscientist, 23*(6), 587-604. doi: 10.1177/1073858417697037

Golkar, A., Bellander, M., Olsson, A., & Ohman, A. (2012). Are fear memories erasable?-reconsolidation of learned fear with fear-relevant and fear-irrelevant stimuli. *Front Behav Neurosci, 6*, 80. doi: 10.3389/fnbeh.2012.00080

Goosens, K. A., Holt, W., & Maren, S. (2000). A role for amygdaloid PKA and PKC in the acquisition of long-term conditional fear memories in rats. *Behav Brain Res, 114*(1-2), 145-152. doi: 10.1016/s0166-4328(00)00224-2

Gottmann, K., Mittmann, T., & Lessmann, V. (2009). BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. *Exp Brain Res, 199*(3-4), 203-234. doi: 10.1007/s00221-009-1994-z

Gotz, R., Koster, R., Winkler, C., Raulf, F., Lottspeich, F., Schartl, M., & Thoenen, H. (1994). Neurotrophin-6 Is a New Member of the Nerve Growth-Factor Family. *Nature, 372*(6503), 266-269. doi: Doi 10.1038/372266a0

Groessl, F., Munsch, T., Meis, S., Griessner, J., Kaczanowska, J., Pliota, P., Kargl, D., Badurek, S., Kraitsy, K., Rassoulpour, A., Zuber, J., Lessmann, V., & Haubensak, W. (2018). Dorsal tegmental dopamine neurons gate associative learning of fear. Nature Neuroscience, 21(7), 952–962. https://doi.org/10.1038/s41593-018-0174-5

Gruart, A., Sciarretta, C., Valenzuela-Harrington, M., Delgado-Garcia, J. M., & Minichiello, L. (2007). Mutation at the TrkB PLC{gamma}-docking site affects hippocampal LTP and associative learning in conscious mice. *Learn Mem, 14*(1), 54-62. doi: 10.1101/lm.428307

Hall, J., Thomas, K. L., & Everitt, B. J. (2000). Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nat Neurosci, 3*(6), 533-535. doi: 10.1038/75698

Harris, A. P., Lennen, R. J., Brydges, N. M., Jansen, M. A., Pernet, C. R., Whalley, H. C., Marshall, I., Baker, S., Basso, A. M., Day, M., Holmes, M. C., & Hall, J. (2016). The role of brain-derived neurotrophic factor in learned fear processing: An awake RAT fmri study. Genes, Brain and Behavior, 15(2), 221–230. https://doi.org/10.1111/gbb.12277

Harris, J. A., & Westbrook, R. F. (1998). Evidence that GABA transmission mediates context-specific extinction of learned fear. *Psychopharmacology (Berl), 140*(1), 105-115. doi: 10.1007/s002130050745

Haubensak, W., Kunwar, P. S., Cai, H., Ciocchi, S., Wall, N. R., Ponnusamy, R., Biag, J., Dong, H.-W., Deisseroth, K., Callaway, E. M., Fanselow, M. S., Lüthi, A., & Anderson, D. J. (2010). Genetic dissection of an amygdala microcircuit that gates conditioned fear. Nature, 468(7321), 270–276. https://doi.org/10.1038/nature09553

Halbach, O. von, & Albrecht, D. (2002). Reciprocal connections of the hippocampal area CA1, the lateral nucleus of the amygdala and cortical areas in a combined horizontal slice preparation. *Neuroscience Research*, *44*(1), 91–100. https://doi.org/10.1016/s0168-0102(02)00092-5

Haubensak, W., Narz, F., Heumann, R., & Lessmann, V. (1998). BDNF-GFP containing secretory granules are localized in the vicinity of synaptic junctions of cultured cortical neurons. *Journal of Cell Science*, *111*(11), 1483–1493. https://doi.org/10.1242/jcs.111.11.1483

Haubrich, J., & Nader, K. (2018). Memory Reconsolidation. *Curr Top Behav Neurosci, 37*, 151-176. doi: 10.1007/7854_2016_463

Hefner, K., Whittle, N., Juhasz, J., Norcross, M., Karlsson, R.-M., Saksida, L. M., Bussey, T. J., Singewald, N., & Holmes, A. (2008). Impaired Fear Extinction Learning and cortico-amygdala circuit abnormalities in a common genetic mouse strain. The Journal of Neuroscience, 28(32), 8074–8085. https://doi.org/10.1523/jneurosci.4904-07.2008.

Hebb, D. O. (1949). The first stage of perception: growth of the assembly. *The Organization of Behavior*, *4*, 60-78.

Heldt, S. A., & Ressler, K. J. (2007). Training-induced changes in the expression of GABAA-associated genes in the amygdala after the acquisition and extinction of Pavlovian fear. *Eur J Neurosci, 26*(12), 3631-3644. doi: 10.1111/j.1460-9568.2007.05970.x

Heldt, S. A., Stanek, L., Chhatwal, J. P., & Ressler, K. J. (2007). Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol Psychiatry, 12*(7), 656-670. doi: 10.1038/sj.mp.4001957

Heldt, S. A., Zimmermann, K., Parker, K., Gaval, M., Weinshenker, D., & Ressler, K. J. (2014). BDNF deletion or TrkB impairment in amygdala inhibits both appetitive and aversive learning. *J Neurosci, 34*(7), 2444-2450. doi: 10.1523/JNEUROSCI.4085-12.2014

Hennigan, A., O'Callaghan, R. M., & Kelly, Á. M. (2007). Neurotrophins and their receptors: Roles in plasticity, neurodegeneration and neuroprotection. *Biochemical Society Transactions*, *35*(2), 424–427. https://doi.org/10.1042/bst0350424

Hermans, D., Craske, M. G., Mineka, S., & Lovibond, P. F. (2006). Extinction in human fear conditioning. *Biol Psychiatry, 60*(4), 361-368. doi: 10.1016/j.biopsych.2005.10.006

Herry, C., Ciocchi, S., Senn, V., Demmou, L., Muller, C., & Luthi, A. (2008). Switching on and off fear by distinct neuronal circuits. *Nature, 454*(7204), 600-606. doi: 10.1038/nature07166

Herry, C., Ferraguti, F., Singewald, N., Letzkus, J. J., Ehrlich, I., & Luthi, A. (2010). Neuronal circuits of fear extinction. *Eur J Neurosci, 31*(4), 599-612. doi: 10.1111/j.1460-9568.2010.07101.x

Herry, C., & Johansen, J. P. (2014). Encoding of fear learning and memory in distributed neuronal circuits. *Nat Neurosci, 17*(12), 1644-1654. doi: 10.1038/nn.3869

Hikind, N., & Maroun, M. (2008). Microinfusion of the D1 receptor antagonist, SCH23390 into the IL but not the BLA impairs consolidation of extinction of auditory fear conditioning. *Neurobiol Learn Mem, 90*(1), 217-222. doi: 10.1016/j.nlm.2008.03.003

Hong, I., Song, B., Lee, S., Kim, J., Kim, J., & Choi, S. (2009). Extinction of cued fear memory involves a distinct form of depotentiation at cortical input synapses onto the lateral amygdala. *Eur J Neurosci, 30*(11), 2089-2099. doi: 10.1111/j.1460-9568.2009.07004.x

Holmes, A., & Singewald, N. (2013). Individual differences in recovery from traumatic fear. Trends in Neurosciences, 36(1), 23–31. https://doi.org/10.1016/j.tins.2012.11.003.

Hopkins, D. A., & Holstege, G. (1978). Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. *Exp Brain Res, 32*(4), 529-547. doi: 10.1007/BF00239551

Huang, Y. Y., Martin, K. C., & Kandel, E. R. (2000). Both protein kinase A and mitogen-activated protein kinase are required in the amygdala for the macromolecular synthesis-dependent late phase of long-term potentiation. *J Neurosci, 20*(17), 6317-6325.

