Aus dem Lehrstuhl
für Psychiatrie und Psychotherapie
Prof. Dr. Rainer Rupprecht
der Fakultät für Medizin
Universität Regensburg

The role of the Translocatorprotein (TSPO) 18 kDa in a psychopathological animal model for anxiety and depression

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

der
Fakultät für Medizin
der Universität Regensburg

vorgelegt von
Lilith Fischer

2024

Aus dem Lehrstuhl
für Psychiatrie und Psychotherapie
Prof. Dr. Rainer Rupprecht
der Fakultät für Medizin
Universität Regensburg

The role of the Translocatorprotein (TSPO) 18 kDa in a psychopathological animal model for anxiety and depression

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

der
Fakultät für Medizin
der Universität Regensburg

vorgelegt von
Lilith Fischer

2024

Dekan: Herr Prof. Dr. Dirk Hellwig

1. Berichterstatter: Herr Prof. Dr. Rainer Rupprecht

2. Berichterstatter: Herr Prof. Dr. Christian Wetzel

Tag der mündlichen Prüfung: 18.07.2024

Das Ziel dieser Studie war es, das Translokatorprotein (TSPO) 18 kDa in einem psychopathologischen Tiermodell für Angst und Depression zu untersuchen, um tiefere Einblicke in die Rolle von TSPO bei psychiatrischen Störungen und deren potenziellen therapeutischen Angriffspunkte zu gewinnen. TSPO ist ein Kanalprotein, das sich in der äusseren Mitochondrienmembran befindet und für die Neurosteroid-Synthese mit limitierenden Eigenschaften wesentlich ist. Neurosteroide erleichtern die Neurotransmission am $GABA_A$-Rezeptor und vermitteln dadurch anxiolytische Eigenschaften. Der TSPO-Ligand Etifoxin wirkt durch verstärkte Neurosteroidsynthese anxiolytisch. In der vorliegenden Studie wurde versucht, weitere Erkenntnisse über die Funktionsweise von Etifoxin anhand der auf hohes bzw. niedriges Angst-ähnliches Verhalten gezüchteter Ratten zu gewinnen. Die vorliegende Studie bestätigt die anxiolytische Wirkung von Etifoxin, deckt aber auch einen Geschlechtsunterschied auf, da Etifoxin nur bei Weibchen mit hohem Angst-ähnlichen Verhalten anxiolytisch wirkte. Bei den männlichen Tieren mit hohem Angst-ähnlichen Verhalten führte Etifoxin nicht zu einer Verringerung des Angst-ähnlichen Verhaltens. Diese Ergebnisse sind im Hinblick auf die höhere Prävalenz psychiatrischer Störungen, insbesondere von Angststörungen, bei Frauen von Bedeutung. Die Studie erweitert frühere Beobachtungen, wonach die therapeutische Wirkung von Etifoxin auf pathologische Zustände mit einem gestörten Neurosteroid-System beschränkt sein könnte. Weitere geschlechtsspezifische Unterschiede müssen bei der Anwendung von TSPO berücksichtigt werden. Die Studie bestätigt ausserdem, dass der TSPO-Ligand Etifoxin in dem Tiermodell der auf hohes bzw. niedriges Angst-ähnliches Verhalten gezüchteter Ratten keine verbessernde Wirkung auf depressives Verhalten hat. In Übereinstimmung mit dem aktuellen Stand der Literatur hatte Etifoxin keine Auswirkungen auf die Stressreaktivität bei Tieren mit pathologischen Zuständen. Die Behandlung mit Etifoxin führte jedoch zu einer erhöhten Kortikosteronreaktion bei Tieren, die nicht auf Angst-ähnliches Verhalten selektiert wurden. Die Ergebnisse unterstreichen, wie wichtig es ist, die Beziehung zwischen TSPO und der Stressreaktion unter pathologischen Bedingungen weiter zu untersuchen, insbesondere im Zusammenhang mit Angststörungen. Darüber hinaus zeigte die Behandlung mit Etifoxin eine Verringerung der Oxytocin-Rezeptorbindung in der zentralen Amygdala von Weibchen mit hohem Angst assoziierten Verhalten. Dies könnte auf eine verstärkte Oxytocin-Signalisierung im Gehirn zurückzuführen sein, bedarf jedoch weiterer Forschung. Bislang gibt es nur wenige Forschungsergebnisse über die Beziehung zwischen TSPO und Oxytocin. Wichtig ist, dass diese Ergebnisse die Bedeutung der Berücksichtigung neuroendokriner Systeme bei der Entstehung von Angststörungen unterstreichen. Die vorliegende Arbeit beweist das Potenzial von TSPO als Ziel für die Behandlung von Angststörungen und zeigt, wie wichtig es ist, weibliche Probanden in die Forschung einzubeziehen, insbesondere in neurobiologischen Bereichen.

Contents

Abbreviations **5**

List of Figures **8**

1 Introduction **9**
- 1.1 General overview . . . 9
- 1.2 Anxiety disorder . . . 9
  - 1.2.1 Definition . . . 9
  - 1.2.2 Epidemiology . . . 12
  - 1.2.3 Pathophysiology and risk factors . . . 12
    - 1.2.3.1 Brain regions . . . 13
    - 1.2.3.2 Neurotransmitters . . . 14
    - 1.2.3.3 Microbiome . . . 16
  - 1.2.4 Animal model of rats selectively bred for extremes in anxiety-related behavior . . . 17
  - 1.2.5 Treatment . . . 20
- 1.3 Depression . . . 22
  - 1.3.1 Definition . . . 22
  - 1.3.2 Epidemiology . . . 22
  - 1.3.3 Etiology and risk factors . . . 23
  - 1.3.4 Treatment . . . 24
- 1.4 Translocator Protein . . . 25
  - 1.4.1 Structure and Function . . . 25
  - 1.4.2 TSPO and neurosteroids . . . 26
  - 1.4.3 TSPO in psychiatric disorders . . . 28
  - 1.4.4 TSPO ligands . . . 29
- 1.5 Aims and Objectives . . . 32

2 Material and Methods **33**
- 2.1 Animals and housing conditions . . . 33
- 2.2 Behavioral tests . . . 33

2.2.1 Light-Dark-Box . . . 34
2.2.2 Forced-Swim-Test . . . 34
2.3 Etifoxine Suspension . . . 35
2.4 Experimental design . . . 35
2.4.1 Experiment 1: Effect of TSPO ligand etifoxine on innate emotionality and stress reactivity . . . 36
2.4.1.1 Behavioral Observations . . . 36
2.4.1.2 Stress reactivity . . . 36
2.4.2 Experiment 2: Impact of peripheral TSPO ligand application on oxytocin receptor binding in selected brain regions . . . 37
2.4.3 Experiment 3: Effect of central infusion of TSPO ligand etifoxine on innate emotionality and stress reactivity . . . 38
2.5 Statistical Analysis . . . 39

3 Results **40**
3.1 Experiment 1 . . . 40
3.1.1 Behavioral observations . . . 40
3.1.2 Stress reactivity . . . 46
3.2 Experiment 2 . . . 47

4 Discussion **49**
4.1 Summary of results . . . 49
4.2 Effect of etifoxine . . . 50
4.3 Oxytocin receptor's density . . . 58
4.4 Limitations and Restrictions . . . 60
4.5 Future Research . . . 60

5 Summary **61**

References **99**

Curriculum vitae **99**

Acknowledgements **101**

Oath **102**

Abbreviations

ACTH	Adrenocorticotropic hormone
ADWA	Adjustment disorder with anxiety
ALLO	Allopregnanolone
ANOVA	Analysis of variance
ANT	Adenine nucleotide translocator
AVP	Arginin-vasopressin
BZ	Benzodiazepines
CBT	Cognitive behavioral therapy
CNS	Central nervous system
CMS	Chronic mild stress
CORT	Cortisol
CRF	Corticotropin-releasing factor
CSF	Cerebrospinal fluid
DALY	Disability-adjusted life year Los
DEHA	Dehydroepiandrosterone
DSM	Diagnostic and statistical manual of mental disorders
EPM	Elevated plusmaze
ELISA	Enzyme-linked immunosorbent assay
FST	Forced Swim Test
GABA	Gamma-aminobutyric acid
GAD	Generalized anxiety disorder
HAB	High anxiety-related behavior
HPA	Hypothalamic–pituitary–adrenal
ICD	International classification of diseases
IP	Intraperitoneal
kDa	Kilo dalton
LAB	Low anxiety-related behavior
LH	Luteinizing hormone