Hubner, C., Bosch, D., Gall, A., Luthi, A., & Ehrlich, I. (2014). Ex vivo dissection of optogenetically activated mPFC and hippocampal inputs to neurons in the basolateral amygdala: implications for fear and emotional memory. *Front Behav Neurosci, 8*, 64. doi: 10.3389/fnbeh.2014.00064

Isaacson, R.L. (1974). The Structure of the Limbic System. In: The Limbic System. Springer, Boston, MA. https://doi.org/10.1007/978-1-4613-4505-3_1

Ji, J., & Maren, S. (2007). Hippocampal involvement in contextual modulation of fear extinction. *Hippocampus, 17*(9), 749-758. doi: 10.1002/hipo.20331

Ji, Y., Lu, Y., Yang, F., Shen, W., Tang, T. T.-T., Feng, L., Duan, S., & Lu, B. (2010). Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nature Neuroscience, 13(3), 302–309. https://doi.org/10.1038/nn.2505.

Johansen, J. P., Cain, C. K., Ostroff, L. E., & LeDoux, J. E. (2011). Molecular mechanisms of fear learning and memory. *Cell, 147*(3), 509-524. doi: 10.1016/j.cell.2011.10.009

Johansen, J. P., Wolff, S. B. E., Lüthi, A., & LeDoux, J. E. (2012). Controlling the elements: An optogenetic approach to understanding the neural circuits of Fear. Biological Psychiatry, 71(12), 1053–1060. https://doi.org/10.1016/j.biopsych.2011.10.023.

Jones, S. V., Stanek-Rattiner, L., Davis, M., & Ressler, K. J. (2007). Differential regional expression of brain-derived neurotrophic factor following olfactory fear learning. *Learn Mem, 14*(12), 816-820. doi: 10.1101/lm.781507

Kandel, E. R. (1997). Genes, synapses, and long-term memory. *J Cell Physiol, 173*(2), 124-125. doi: 10.1002/(SICI)1097-4652(199711)173:2<124::AID-JCP6>3.0.CO;2-P

Krause, S., Schindowski, K., Zechel, S., & von Bohlen und Halbach, O. (2008). Expression of trkb and trkc receptors and their ligands brain-derived neurotrophic factor and neurotrophin-3 in the murine amygdala. *Journal of Neuroscience Research*, *86*(2), 411–421. https://doi.org/10.1002/jnr.21490

Karpova, N. N., Pickenhagen, A., Lindholm, J., Tiraboschi, E., Kulesskaya, N., Agustsdottir, A., Castren, E. (2011). Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science, 334*(6063), 1731-1734. doi: 10.1126/science.1214592

Kawamoto, Y., Nakamura, S., Nakano, S., Oka, N., Akiguchi, I., & Kimura, J. (1996). Immunohistochemical localization of brain-derived neurotrophic factor in adult rat brain. *Neuroscience, 74*(4), 1209-1226. doi: 10.1016/0306-4522(96)00245-x

Kent, J. M., & Rauch, S. L. (2003). Neurocircuitry of anxiety disorders. *Curr Psychiatry Rep, 5*(4), 266-273. doi: 10.1007/s11920-003-0055-8

Kemp, A., & Manahan-Vaughan, D. (2004). Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. *Proceedings of the National Academy of Sciences*, *101*(21), 8192–8197. https://doi.org/10.1073/pnas.0402650101

Kim, J., An, B., Kim, J., Park, S., Park, S., Hong, I., Lee, S., Park, K., & Choi, S. (2015). MGluR2/3 in the lateral amygdala is required for fear extinction: Cortical input synapses onto the lateral amygdala as a target site of the mGluR2/3 Action. Neuropsychopharmacology, 40(13), 2916–2928. https://doi.org/10.1038/npp.2015.145.

Kim, J., Lee, S., Park, H., Song, B., Hong, I., Geum, D., Shin, K., & Choi, S. (2007). Blockade of amygdala metabotropic glutamate receptor subtype 1 impairs fear extinction. Biochemical and Biophysical Research Communications, 355(1), 188–193. https://doi.org/10.1016/j.bbrc.2007.01.125

Kim, J., Lee, S., Park, K., Hong, I., Song, B., Son, G., Park, H., Kim, W. R., Park, E., Choe, H. K., Kim, H., Lee, C., Sun, W., Kim, K., Shin, K. S., & Choi, S. (2007). Amygdala depotentiation and fear extinction. Proceedings of the National Academy of Sciences, 104(52), 20955–20960. https://doi.org/10.1073/pnas.0710548105.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*(5057), 675-677. doi: 10.1126/science.1585183

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. *Neurosci Biobehav Rev, 30*(2), 188-202. doi: 10.1016/j.neubiorev.2005.06.005

Kim, W. B., & Cho, J. H. (2017). Synaptic Targeting of Double-Projecting Ventral CA1 Hippocampal Neurons to the Medial Prefrontal Cortex and Basal Amygdala. *J Neurosci, 37*(19), 4868-4882. doi: 10.1523/JNEUROSCI.3579-16.2017

Kirtley, A., & Thomas, K. L. (2010). The exclusive induction of extinction is gated by BDNF. *Learn Mem, 17*(12), 612-619. doi: 10.1101/lm.1877010

Kishioka, A., Fukushima, F., Ito, T., Kataoka, H., Mori, H., Ikeda, T., Itohara, S., Sakimura, K., & Mishina, M. (2009). A novel form of memory for auditory fear conditioning at a low-intensity unconditioned stimulus. PLoS ONE, 4(1). https://doi.org/10.1371/journal.pone.0004157

Klavir, O., Prigge, M., Sarel, A., Paz, R., & Yizhar, O. (2017). Manipulating fear associations via optogenetic modulation of amygdala inputs to prefrontal cortex. *Nat Neurosci, 20*(6), 836-844. doi: 10.1038/nn.4523

Knapska, E., & Maren, S. (2009). Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. *Learn Mem, 16*(8), 486-493. doi: 10.1101/lm.1463909

Korsching, S. (1993). The Neurotrophic Factor Concept: A reexamination. The Journal of Neuroscience, 13(7), 2739–2748. https://doi.org/10.1523/jneurosci.13-07-02739.1993

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., & Bonhoeffer, T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A, 92*(19), 8856-8860. doi: 10.1073/pnas.92.19.8856

Koutsikou, S., Crook, J. J., Earl, E. V., Leith, J. L., Watson, T. C., Lumb, B. M., & Apps, R. (2014). Neural substrates underlying fear-evoked freezing: the periaqueductal grey-cerebellar link. *J Physiol, 592*(10), 2197-2213. doi: 10.1113/jphysiol.2013.268714

Kuczewski, N., Porcher, C., & Gaiarsa, J. L. (2010). Activity-dependent dendritic secretion of brain-derived neurotrophic factor modulates synaptic plasticity. *Eur J Neurosci, 32*(8), 1239-1244. doi: 10.1111/j.1460-9568.2010.07378.x

Laurent, V., Marchand, A. R., & Westbrook, R. F. (2008). The basolateral amygdala is necessary for learning but not relearning extinction of context conditioned fear. *Learn Mem, 15*(5), 304-314. doi: 10.1101/lm.928208

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annu Rev Neurosci, 23*, 155-184. doi: 10.1146/annurev.neuro.23.1.155.

LeDoux, J. E., Sakaguchi, A., & Reis, D. J. (1984). Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. The Journal of Neuroscience, 4(3), 683–698. https://doi.org/10.1523/jneurosci.04-03-00683.1984.

LeDoux, J. (2014). Coming to terms with fear. *Proceedings Of The National Academy Of Sciences*, *111*(8), 2871-2878. doi: 10.1073/pnas.1400335111

Lee, R., Kermani, P., Teng, K. K., & Hempstead, B. L. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*(5548), 1945-1948. doi: 10.1126/science.1065057.