LDB	Light-dark-box
NAB	Non-selected for anxiety-related behavior
MDD	Major depressive disorder
MPTP	Mitochondrial permeability transition pore
mRNA	Messenger ribonucleic acid
OCD	Obsessive-compulsive disorder
OXT	Oxytocin
PET	Positron emission tomography
PFC	Prefrontal cortex
POMC	Proopiomelanocortin
PREG	Pregnanolone
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus
SAD	Social anxiety disorder
SEM	Standard error of the mean
SNRIs	Serotonin-noradrenaline reuptake inhibitors
SON	Supraoptic nucleus
SSRIs	Serotonin reuptake inhibitors
STAR	Steroidogenic acute regulatory protein
TCA	Tricyclic antidepressants
THDOC	Allotetrahydrodeoxycorticosterone
TTN	Triakontatetraneuropeptide
TSPO	Translocator Protein
VAS	Visual analog scale
VDAC	Voltage-dependent anion channel
VGT	Vogel´s conflict test
WHO	World Health Organisation

List of Figures

1.2.1 Key neuronal structures involved in generating and regulating anxiety . . . 13
1.4.1 Structure of TSPO . . . 25
1.4.2 Neurosteroid synthesis . . . 27
1.4.3 Schematic action mechanism of etifoxine . . . 30

2.4.1 Experiments timeline . . . 35

3.1.1 Effects of the etifoxine treatment on anxiety-related behavior . . . 41
3.1.2 Effect of the TSPO ligand etifoxine on anxiety-related behavior of female control rats . . . 41
3.1.3 Effect of the TSPO ligand etifoxine on locomotion in the LDB . . . 42
3.1.4 Effect of the TSPO ligand etifoxine on anxiety-related behavior of both sexes in the LDB . . . 43
3.1.5 Effect of the TSPO ligand etifoxine on passive stress-coping behavior in the FST . . . 44
3.1.6 Effect of the TSPO ligand etifoxine on active stress-coping behavior in the FST . . . 45
3.1.7 Effect of the TSPO ligand etifoxine on stress reactivity in the plasma . . . 46
3.2.1 Effect of the TSPO ligand etifoxine on OXT receptor binding in the central amygdala of female rats . . . 47
3.2.2 Effect of the TSPO ligand etifoxine on OXT receptor binding in the central amygdala of male rats . . . 48

Chapter 1

Introduction

1.1 General overview

Anxiety disorders are among the most prevalent psychiatric disorders and ranked as the sixth most prominent cause of disability worldwide (World-Health-Organization, 2017). The global prevalence of anxiety disorders and depressions is estimated at around 300 million (World-Health-Organization, 2017). These disorders result in a tremendous personal burden and affect the economy and health systems with an enormous cost factor (Gustavsson et al., 2011). In Germany, mental disorders are the third most common cause of the incapacity of work (DAK-Gesundheit, 2019). In this context, the disability-adjusted- life years lost (DALY) is an important tool to estimate the disease burden. DALY considers the number of years lost due to illness, disability, or death. The World-Health-Organization (2019a) attributes a DALY of 46 years to depression and 28 years to anxiety disorders.

1.2 Anxiety disorder

1.2.1 Definition

In order to understand anxiety disorders, it is important to distinguish fear and anxiety (Davis et al., 2010). Fear is the emotional reaction to a distinctly real and present threat, whereas anxiety is the expectation of rather undefined and unpredictable threats.

These two sensations have some overlap, yet fear is rather associated with the autonomic nervous systems response of fight-or-flight, whereas anxiety is expressed through vigilant, preparatory, and cautious behavior (American-Psychiatric-Association, 2013). Persistent, maladaptive, and excessive anxiety behavior is considered a pathological form of anxiety. Typically, the pathological state of anxiety lasts for around six months (Bandelow, 2014). The focus of apprehension of anxiety is either the stimulus or the circumstances that activate fear or anxiety (Kogan et al., 2016). This focus can have an external origin, such as social situations, or an internal origin, such as emotions and feelings. The symptoms of excessive anxiety include feelings of enormous and enduring fear, somatic reactions like raised heart rate and sweating, or psychological symptoms like discomfort and restlessness. Further, panic attacks and avoidance behavior can be part of some anxiety disorders (Craske et al., 2017).