Lee, J. L., Everitt, B. J., & Thomas, K. L. (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science, 304*(5672), 839 843. doi: 10.1126/science.1095760

Lessmann, V., & Brigadski, T. (2009). Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update. *Neurosci Res, 65*(1), 11-22. doi: 10.1016/j.neures.2009.06.004

Lessmann, V., Gottmann, K., & Malcangio, M. (2003). Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol, 69*(5), 341-374. doi: 10.1016/s0301-0082(03)00019-4

Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. *Science, 237*(4819), 1154-1162. doi: 10.1126/science.3306916

Lewin, G. R., & Barde, Y. A. (1996). Physiology of the neurotrophins. *Annu Rev Neurosci, 19*, 289-317. doi: 10.1146/annurev.ne.19.030196.001445

Li, Y., Meloni, E. G., Carlezon, W. A., Jr., Milad, M. R., Pitman, R. K., Nader, K., & Bolshakov, V. Y. (2013). Learning and reconsolidation implicate different synaptic mechanisms. *Proc Natl Acad Sci U S A, 110*(12), 4798-4803. doi: 10.1073/pnas.1217878110.

Li, Y., Wang, D., Li, Y., Chu, H., Zhang, L., Hou, M., Jiang, X., Chen, Z., Su, B., & Sun, T. (2017). Pre-synaptic trkb in basolateral amygdala neurons mediates BDNF signaling transmission in memory extinction. Cell Death & Disease, 8(7). https://doi.org/10.1038/cddis.2017.302.

Likhtik, E., Pelletier, J. G., Paz, R., & Paré, D. (2005). Prefrontal control of the amygdala. *Journal of Neuroscience*, *25*(32), 7429-7437.

Likhtik, E., Popa, D., Apergis-Schoute, J., Fidacaro, G. A., & Pare, D. (2008). Amygdala intercalated neurons are required for expression of fear extinction. *Nature, 454*(7204), 642-645. doi: 10.1038/nature07167

Lin, C. H., Lee, C. C., & Gean, P. W. (2003). Involvement of a calcineurin cascade in amygdala depotentiation and quenching of fear memory. *Mol Pharmacol, 63*(1), 44-52. doi: 10.1124/mol.63.1.44

Lin, C. H., Yeh, S. H., Leu, T. H., Chang, W. C., Wang, S. T., & Gean, P. W. (2003). Identification of calcineurin as a key signal in the extinction of fear memory. Journal of Neuroscience, 23(5), 1574-1579. https://doi.org/10.1523/jneurosci.23-05-01574.2003.

Lin, C. H., Yeh, S. H., Lin, C. H., Lu, K. T., Leu, T. H., Chang, W. C., & Gean, P. W. (2001). A role for the PI-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. *Neuron, 31*(5), 841-851. doi: 10.1016/s0896-6273(01)00433-0

Liu, J. L., Li, M., Dang, X. R., Wang, Z. H., Rao, Z. R., Wu, S. X., Li, Y, Q., & Wang, W. (2009). A NMDA receptor antagonist, MK-801 impairs consolidating extinction of auditory conditioned fear responses in a Pavlovian model. *PLoS One, 4*(10), e7548. doi: 10.1371/journal.pone.0007548

Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nat Rev Neurosci, 6*(8), 603-614. doi: 10.1038/nrn1726

Lu, K.T., Walker, D. L., & Davis, M. (2001). Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. The Journal of Neuroscience, 21(16).

Lubin, F. D., Roth, T. L., & Sweatt, J. D. (2008). Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *J Neurosci, 28*(42), 10576-10586. doi: 10.1523/JNEUROSCI.1786-08.2008.

Luchkina, N. V., & Bolshakov, V. Y. (2019). Mechanisms of fear learning and extinction: synaptic plasticity-fear memory connection. *Psychopharmacology (Berl), 236*(1), 163-182. doi: 10.1007/s00213-018-5104-4

Lugo, J. N., Smith, G. D., & Holley, A. J. (2014). Trace fear conditioning in mice. *J Vis Exp*(85). doi: 10.3791/51180

Martínez-Laorden, E., Navarro-Zaragoza, J., Milanés, M. V., Laorden, M. L., & Almela, P. (2019). Conditioned aversive memory associated with morphine withdrawal increases brain-derived neurotrophic factor in dentate gyrus and basolateral amygdala. *Addiction Biology*, *25*(4). https://doi.org/10.1111/adb.12792

Malenka, R. C., & Nicoll, R. A. (1999). Long-term potentiation--a decade of progress? *Science, 285*(5435), 1870-1874. doi: 10.1126/science.285.5435.1870

Ma, X., Vuyyuru, H., Munsch, T., Endres, T., Lessmann, V., & Meis, S. (2021). ProBDNF Dependence of LTD and Fear Extinction Learning in the Amygdala of Adult Mice. *Cerebral Cortex*, *32*(7), 1350-1364. doi: 10.1093/cercor/bhab265

Mamiya, N., Fukushima, H., Suzuki, A., Matsuyama, Z., Homma, S., Frankland, P. W., & Kida, S. (2009). Brain region-specific gene expression activation required for reconsolidation and extinction of contextual fear memory. *J Neurosci, 29*(2), 402-413. doi: 10.1523/JNEUROSCI.4639-08.2009

Mao, S. C., Chang, C. H., Wu, C. C., Orejarena, M. J., Manzoni, O. J., & Gean, P. W. (2013). Inhibition of spontaneous recovery of fear by mGluR5 after prolonged extinction training. *PLoS One, 8*(3), e59580. doi: 10.1371/journal.pone.0059580

Mao, S. C., Hsiao, Y. H., & Gean, P. W. (2006). Extinction training in conjunction with a partial agonist of the glycine site on the NMDA receptor erases memory trace. *J Neurosci, 26*(35), 8892-8899. doi: 10.1523/JNEUROSCI.0365-06.2006

Marek, R., Sun, Y., & Sah, P. (2019). Neural circuits for a top-down control of fear and extinction. *Psychopharmacology (Berl), 236*(1), 313-320. doi: 10.1007/s00213-018-5033-2

Maren, S. (2011). Seeking a spotless mind: extinction, deconsolidation, and erasure of fear memory. *Neuron, 70*(5), 830-845. doi: 10.1016/j.neuron.2011.04.023

Maren, S., & Holmes, A. (2016). Stress and Fear Extinction. *Neuropsychopharmacology, 41*(1), 58-79. doi: 10.1038/npp.2015.180

Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci, 14*(6), 417-428. doi: 10.1038/nrn3492

Maren, S., & Quirk, G. J. (2004). Neuronal signalling of fear memory. *Nat Rev Neurosci, 5*(11), 844-852. doi: 10.1038/nrn1535

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. *Prog Neurobiol, 55*(3), 257-332. doi: 10.1016/s0301-0082(98)00003-3

McDonald, A. J., Mascagni, F., & Guo, L. (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience, 71*(1), 55-75. doi: 10.1016/0306-4522(95)00417-3

McGarry, L. M., & Carter, A. G. (2017). Prefrontal Cortex Drives Distinct Projection Neurons in the Basolateral Amygdala. *Cell Rep, 21*(6), 1426-1433. doi: 10.1016/j.celrep.2017.10.046.

McKernan, M. G., & Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature, 390*(6660), 607-611. doi: 10.1038/37605

Meis, S., Endres, T., & Lessmann, V. (2020). Neurotrophin signalling in amygdala-dependent cued fear learning. *Cell Tissue Res, 382*(1), 161-172. doi: 10.1007/s00441-020-03260-3

Meis, S., Endres, T., Munsch, T., & Lessmann, V. (2018). The Relation Between Long-Term Synaptic Plasticity at Glutamatergic Synapses in the Amygdala and Fear Learning in Adult Heterozygous BDNF-Knockout Mice. *Cereb Cortex, 28*(4), 1195-1208. doi: 10.1093/cercor/bhx032

Melzer, S., & Monyer, H. (2020). Diversity and function of corticopetal and corticofugal GABAergic projection neurons. *Nature Reviews Neuroscience*, *21*(9), 499–515. https://doi.org/10.1038/s41583-020-0344-9

Melzer, S., Newmark, E., Mizuno, G. O., Hyun, M., Philson, A. C., Quiroli, E., Righetti, B., Gregory, M. R., Huang, K. W., Levasseur, J., Tian, L., & Sabatini, B. L. (2020). Bombesin-like peptide recruits disinhibitory cortical circuits and enhances fear memories. https://doi.org/10.1101/2020.10.26.355123

Michalski, B., & Fahnestock, M. (2003). Pro-brain-derived neurotrophic factor is decreased in parietal cortex in Alzheimer's disease. *Brain Res Mol Brain Res, 111*(1-2), 148-154. doi: 10.1016/s0169-328x(03)00003-2

Milad, M. R., & Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. Nature, 420(6911), 70–74. https://doi.org/10.1038/nature01138.