The term anxiety disorder stands for various illnesses that differ in the type of anxiety trigger, associated reactions, and content of thoughts (see table 1.2.1). These include separation anxiety disorder, selective mutism, specific phobia, social anxiety disorder (SAD), panic disorder, agoraphobia, substance- or medication-induced, and general anxiety disorder (GAD) (Craske et al., 2017). Obsessive-compulsive disorder (OCD) and Post-traumatic stress disorder (PTSD) are classified separately in the Diagnostic and statistical manual of mental disorders 5 (DSM-5).

Table 1.2.1: Classification of anxiety disorder

Anxiety Disorder	Definition
Generalized anxiety disorder	Characterized by excessive and persistent fear in different fields. Physical symptoms are restlessness, concentration difficulties, inability to cope with certain situations, rapid fatigue, and sleep disturbances.
Panic disorder	Experience of recurrent, unexpected, or expected (specific stimulus) panic attacks, leading to a persistent worry of further panic attacks. Enormous fear, increasing within minutes and accompanied by physical and cognitive symptoms, characterizes the panic attack.
Social anxiety disorder (Social phobia)	Social situations in which the individual is examined are feared. Such situations can vary from eating or drinking in public to performing in front of others. Humiliation, negative evaluation, and rejection are expected.
Agoraphobia	Certain situations like using public transport, being in enclosed spaces, or a crowd lead to excessive fear. The individual avoids mentioned situations, as he fears that help is not accessible.
Specific phobia	Individuals are anxious and fearful about a specific stimulus or situation. An almost immediate anxious reaction follows the stimulus, accompanied by avoidance of the stimulus. The phobia-inducing stimulus can be an animal, a natural environment, a doctor consultation, or other situations.
Separation anxiety disorder	Excessive fear of losing attached figures or homes, with massive distress and worries when separated from these reference points.
Selective mutism	Failure to speak in specific situations, e.g., school, wherein other conditions, no problems with speech occur. The inability to talk in special situations leads to a disadvantage in the academic and social sense.
Substance/medication-induced anxiety disorder	Symptoms of anxiety disorder occur due to substance intoxication or another medical condition.

Note. Definition and diagnosis of anxiety disorders adapted from the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American-Psychiatric-Association, 2013).

1.2.2 Epidemiology

According to the World Mental Health Survey (Kessler et al., 2005), and in line with other community surveys (Wittchen & Jacobi, 2005; Alonso et al., 2007), almost one in four people have or previously suffered from an anxiety disorder in the past. The estimated lifetime prevalence of anxiety disorders varies between 10% and 15%. Most anxiety disorders start early in life, with an incidence increase during childhood and adolescence, followed by a decrease in older age groups. Jacobi et al. (2014) show that the prevalence of SAD, GAD, and specific phobia peak in the 18-34 year group, whereas the prevalence for panic disorder was highest in the 35-49 year group. The episode of anxiety disorders appears to be persistent throughout its lifetime, yet the duration of the disease is around an average of 11 years (Kessler et al., 2005).

1.2.3 Pathophysiology and risk factors

The pathogenesis of anxiety disorders is a complex combination of genetic, environmental, and metabolic factors. Due to the variety and heterogeneity of anxiety disorders, the complete mechanism is not comprehended. So far, researchers have agreed on some general aspects of the development of anxiety disorders. Genetics and environment are essential for the emergence of anxiety disorders. Risk factors for developing anxiety disorders are family history and a female gender (Bangasser & Cuarenta, 2021; Craske et al., 2017). Additionally, the genetic risk for anxiety disorders is approximately 35-50 % (Shimada-Sugimoto et al., 2015). Several candidate genes for anxiety-related traits are identified (Gottschalk & Domschke, 2017). Further, a strong interaction of genes and environmental factors is suggested (Bartlett et al., 2017). An example is the interaction of separation life events with variations in the 5-HTTLPR serotonin transporter gene, connected with an increased risk for panic disorder (Choe et al., 2013). Family studies show that several life events, such as sexual violence, abuse, or traumatic injuries, increase the risk for anxiety disorders (Ströhle et al., 2018). Particularly early-life stress is associated with the development of anxiety disorder (Hunter & McEwen, 2013). Concurrent with these findings, partnership and higher socioeconomic status reduce the prevalence of anxiety

disorders (Jacobi et al., 2014).

1.2.3.1 Brain regions

Brain regions that play a role in the development of anxiety disorders include the amygdala, hippocampus, prefrontal cortex, anterior cingulate cortex, insular cortex, and stria terminalis of the brain stem (Craske et al., 2017). Brain network dysfunction is associated with developing anxiety disorders (Brühl et al., 2014; Lai, 2020). The amygdala is the center point of the neuronal circuitry in response to an immediate threat (LeDoux & Pine, 2016). In fear-conditioned animals, lesions in the amygdala obliterate anxiety-related behavior (Blanchard & Blanchard, 1972). Healthy humans respond with the activation of the amygdala to the presentation of a threat (Dolan & Vuilleumier, 2003; Phelps, 2005). Furthermore, Phelps (2005) and Adolphs (2008) show that people with a damaged amygdala do not respond adequately to a threat.

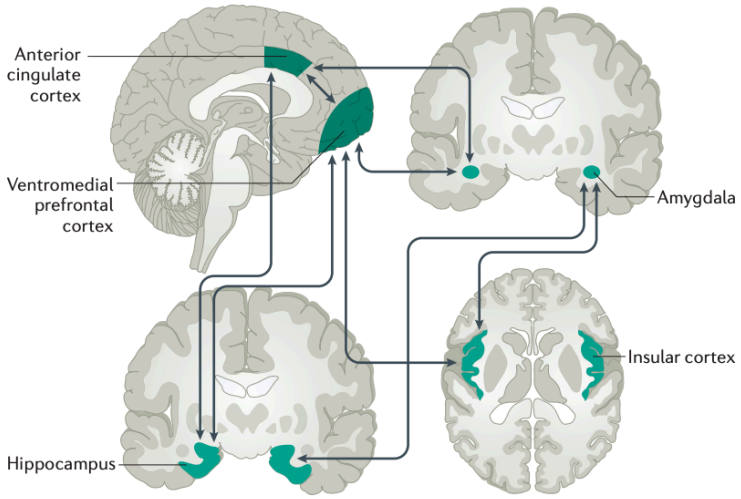

Figure 1.2.1: Amygdala-driven anxiety-circuit: the amygdala is bidirectionally connected and controlled with the anterior cingulate cortex and the ventromedial cortex. In addition, functional interaction between these regions and the hippocampus is shown (Craske et al., 2017; Milad & Quirk, 2012).

The prefrontal cortex, ventrolateral prefrontal cortex, and the anterior cingulate cortex, however, have an inhibitory effect on the development of anxiety (Bandelow et al., 2017; Pillay et al., 2006). The idea of an amygdala-driven anxiety circuit is consistent with findings in rodent and human studies. Differences in the activity and coordination

of the amygdala, prefrontal cortex, and hippocampus are associated with the ability to suppress anxiety and fear (Milad & Quirk, 2012; Robinson et al., 2014). In line with these findings, Cremers et al. (2015) found correlations between cortical-amygdala connectivity and symptoms in SAD. An early-life model of anxiety in primates highlights the importance of the functional connectivity between the frontal regions and the amygdala. Reduced connectivity between these two brain areas is associated with elevated anxiety (Birn et al., 2014). However, distinguishing different anxiety disorders according to brain regions in neuroimaging techniques is not possible yet (Craske et al., 2017).