Milad, M. R., & Quirk, G. J. (2012). Fear extinction as a model for translational neuroscience: ten years of progress. *Annu Rev Psychol, 63*, 129-151. doi: 10.1146/annurev.psych.121208.131631

Millhouse, O. E. (1986). The intercalated cells of the amygdala. *J Comp Neurol, 247*(2), 246-271. doi: 10.1002/cne.902470209

Milner, B., Squire, L. R., & Kandel, E. R. (1998). Cognitive neuroscience and the study of memory. *Neuron, 20*(3), 445-468. doi: 10.1016/s0896-6273(00)80987-3

Milner, P. M. (1957). The cell assembly: Mark II. *Psychol Rev, 64*(4), 242-252. doi: 10.1037/h0042287

Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *Am Psychol, 61*(1), 10-26. doi: 10.1037/0003-066X.61.1.10

Minichiello, L., Calella, A. M., Medina, D. L., Bonhoeffer, T., Klein, R., & Korte, M. (2002). Mechanism of TrkB-mediated hippocampal long-term potentiation. *Neuron, 36*(1), 121-137. doi: 10.1016/s0896-6273(02)00942-x

Minichiello, L., Korte, M., Wolfer, D., Kühn, R., Unsicker, K., Cestari, V., Rossi-Arnaud, C., Lipp, H.-P., Bonhoeffer, T., & Klein, R. (1999). Essential role for trkb receptors in Hippocampus-mediated learning. Neuron, 24(2), 401–414. https://doi.org/10.1016/s0896-6273(00)80853-3.

Mirante, O., Brandalise, F., Bohacek, J., & Mansuy, I. M. (2014). Distinct molecular components for thalamic- and cortical-dependent plasticity in the lateral amygdala. *Front Mol Neurosci, 7*, 62. doi: 10.3389/fnmol.2014.00062

Miserendino, M. J., Sananes, C. B., Melia, K. R., & Davis, M. (1990). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature, 345*(6277), 716-718. doi: 10.1038/345716a0.

Mizuno, K., Dempster, E., Mill, J., & Giese, K. P. (2012). Long-lasting regulation of hippocampal Bdnf gene transcription after contextual fear conditioning. *Genes Brain Behav, 11*(6), 651-659. doi: 10.1111/j.1601-183X.2012.00805.x.

Mizutani, A., Saito, H., & Matsuki, N. (1996). Possible involvement of plasmin in long-term potentiation of rat hippocampal slices. *Brain Res, 739*(1-2), 276-281. doi: 10.1016/s0006-8993(96)00834-7.

Monfils, M. H., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2009). Extinction-Reconsolidation Boundaries: Key to Persistent Attenuation of Fear Memories. *Science*, *324*(5929), 951–955. https://doi.org/10.1126/science.1167975

Montag, C., Weber, B., Fliessbach, K., Elger, C., & Reuter, M. (2009). The BDNF Val66Met polymorphism impacts parahippocampal and amygdala volume in healthy humans: incremental support for a genetic risk factor for depression. *Psychol Med, 39*(11), 1831-1839. doi: 10.1017/S0033291709005509

Mowla, S. J., Farhadi, H. F., Pareek, S., Atwal, J. K., Morris, S. J., Seidah, N. G., & Murphy, R. A. (2001). Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *J Biol Chem, 276*(16), 12660-12666. doi: 10.1074/jbc.M008104200

Mowla, S. J., Pareek, S., Farhadi, H. F., Petrecca, K., Fawcett, J. P., Seidah, N. G., Morris, S. J., Sossin, W. S., & Murphy, R. A. (1999). Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. The Journal of Neuroscience, 19(6), 2069–2080. https://doi.org/10.1523/jneurosci.19-06-02069.1999

Murphy, R. A., Acheson, A., Hodges, R., Haskins, J., Richards, C., Reklow, E., Chlumecky, V., Barker, P. A., Alderson, R. F., & Lindsay, R. M. (1993). Immunological relationships of NGF, BDNF, and NT-3: Recognition and functional inhibition by antibodies to NGF. The Journal of Neuroscience, 13(7), 2853–2862. https://doi.org/10.1523/jneurosci.13-07-02853.1993.

Musumeci, G., & Minichiello, L. (2011). BDNF-TrkB signalling in fear learning: from genetics to neural networks. *Rev Neurosci, 22*(3), 303-315. doi: 10.1515/RNS.2011.031

Musumeci, G., Sciarretta, C., Rodríguez-Moreno, A., Al Banchaabouchi, M., Negrete-Díaz, V., Costanzi, M., Berno, V., Egorov, A. V., von Bohlen und Halbach, O., Cestari, V., Delgado-García, J. M., & Minichiello, L. (2009). TrkB modulates fear learning and amygdalar synaptic plasticity by specific docking sites. The Journal of Neuroscience, 29(32), 10131–10143. https://doi.org/10.1523/jneurosci.1707-09.2009.

Myers, K. M., & Davis, M. (2007). Mechanisms of fear extinction. *Mol Psychiatry, 12*(2), 120-150. doi: 10.1038/sj.mp.4001939.

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*(6797), 722-726. doi: 10.1038/35021052

Nader, K., & Einarsson, E. Ö. (2010). Memory reconsolidation: an update. *Annals of the New York Academy of Sciences*, *1191*(1), 27–41. https://doi.org/10.1111/j.1749-6632.2010.05443.x

Nicoll, R. A., & Malenka, R. C. (1999). Expression mechanisms underlying NMDA receptor-dependent long-term potentiation. *Ann N Y Acad Sci, 868*, 515-525. doi: 10.1111/j.1749-6632.1999.tb11320.x

Nilsson, A. S., Fainzilber, M., Falck, P., & Ibanez, C. F. (1998). Neurotrophin-7: a novel member of the neurotrophin family from the zebrafish. *Febs Letters, 424*(3), 285-290. doi: Doi 10.1016/S0014-5793(98)00192-6

Nonaka, A., Toyoda, T., Miura, Y., Hitora-Imamura, N., Naka, M., Eguchi, M., Yamaguchi, S., Ikegaya, Y., Matsuki, N., & Nomura, H. (2014). Synaptic plasticity associated with a memory engram in the basolateral amygdala. Journal of Neuroscience, 34(28), 9305–9309. https://doi.org/10.1523/jneurosci.4233-13.2014.

Oler, I. D., & Baum, M. (1968). Facilitated extinction of an avoidance response through shortening of the inter-trial interval. Psychonomic Science, 11(9), 323–324. https://doi.org/10.3758/bf03328213

Olsen, D., Kaas, M., Schwartz, O., Nykjaer, A., & Glerup, S. (2013). Loss of BDNF or its receptors in three mouse models has unpredictable consequences for anxiety and fear acquisition. Learning & Memory, 20(9), 499–504. https://doi.org/10.1101/lm.032045.113 .