1.2.3.2 Neurotransmitters

Furthermore, neurotransmitters have long been the focus of the development of anxiety disorders (Bandelow et al., 2017). Various neurotransmitters are modulators of different emotional processes. Reward, for instance, is driven by endogenous dopamine and opioids (Le Merrer et al., 2009), whereas punishment is modulated via serotonin (Jean-Richard-Dit-Bressel et al., 2018). Serotonin, mainly released from the raphe nuclei in the brainstem, has a dual role in aversive behaviors. On the one hand, it enhances the anxiety response of the amygdala (Deakin & Graeff, 1991; Deakin, 2013) and at the same time, it inhibits the fight-or-flight response within the brainstem (Mobbs et al., 2007). Deakin and Graeff proposed in their serotonin hypotheses that serotonergic neurons modulate the response to chronic and acute stressors. Moreover, a disruption of these neurons can enhance vulnerability to anxiety disorder (Paul et al., 2014). In line with this finding Corchs et al. (2015) reported a negative effect of decreased serotonin depletion on the symptoms of SAD and PTSD. The role of serotonin and its receptors in anxiety is studied widely and reflected in the successful serotonergic treatment (Baldwin & Polkinghorn, 2005; Durant et al., 2010). Another neurotransmitter system involved in anxiety is the Gamma-aminobutyric acid (GABA) system. GABA is the main inhibitory neurotransmitter of the brain. It binds either the rapid acting ion-gated or ionotropic receptors ($GABA_A$ and $GABA_C$) or the slower acting G-coupled metabotropic receptor ($GABA_B$). The most prominent GABA receptor in the brain is the $GABA_A$ receptor, a chloride

channel (Bormann, 2000). The influx of chloride ions stabilizes the membrane potential, exerting an inhibitory effect. Early behavioral studies in animals with GABAergic drugs promote the role of GABA in anxiety disorders (Durant et al., 2010; Nutt, 1990). A disbalance in the GABAergic system can lead to a decreased inhibitory action of neuronal circuits and the manifestation of anxiety disorders (Luscher et al., 2011). Some experimental research in animals provides evidence for this inhibitory purpose of GABA on the neuronal circuits. Barbalho et al. (2009) and Sanders & Shekhar (1995) showed that infusions of GABA or GABA receptor agonists into the amygdala have anxiolytic effects in rodents, whereas infusions of GABA antagonists increases anxious behavior. For the role of GABAa in treatment options see below.

Neuroendocrine systems, precisely the hypothalamic-pituitary-adrenal (HPA) axis, are also crucial for developing anxiety disorders. In terms of an efficient response to a stressor, the body must immediately unravel resources. Partly, the activation of the HPA axis obtains this balance. The HPA axis is a negative feedback loop. It begins with the release of corticotropin-releasing factor (CRF) and arginine-vasopressin in the paraventricular nucleus (PVN) of the hypothalamus. Thereby the production of proopiomelanocortin (POMC) in the pituitary is stimulated. After the conversion of POMC into adrenocorticotropic hormone (ACTH), it is released into the blood. ACTH promotes the production of corticosteroids in the cortex of the adrenal gland. After release into the bloodstreams the corticosteroids (CORT) bind to mineralocorticoid and glucocorticoid receptors. They can further inhibit CRF and ACTH production as a negative feedback mechanism. ACTH further stimulates the production of epinephrine and norepinephrine. Together with CORT, these catecholamines control the stress response of the autonomic nervous system, such as the reduction of digestion and immune function and the increase of heart rate and blood pressure (Bartlett et al., 2017). Further, cortisol is involved in metabolism and plays a vital role in maintaining homeostasis in stress. The dysregulation of the HPA axis, caused by chronic stress, can lead to the development of anxiety and other mental disorders (Kinlein et al., 2019). This dysregulation is characterized by enhanced activity of the HPA axis, hypercortisolemia, and diminished inhibitory feedback

(Juruena et al., 2017; Saridjan et al., 2010).

Neuropeptides, like the nonapeptide oxytocin (OXT), are also connected with anxiety disorders. OXT is synthesized in magnocellular neurons within the supraoptic (SON), paraventricular (PVN), and accessory nuclei of the hypothalamus. It is secreted into the bloodstream, facilitating uterus contractions and the milk ejection reflex during labor. However, OXT is also released within the brain, where it can modulate social behavior and emotionality (Landgraf & Neumann, 2004; Rhodes et al., 1981). OXT increases not only maternal aggression (Bosch & Neumann, 2012) but also acts anxiolytic (Neumann et al., 2000). This effect was especially mediated within the amygdala (Bale et al., 2001). Anxiolytic effects are apparent after acute administration of synthetic and endogenous OXT (Blume et al., 2008). This anxiolytic effect is mainly present in stressful situations or increased release during lactation when the OXT system is physiologically active (Neumann et al., 2000). Only a few studies investigated the chronic effect of OXT treatment in humans. However, a beneficial effect of intranasal OXT administration exists in anticipatory anxiety (de Oliveira et al., 2012) and the promotion of trust (Kosfeld et al., 2005; Zak et al., 2005). Additionally, evidence suggests an imbalance of the endogenous OXT system in animal models and humans (Neumann & Slattery, 2016).