Orsini, C. A., & Maren, S. (2012). Neural and cellular mechanisms of fear and extinction memory formation. *Neurosci Biobehav Rev, 36*(7), 1773-1802. doi: 10.1016/j.neubiorev.2011.12.014

Ou, L. C., & Gean, P. W. (2006). Regulation of amygdala-dependent learning by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol-3-kinase. *Neuropsychopharmacology, 31*(2), 287-296. doi: 10.1038/sj.npp.1300830

Ou, L. C., & Gean, P. W. (2007). Transcriptional regulation of brain-derived neurotrophic factor in the amygdala during consolidation of fear memory. *Mol Pharmacol, 72*(2), 350-358. doi: 10.1124/mol.107.034934

Ou, L. C., Yeh, S. H., & Gean, P. W. (2010). Late expression of brain-derived neurotrophic factor in the amygdala is required for persistence of fear memory. *Neurobiol Learn Mem, 93*(3), 372-382. doi: 10.1016/j.nlm.2009.12.003

Pang, P. T., Teng, H. K., Zaitsev, E., Woo, N. T., Sakata, K., Zhen, S., Teng, K. K., Yung, W.-H., Hempstead, B. L., & Lu, B. (2004). Cleavage of probdnf by TPA/plasmin is essential for long-term hippocampal plasticity. Science, 306(5695), 487–491. https://doi.org/10.1126/science.1100135.

Paré, D., & Smith, Y. (1993). The intercalated cell masses project to the central and medial nuclei of the amygdala in cats. *Neuroscience*, *57*(4), 1077-1090.

Pape, H. C., & Pare, D. (2010). Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol Rev, 90*(2), 419-463. doi: 10.1152/physrev.00037.2009

Pare, D., Quirk, G. J., & Ledoux, J. E. (2004). New vistas on amygdala networks in conditioned fear. *J Neurophysiol, 92*(1), 1-9. doi: 10.1152/jn.00153.2004

Patterson, S. L., Pittenger, C., Morozov, A., Martin, K. C., Scanlin, H., Drake, C., & 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*(1), 123-140. doi: 10.1016/s0896-6273(01)00443-3

Pavlov, I. P. (1927). Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex (G. V. Anrep, Trans.). New York: Dover.

Pavlov, P. I. (2010). Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. *Ann Neurosci, 17*(3), 136-141. doi: 10.5214/ans.0972-7531.1017309

Paylor, R., Tracy, R., Wehner, J., & Rudy, J. W. (1994). DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning. *Behav Neurosci, 108*(4), 810-817. doi: 10.1037//0735-7044.108.4.810

Pearn, M. L., Hu, Y., Niesman, I. R., Patel, H. H., Drummond, J. C., Roth, D. M., Akassoglou, K., Patel, P. M., & Head, B. P. (2012). Propofol neurotoxicity is mediated by P75 neurotrophin receptor activation. Anesthesiology, 116(2), 352–361. https://doi.org/10.1097/aln.0b013e318242a48c.

Penzo, M. A., Robert, V., Tucciarone, J., De Bundel, D., Wang, M., Van Aelst, L., Darvas, M., Parada, L. F., Palmiter, R. D., He, M., Huang, Z. J., & Li, B. (2015). The paraventricular thalamus controls a central amygdala fear circuit. Nature, 519(7544), 455–459. https://doi.org/10.1038/nature13978

Peters, J., Dieppa-Perea, L. M., Melendez, L. M., & Quirk, G. J. (2010). Induction of fear extinction with hippocampal-infralimbic BDNF. *Science, 328*(5983), 1288-1290. doi: 10.1126/science.1186909

Phelps, E. A. (2006). Emotion and cognition: insights from studies of the human amygdala. *Annu Rev Psychol, 57*, 27-53. doi: 10.1146/annurev.psych.56.091103.070234

Phelps, E. A., & LeDoux, J. E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron, 48*(2), 175-187. doi: 10.1016/j.neuron.2005.09.025

Pliota, P., Groessl, F., Griessner, J., Pasieka, M., Deussing, J., & Haubensak, W. (2018). Stress peptides sensitize fear circuitry to promote passive coping. https://doi.org/10.26226/morressier.5b31ec542afeeb001345b89c

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci, 106*(2), 274-285. doi: 10.1037//0735-7044.106.2.274

Pitkanen, A., Savander, V., & LeDoux, J. E. (1997). Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. *Trends Neurosci, 20*(11), 517-523. doi: 10.1016/s0166-2236(97)01125-9

Pruunsild, P., Kazantseva, A., Aid, T., Palm, K., & Timmusk, T. (2007). Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. *Genomics, 90*(3), 397-406. doi: 10.1016/j.ygeno.2007.05.004

Psotta, L., Lessmann, V., & Endres, T. (2013). Impaired fear extinction learning in adult heterozygous BDNF knock-out mice. *Neurobiol Learn Mem, 103*, 34-38. doi: 10.1016/j.nlm.2013.03.003

Quillfeldt, J. A. (2016). Behavioral methods to study learning and memory in rats. Rodent Model as Tools in Ethical Biomedical Research, 271–311. https://doi.org/10.1007/978-3-319-11578-8_17

Quirk, G., Russo, G., Barron, J., & Lebron, K. (2000). The Role of Ventromedial Prefrontal Cortex in the Recovery of Extinguished Fear. *The Journal Of Neuroscience*, *20*(16), 6225-6231. doi: 10.1523/jneurosci.20-16-06225.2000

Quirk, G. J., Likhtik, E., Pelletier, J. G., & Paré, D. (2003). Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. The Journal of Neuroscience, 23(25), 8800–8807. https://doi.org/10.1523/jneurosci.23-25-08800.2003.

Quirk, G. J., Garcia, R., & Gonzalez-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biol Psychiatry, 60*(4), 337-343. doi: 10.1016/j.biopsych.2006.03.010

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology, 33*(1), 56-72. doi: 10.1038/sj.npp.1301555

Quirk, G. J., Pare, D., Richardson, R., Herry, C., Monfils, M. H., Schiller, D., & Vicentic, A. (2010). Erasing fear memories with extinction training. *J Neurosci, 30*(45), 14993-14997. doi: 10.1523/JNEUROSCI.4268-10.2010

Ramón y Cajal, S. (1894). The Croonian lecture.La fine structure des centres nerveux. *Proceedings of the Royal Society of London*, *55*(331-335), 444-468.

Rattiner, L. M., Davis, M., & Ressler, K. J. (2004). Differential regulation of brain-derived neurotrophic factor transcripts during the consolidation of fear learning. *Learn Mem, 11*(6), 727-731. doi: 10.1101/lm.83304

Rattiner, L. M., Davis, M., French, C. T., & Ressler, K. J. (2004). Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. The Journal of Neuroscience, 24(20), 4796–4806. https://doi.org/10.1523/jneurosci.5654-03.2004.

Rattiner, L. M., Davis, M., & Ressler, K. J. (2005). Brain-derived neurotrophic factor in amygdala-dependent learning. *Neuroscientist, 11*(4), 323-333. doi: 10.1177/1073858404272255.

Radiske, A., Rossato, J. I., Kohler, C. A., Gonzalez, M. C., Medina, J. H., & Cammarota, M. (2015). Requirement for BDNF in the reconsolidation of fear extinction. *Journal of Neuroscience*, *35*(16), 6570–6574. https://doi.org/10.1523/jneurosci.4093-14.2015

Reichardt, L. F. (2006). Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1473), 1545–1564. https://doi.org/10.1098/rstb.2006.1894

Ren, J., Li, X., Zhang, X., Li, M., Wang, Y., & Ma, Y. (2013). The effects of intra-hippocampal microinfusion of D-cycloserine on fear extinction, and the expression of NMDA receptor subunit NR2B and neurogenesis in the hippocampus in rats. *Prog Neuropsychopharmacol Biol Psychiatry, 44*, 257-264. doi: 10.1016/j.pnpbp.2013.02.017.

Rescorla, R. A. (1968). Probability of shock in the presence and absence of CS in fear conditioning. *J Comp Physiol Psychol, 66*(1), 1-5. doi: 10.1037/h0025984

Ressler, K. J., Rothbaum, B. O., Tannenbaum, L., Anderson, P., Graap, K., Zimand, E., Hodges, L., & Davis, M. (2004). Cognitive enhancers as adjuncts to psychotherapy. Archives of General Psychiatry, 61(11), 1136. https://doi.org/10.1001/archpsyc.61.11.1136.

Reynolds, B. R. A. D. L. E. Y. (1945). Extinction of trace conditioned responses as a function of the spacing of trials during the acquisition and extinction series. *Journal of Experimental Psychology*, *35*(2), 81.

Rizvi, T. A., Ennis, M., Behbehani, M. M., & Shipley, M. T. (1991). Connections between the central nucleus of the amygdala and the midbrain periaqueductal gray: topography and reciprocity. *J Comp Neurol, 303*(1), 121-131. doi: 10.1002/cne.903030111

Royer, S., Martina, M., & Paré, D. (1999). An Inhibitory Interface Gates Impulse Traffic between the Input and Output Stations of the Amygdala. *The Journal Of Neuroscience*, *19*(23), 10575-10583. doi: 10.1523/jneurosci.19-23-10575.1999

Rodriguez-Tebar, A., Dechant, G., & Barde, Y. A. (1990). Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron, 4*(4), 487-492. doi: 10.1016/0896-6273(90)90107-q

Rodrigues, S. M., Schafe, G. E., & LeDoux, J. E. (2001). Intra-amygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. The Journal of Neuroscience, 21(17), 6889–6896. https://doi.org/10.1523/jneurosci.21-17-06889.2001.

Rodrigues, S. M., Bauer, E. P., Farb, C. R., Schafe, G. E., & LeDoux, J. E. (2002). The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. The Journal of Neuroscience, 22(12), 5219–5229. https://doi.org/10.1523/jneurosci.22-12-05219.2002.

Rodrigues, S. M., Farb, C. R., Bauer, E. P., LeDoux, J. E., & Schafe, G. E. (2004). Pavlovian fear conditioning regulates Thr286 autophosphorylation of Ca2+/calmodulin-dependent protein kinase II at lateral amygdala synapses. *J Neurosci, 24*(13), 3281-3288. doi: 10.1523/JNEUROSCI.5303-03.2004

Rodrigues, S. M., Schafe, G. E., & LeDoux, J. E. (2004). Molecular mechanisms underlying emotional learning and memory in the lateral amygdala. *Neuron, 44*(1), 75-91. doi: 10.1016/j.neuron.2004.09.014

Rogan, M. T., Staubli, U. V., & LeDoux, J. E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. *Nature, 390*(6660), 604-607. doi: 10.1038/37601

Romanski, L. M., & LeDoux, J. E. (1993). Information cascade from primary auditory cortex to the amygdala: corticocortical and corticoamygdaloid projections of temporal cortex in the rat. *Cereb Cortex, 3*(6), 515-532. doi: 10.1093/cercor/3.6.515

Rosas-Vidal, L. E., Do-Monte, F. H., Sotres-Bayon, F., & Quirk, G. J. (2014). Hippocampal--prefrontal BDNF and memory for fear extinction. *Neuropsychopharmacology, 39*(9), 2161-2169. doi: 10.1038/npp.2014.64

Rosas-Vidal, L. E., Lozada-Miranda, V., Cantres-Rosario, Y., Vega-Medina, A., Melendez, L., & Quirk, G. J. (2018). Alteration of BDNF in the medial prefrontal cortex and the ventral hippocampus impairs extinction of avoidance. *Neuropsychopharmacology, 43*(13), 2636-2644. doi: 10.1038/s41386-018-0176-8

Rosch, H., Schweigreiter, R., Bonhoeffer, T., Barde, Y. A., & Korte, M. (2005). The neurotrophin receptor p75NTR modulates long-term depression and regulates the expression of AMPA receptor subunits in the hippocampus. *Proc Natl Acad Sci U S A, 102*(20), 7362-7367. doi: 10.1073/pnas.0502460102

Ryu, J., Futai, K., Feliu, M., Weinberg, R., & Sheng, M. (2008). Constitutively active Rap2 transgenic mice display fewer dendritic spines, reduced extracellular signal-regulated kinase signaling, enhanced long-term depression, and impaired spatial learning and fear extinction. *J Neurosci, 28*(33), 8178-8188. doi: 10.1523/JNEUROSCI.1944-08.2008

Sah, P., Westbrook, R. F., & Lüthi, A. (2008). fear conditioning and long-term potentiation in the amygdala. Annals of the New York Academy of Sciences, 1129(1), 88–95. https://doi.org/10.1196/annals.1417.020.

Sakata, K., Martinowich, K., Woo, N. H., Schloesser, R. J., Jimenez, D. V., Ji, Y., . . . Lu, B. (2013). Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. *Proc Natl Acad Sci U S A, 110*(37), 15103-15108. doi: 10.1073/pnas.1222872110

Sangha, S., Narayanan, R. T., Bergado-Acosta, J. R., Stork, O., Seidenbecher, T., & Pape, H. C. (2009). Deficiency of the 65 kDa isoform of glutamic acid decarboxylase impairs extinction of cued but not contextual fear memory. *J Neurosci, 29*(50), 15713-15720. doi: 10.1523/JNEUROSCI.2620-09.2009

Sangha, S. (2015). Plasticity of Fear and Safety Neurons of the Amygdala in Response to Fear Extinction. *Front Behav Neurosci, 9*, 354. doi: 10.3389/fnbeh.2015.00354

Sangha, S., Diehl, M. M., Bergstrom, H. C., & Drew, M. R. (2020). Know safety, no fear. *Neurosci Biobehav Rev, 108*, 218-230. doi: 10.1016/j.neubiorev.2019.11.006

Santini, E., Muller, R. U., & Quirk, G. J. (2001). Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. The Journal of Neuroscience, 21(22), 9009–9017. https://doi.org/10.1523/jneurosci.21-22-09009.2001.

Santini, E., Ge, H., Ren, K., Pena de Ortiz, S., & Quirk, G. J. (2004). Consolidation of fear extinction requires protein synthesis in the medial prefrontal cortex. *J Neurosci, 24*(25), 5704-5710. doi: 10.1523/JNEUROSCI.0786-04.2004

Schafe, G. E., Atkins, C. M., Swank, M. W., Bauer, E. P., Sweatt, J. D., & LeDoux, J. E. (2000). Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of Pavlovian Fear Conditioning. The Journal of Neuroscience, 20(21), 8177–8187. https://doi.org/10.1523/jneurosci.20-21-08177.2000.

Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of Auditory Pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. The Journal of Neuroscience, 20(18). https://doi.org/10.1523/jneurosci.20-18-j0003.2000.

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. *Trends Neurosci, 24*(9), 540-546. doi: 10.1016/s0166-2236(00)01969-x

Schildt, S., Endres, T., Lessmann, V., & Edelmann, E. (2013). Acute and chronic interference with BDNF/TrkB-signaling impair LTP selectively at mossy fiber synapses in the CA3 region of mouse hippocampus. *Neuropharmacology, 71*, 247-254. doi: 10.1016/j.neuropharm.2013.03.041

Schulz-Klaus, B., Lessmann, V., & Endres, T. (2013). BDNF-dependent consolidation of fear memories in the perirhinal cortex. *Front Behav Neurosci, 7*, 205. doi: 10.3389/fnbeh.2013.00205

Seidah, N. G., Benjannet, S., Pareek, S., Chrétien, M., & Murphy, R. A. (1996). Cellular processing of the neurotrophin precursors of nt3 and BDNF by the mammalian proprotein convertases. FEBS Letters, 379(3), 247–250. https://doi.org/10.1016/0014-5793(95)01520-5.

Seidah, N. G., Mowla, S. J., Hamelin, J., Mamarbachi, A. M., Benjannet, S., Touré, B. B., Basak, A., Munzer, J. S., Marcinkiewicz, J., Zhong, M., Barale, J. C., Lazure, C., Murphy, R. A., Chrétien, M., & Marcinkiewicz, M. (1999). Mammalian subtilisin/kexin isozyme ski-1: A widely expressed proprotein convertase with a unique cleavage specificity and cellular localization. Proceedings of the National Academy of Sciences, 96(4), 1321–1326. https://doi.org/10.1073/pnas.96.4.1321.