1.2.3.3 Microbiome

Moreover, the microbiome, as the composition of all bacteria and microorganisms found in and on the body, received greater attention in the context of the pathophysiology of mental illnesses in the last years (Frankiensztajn et al., 2020). The influence of a disrupted microbiome as a potential link to mental illnesses is described (Clapp et al., 2017). For example, an altered microbiome was found in patients with major depressive disorder (Bastiaanssen et al., 2020; Foster et al., 2021). Preclinical studies revealed a connection between microbiome and anxiety (Clarke et al., 2013; Bastiaanssen et al., 2020). For germ-free mice, for example, the absence of gut microbiome reduces anxiety-related behavior (Pan et al., 2019). Further, the microbiome regulates CORT levels, as Jašarević et al. (2018) found that the transfer of vaginal microbiome from stressed mothers to offspring

that were not stressed results in higher CORT levels in this group. In addition, prenatally stressed animals show a significantly different microbiome composition than their controls (Gur et al., 2019). This might affect the disruption of the HPA axis through modifications of central gene expression in the hippocampus and hypothalamus, as mentioned above (Frankiensztajn et al., 2020). However, precise information about the microbiome still needs to be provided (Clapp et al., 2017).

1.2.4 Animal model of rats selectively bred for extremes in anxiety-related behavior

Animal models are essential in terms of understanding the pathology of anxiety disorders (Bartlett et al., 2017). Creating animal models for psychiatric disorders presents considerable challenges due to the diverse range of psychological and physiological symptoms that are difficult to replicate in animals. Additionally, there is a notable absence of objective biomarkers and diagnostic tests for clinical situations (Nestler & Hyman, 2010). Essential criteria for adequate animal models are aetiological, face, predictive, and construct validity. Aetiological validity indicates that identical conditions cause the phenotype of the disorder. Face validity means the model's phenotype is similar to the imitated human syndrome regarding symptoms. Predictive validity indicates that known human pathological manipulation, such as exposure to stressful events, have equal consequences in humans and animals. Finally, construct validity ensures that both animal model and human diseases share similar pathological substrates, which explain the pathology in both models, thereby generating an understanding of general theories and enabling clinical investigations (Berton et al., 2012; Neumann et al., 2011). These criteria are difficult to meet regarding the complexity of symptoms in psychiatric diseases such as anxiety and depression. Therefore it has become clear that with animal models, functional dimensions can be assessed rather than the disease as such (Gururajan et al., 2019). In general, three strategies have been used to develop sufficient animal models. These are genetic models, such as gene knockouts (Scherma et al., 2019); animal models with environmental manipulations, such as maternal separation (Rincel & Darnaudéry, 2020) and animal models with selective breeding for extremes in a particular behavioral phenotype (Neumann et al.,

2011).

An important example of an animal models are Wistar rats bred for high (HAB) and low (LAB) anxiety-related behavior, and non-selected (NAB) for anxiety-related behavior. These models present a unique opportunity to study trait anxiety and depression in a translational aspect. The animal model was established in Leipzig and later transferred to Regensburg with identical selection criteria. Animals at the age of 9 weeks are tested in the elevated plusmaze (EPM), an unconditioned test for anxiety. The test creates a conflict between the animal's exploratory drive and fear of bright and open areas. Regarding their behavior in the trial, the animals are divided into two groups. Animals that explore the EPM's open and closed arms are grouped as LAB animals. Animals, which mainly stay in the closed arms, are grouped as HAB animals. Initially, animals with less than 5% (HAB) or more than 40% (LAB) of time spent on the light arm were cautiously paired without pairing close relatives. (Landgraf & Wigger, 2002).