Sears, R. M., Schiff, H. C., & LeDoux, J. E. (2014). Molecular mechanisms of threat learning in the lateral nucleus of the amygdala. Progress in Molecular Biology and Translational Science, 263–304. https://doi.org/10.1016/b978-0-12-420170-5.00010-6

Segal, R. A., & Greenberg, M. E. (1996). Intracellular signaling pathways activated by neurotrophic factors. *Annu Rev Neurosci, 19*, 463-489. doi: 10.1146/annurev.ne.19.030196.002335.

Sejnowski, T. J. (1999). The book of Hebb. *Neuron, 24*(4), 773-776. doi: 10.1016/s0896-6273(00)81025-9

Shelton, D. L., Sutherland, J., Gripp, J., Camerato, T., Armanini, M. P., Phillips, H. S., Carroll, K., Spencer, S. D., & Levinson, A. D. (1995). Human trks: Molecular cloning, tissue distribution, and expression of extracellular domain immunoadhesins. The Journal of Neuroscience, 15(1), 477–491. https://doi.org/10.1523/jneurosci.15-01-00477.1995.

Shen, K., & Meyer, T. (1999). Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. *Science, 284*(5411), 162-166. doi: 10.1126/science.284.5411.162

Shi, C.-J., & Cassell, M. D. (1997). Cortical, thalamic, and amygdaloid projections of rat temporal cortex. The Journal of Comparative Neurology, 382(2), 153–175. https://doi.org/10.1002/(sici)1096-9861(19970602)382:2<153::aid-cne2>3.0.co;2-2.

Sierra-Mercado, D., Padilla-Coreano, N., & Quirk, G. J. (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology, 36*(2), 529-538. doi: 10.1038/npp.2010.184

Sigurdsson, T., Doyère, V., Cain, C. K., & LeDoux, J. E. (2007). Long-term potentiation in the amygdala: A cellular mechanism of Fear Learning and Memory. Neuropharmacology, 52(1), 215–227. https://doi.org/10.1016/j.neuropharm.2006.06.022.

Singewald, N., Schmuckermair, C., Whittle, N., Holmes, A., & Ressler, K. J. (2015). Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. *Pharmacol Ther, 149*, 150-190. doi: 10.1016/j.pharmthera.2014.12.004

Singewald, N., & Holmes, A. (2019). Rodent models of impaired fear extinction. *Psychopharmacology (Berl), 236*(1), 21-32. doi: 10.1007/s00213-018-5054-x

Singewald, N., Schmuckermair, C., Whittle, N., Holmes, A., & Ressler, K. J. (2015). Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. Pharmacology & Therapeutics, 149, 150–190. https://doi.org/10.1016/j.pharmthera.2014.12.004.

Skrzypiec, A. E., Buczko, W., & Pawlak, R. (2008). Tissue plasminogen activator in the amygdala: a new role for an old protease. *J Physiol Pharmacol, 59 Suppl 8*, 135-146.

Skaper, S. D. (2012). The neurotrophin family of neurotrophic factors: an overview. *Methods Mol Biol, 846*, 1-12. doi: 10.1007/978-1-61779-536-7_1

Smith, Y., Pare, J. F., & Pare, D. (1998). Cat intraamygdaloid inhibitory network: ultrastructural organization of parvalbumin-immunoreactive elements. *J Comp Neurol, 391*(2), 164-179. doi: 10.1002/(sici)1096-9861(19980209)391:2<164::aid-cne2>3.0.co;2-0

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., Voss, H. U., Glover, G., Ballon, D. J., Liston, C., Teslovich, T., Van Kempen, T., Lee, F. S., & Casey, B. J. (2010). A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. Science, 327(5967), 863–866. https://doi.org/10.1126/science.1181886.

Song, M., Martinowich, K., & Lee, F. S. (2017). BDNF at the synapse: why location matters. *Mol Psychiatry, 22*(10), 1370-1375. doi: 10.1038/mp.2017.144

Sotres-Bayon, F., Bush, D. E., & LeDoux, J. E. (2007). Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. *Neuropsychopharmacology, 32*(9), 1929-1940. doi: 10.1038/sj.npp.1301316

Sotres-Bayon, F., Diaz-Mataix, L., Bush, D. E., & LeDoux, J. E. (2009). Dissociable roles for the ventromedial prefrontal cortex and amygdala in fear extinction: NR2B contribution. *Cereb Cortex, 19*(2), 474-482. doi: 10.1093/cercor/bhn099

Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E., & Quirk, G. J. (2012). Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. *Neuron, 76*(4), 804-812. doi: 10.1016/j.neuron.2012.09.028

Stacho, M., & Manahan-Vaughan, D. (2022). The intriguing contribution of hippocampal long-term depression to spatial learning and long-term memory. *Frontiers in Behavioral Neuroscience*, *16*. https://doi.org/10.3389/fnbeh.2022.806356

Strobel, C., Marek, R., Gooch, H. M., Sullivan, R. K. P., & Sah, P. (2015). Prefrontal and Auditory Input to Intercalated Neurons of the Amygdala. *Cell Rep, 10*(9), 1435-1442. doi: 10.1016/j.celrep.2015.02.008

Sun, W., Li, X., & An, L. (2018). Distinct roles of prelimbic and infralimbic proBDNF in extinction of conditioned fear. *Neuropharmacology, 131*, 11-19. doi: 10.1016/j.neuropharm.2017.12.018

Timmusk, T., Lendahl, U., Funakoshi, H., Arenas, E., Persson, H., & Metsis, M. (1995). Identification of brain-derived neurotrophic factor promoter regions mediating tissue-specific, axotomy-, and neuronal activity-induced expression in transgenic mice. *J Cell Biol, 128*(1-2), 185-199. doi: 10.1083/jcb.128.1.185

Timmusk, T., Palm, K., Metsis, M., Reintam, T., Paalme, V., Saarma, M., & Persson, H. (1993). Multiple promoters direct tissue-specific expression of the rat BDNF gene. *Neuron, 10*(3), 475-489. doi: 10.1016/0896-6273(93)90335-o

Thomas, A. X., Cruz Del Angel, Y., Gonzalez, M. I., Carrel, A. J., Carlsen, J., Lam, P. M., Hempstead, B. L., Russek, S. J., & Brooks-Kayal, A. R. (2016). Rapid increases in PROBDNF after pilocarpine-induced status epilepticus in mice are associated with reduced probdnf cleavage machinery. Eneuro, 3(1). https://doi.org/10.1523/eneuro.0020-15.2016.

Thomas, K., & Davies, A. (2005). Neurotrophins: a ticket to ride for BDNF. *Curr Biol, 15*(7), R262-264. doi: 10.1016/j.cub.2005.03.023

Trent, S., Barnes, P., Hall, J., & Thomas, K. L. (2017). AMPA receptors control fear extinction through an Arc-dependent mechanism. *Learn Mem, 24*(8), 375-380. doi: 10.1101/lm.045013.117

Tovote, P., Esposito, M. S., Botta, P., Chaudun, F., Fadok, J. P., Markovic, M., Wolff, S. B., Ramakrishnan, C., Fenno, L., Deisseroth, K., Herry, C., Arber, S., & Lüthi, A. (2016). Midbrain circuits for defensive behaviour. Nature, 534(7606), 206–212. https://doi.org/10.1038/nature17996.

Tovote, P., Fadok, J. P., & Luthi, A. (2015). Neuronal circuits for fear and anxiety. *Nat Rev Neurosci, 16*(6), 317-331. doi: 10.1038/nrn3945.

Underwood, C. K., & Coulson, E. J. (2008). The p75 neurotrophin receptor. *Int J Biochem Cell Biol, 40*(9), 1664-1668. doi: 10.1016/j.biocel.2007.06.010.

Uys, J. D., Stein, D. J., Daniels, W. M., & Harvey, B. H. (2003). Animal models of anxiety disorders. *Curr Psychiatry Rep, 5*(4), 274-281. doi: 10.1007/s11920-003-0056-7.

Vervliet, B., Craske, M. G., & Hermans, D. (2013). Fear extinction and relapse: state of the art. *Annu Rev Clin Psychol, 9*, 215-248. doi: 10.1146/annurev-clinpsy-050212-185542

von Bohlen und Halbach, O. (2010). Involvement of BDNF in age-dependent alterations in the hippocampus. *Frontiers in Aging Neuroscience*. https://doi.org/10.3389/fnagi.2010.00036.

von Bohlen und Halbach, O., & von Bohlen und Halbach, V. (2018). BDNF effects on dendritic spine morphology and hippocampal function. *Cell and Tissue Research*, *373*(3), 729–741. https://doi.org/10.1007/s00441-017-2782-x.

von Bohlen und Halbach, O., Minichiello, L., & Unsicker, K. (2003). Haploinsufficiency in trkb and/or trkc neurotrophin receptors causes structural alterations in the aged hippocampus and amygdala. *European Journal of Neuroscience*, *18*(8), 2319–2325. https://doi.org/10.1046/j.1460-9568.2003.02953.x

Walker, D. L., & Davis, M. (2002). The role of amygdala glutamate receptors in fear learning, fear-potentiated startle, and extinction. *Pharmacol Biochem Behav, 71*(3), 379-392. doi: 10.1016/s0091-3057(01)00698-0.

Walker, D. L., Ressler, K. J., Lu, K. T., & Davis, M. (2002). Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. The Journal of Neuroscience, 22(6), 2343–2351. https://doi.org/10.1523/jneurosci.22-06-02343.2002.

Washburn, M. S., & Moises, H. C. (1992). Electrophysiological and morphological properties of rat basolateral amygdaloid neurons in vitro. The Journal of Neuroscience, 12(10), 4066–4079. https://doi.org/10.1523/jneurosci.12-10-04066.1992.

Wank, I., Pliota, P., Badurek, S., Kraitsy, K., Kaczanowska, J., Griessner, J., Kreitz, S., Hess, A., & Haubensak, W. (2021). Central amygdala circuitry modulates nociceptive processing through differential hierarchical interaction with affective network dynamics. *Communications Biology*, *4*(1). https://doi.org/10.1038/s42003-021-02262-3.

Wetmore, C., Cao, Y. H., Pettersson, R. F., & Olson, L. (1991). Brain-derived neurotrophic factor: subcellular compartmentalization and interneuronal transfer as visualized with anti-peptide antibodies. *Proc Natl Acad Sci U S A, 88*(21), 9843-9847. doi: 10.1073/pnas.88.21.9843.

Woo, N. H., Teng, H. K., Siao, C. J., Chiaruttini, C., Pang, P. T., Milner, T. A., Hempstead, B. L., & Lu, B. (2005). Activation of p75ntr by probdnf facilitates hippocampal long-term depression. Nature Neuroscience, 8(8), 1069–1077. https://doi.org/10.1038/nn1510.

Wood, M., Adil, O., Wallace, T., Fourman, S., Wilson, S. P., Herman, J. P., & Myers, B. (2019). Infralimbic prefrontal cortex structural and functional connectivity with the limbic forebrain: a combined viral genetic and optogenetic analysis. *Brain Struct Funct, 224*(1), 73-97. doi: 10.1007/s00429-018-1762-6.

Xin, J., Ma, L., Zhang, T. Y., Yu, H., Wang, Y., Kong, L., & Chen, Z. Y. (2014). Involvement of BDNF signaling transmission from basolateral amygdala to infralimbic prefrontal cortex in conditioned taste aversion extinction. *J Neurosci, 34*(21), 7302-7313.

Xue, Z., Shui, M., Lin, X., Sun, Y., Liu, J., Wei, C., Wu, A., & Li, T. (2022). Role of BDNF/PROBDNF imbalance in postoperative cognitive dysfunction by modulating synaptic plasticity in aged mice. *Frontiers in Aging Neuroscience*, *14*. https://doi.org/10.3389/fnagi.2022.780972

Yamashita, T., & Tohyama, M. (2003). The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. *Nat Neurosci, 6*(5), 461-467. doi: 10.1038/nn1045

Yan, Q., Rosenfeld, R. D., Matheson, C. R., Hawkins, N., Lopez, O. T., Bennett, L., & Welcher, A. A. (1997). Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience, 78*(2), 431-448. doi: 10.1016/s0306-4522(96)00613-6

Yang, J., Siao, C.-J., Nagappan, G., Marinic, T., Jing, D., McGrath, K., Chen, Z.-Y., Mark, W., Tessarollo, L., Lee, F. S., Lu, B., & Hempstead, B. L. (2009). Neuronal release of probdnf. Nature Neuroscience, 12(2), 113–115. https://doi.org/10.1038/nn.2244.

Yang, F., Je, H.-S., Ji, Y., Nagappan, G., Hempstead, B., & Lu, B. (2009). Pro-BDNF–induced synaptic depression and retraction at developing neuromuscular synapses. *Journal of Cell Biology*, *185*(4), 727–741. https://doi.org/10.1083/jcb.200811147

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., Ballon, D., Lee, F. S., Scharfman, H. E., & Hempstead, B. L. (2014). ProBDNF negatively regulates neuronal remodeling, synaptic transmission, and synaptic plasticity in Hippocampus. *Cell Reports*, *7*(3), 796–806. https://doi.org/10.1016/j.celrep.2014.03.040 .

Young, M. B., Andero, R., Ressler, K. J., & Howell, L. L. (2015). 3,4 Methylenedioxymethamphetamine facilitates fear extinction learning. *Transl Psychiatry, 5*, e634. doi: 10.1038/tp.2015.138

Yuan, J., Yan, M., Xu, Y., Chen, W., & Wang, X. (2018). Social Company Disrupts Fear Memory Renewal: Evidence From Two Rodent Studies. *Front Neurosci, 12*, 565. doi: 10.3389/fnins.2018.00565

Zettler, C., Bridges, D. C., Zhou, X. F., & Rush, R. A. (1996). Detection of increased tissue concentrations of nerve growth factor with an improved extraction procedure. *J Neurosci Res, 46*(5), 581-594. doi: 10.1002/(SICI)1097-4547(19961201)46:5<581::AID-JNR7>3.0.CO;2-F

Zhou, X. F., Song, X. Y., Zhong, J. H., Barati, S., Zhou, F. H., & Johnson, S. M. (2004). Distribution and localization of pro-brain-derived neurotrophic factor-like immunoreactivity in the peripheral and central nervous system of the adult rat. *J Neurochem, 91*(3), 704-715. doi: 10.1111/j.1471-4159.2004.02775.x

Zhou, X. F., Zettler, C., & Rush, R. A. (1994). An improved procedure for the immunohistochemical localization of nerve growth factor-like immunoreactivity. *J Neurosci Methods, 54*(1), 95-102. doi: 10.1016/0165-0270(94)90163-5

Zimmerman, J. M., & Maren, S. (2010). NMDA receptor antagonism in the basolateral but not central amygdala blocks the extinction of Pavlovian fear conditioning in rats. *Eur J Neurosci, 31*(9), 1664-1670. doi: 10.1111/j.1460-9568.2010.07223.x.

7. Appendix

A. Declaration of Honour

“I hereby declare that I prepared this thesis without the impermissible help of third parties and that none other than the aids indicated have been used; all sources of information are clearly marked, including my own publications.

In particular, I have not consciously:

- fabricated data or rejected undesirable results,
- misused statistical methods with the aim of drawing other conclusions than those warranted by the available data,
- plagiarized external data or publications,
- presented the results of other researchers in a distorted way.

I am aware that violations of copyright may lead to an injunction and damage claims by the author and also to prosecution by law enforcement authorities. I hereby agree that the thesis may be electronically reviewed with the aim of identifying plagiarism.

This work has not been submitted as a doctoral thesis in the same or a similar form in Germany, nor in any other country. It has not yet been published as a whole.”

(Place, date) (Signature)