The line-specific differences of anxiety-related behavior found in the EPM were consistent in both genders and several various experimental settings such as different time settings and countries (Carnevali et al., 2013; Salomé et al., 2002; Slattery & Neumann, 2010). Various other behavioral tests confirm line-specific differences in anxiety-related behavior, such as the light-dark box (LDB), the open field, and the hole board test. HAB animals spent less time in the light part of the LDB, indicating higher anxiety-related behavior (Ohl et al., 2001a; Landgraf & Wigger, 2002; Slattery & Neumann, 2010). Of utmost importance is that the line-specific alterations of emotional behavior were stable throughout their lifetime. These started, for example, with altered ultrasound reactions in the prenatal period and were also present throughout pregnancy and lactation (Bosch et al., 2006; Bosch, 2011; Wigger et al., 2001). HAB animals show other functional changes, such as alternation in respiration rates, similar to anxious patients (Carnevali et al., 2013). High anxiety levels in the HAB animals are accompanied by reduced locomotion, thus indicating passive stress-coping mechanisms (Landgraf & Wigger, 2002). Furthermore, the animals with high anxiety-related behavior are associated with increased passive stress-coping mechanisms in the FST, a test more specific for depressive-like behav-

ior (Slattery & Cryan, 2012). This co-appearance of anxiety-related and depressive-like behavior is especially important since both pathological conditions are often linked to each other (Keck et al., 2003; Slattery & Neumann, 2010).

Genetic alterations were determined between the two breeding lines. Single-nucleotide polymorphisms (SNP) in the region coding for arginine-vasopressin (AVP) are found in HAB and LAB animals (Murgatroyd et al., 2004). The alterations are associated with changes in emotional and social behavior. AVP is a neurohypophysial hormone, which is closely related to OXT and also involved in the regulation of emotional behavior. Changes in the promoter region of the AVP gene in HAB animals led to over-expression of AVP, which resulted in variations of the phenotype (Landgraf & Wigger, 2003; Murgatroyd et al., 2004). In line with these findings, various behavioral, neuroendocrine, and neuropharmacological studies highlighted the role of the AVP gene in trait anxiety (Engelmann et al., 2004; Landgraf & Neumann, 2004). Alterations in central OXT genes were not found to be equally involved in the expression of the anxiolytic HAB phenotype (Murgatroyd et al., 2004); however chronic icv infusions of OXT affected anxiety-related behavior (Slattery & Neumann, 2010).

Animals of the HAB-LAB model have a disrupted HPA axis. Under basal conditions, levels of CORT and ACTH do not differ between the two breeding lines. This also applies to the HPA response to mild stressors (Frank et al., 2006; Keck et al., 2002; Landgraf et al., 1999; Liebsch et al., 1998). However, HAB animals show more vigorous HPA axis response to stronger stressors than LAB animals, as more CORT and ACTH release was detected (Landgraf et al., 1999; Liebsch et al., 1998). In addition, HAB animals are non-suppressors of dexamethasone in the dexamethasone test, similar to effects seen in depressed patients (Landgraf & Wigger, 2002). Interestingly, the microbiome of HAB animals is remarkably different from NAB animals. In a recent study, modifying the microbiome with an antibiotic shows reduced depressive-like behavior (Schmidtner et al., 2019).

Several neuropharmacological treatments were shown to reduce anxiety- and depressive-like behaviors in HAB animals, providing predictive and face validity. For example,

administering the known anxiolytic benzodiazepine diazepam reduces anxiety-related behavior. In addition, administering the SSRI antidepressant agent paroxetine improves depressive-like behavior (Liebsch et al., 1998; Muigg et al., 2007).

1.2.5 Treatment

Despite effective treatment for mental disorders, substantial undertreatment is reported. This phenomenon is called the "treatment gap" (Bandelow et al., 2015; Kohn et al., 2004). A large European study estimates that only 21% of patients with anxiety disorder demanded professional help, and within this group, 23% did not receive treatment at all (Alonso et al., 2002). This is due to various reasons. For example, personal issues, like one person's displacement of the existence of the diseases or the belief that the problem will solve itself (Kessler et al., 2001). Further, it is essential to distinguish between different anxiety disorders, as the numbers of patients receiving health care differs within the diseases. This might be explained by other approaches that patients with various anxiety disorders have. Patients with panic disorders, for instance, repeatedly worry that they suffer a severe somatic disorder, such as a cardiac event or the development of tumors, therefore utilizing more medical health care. In contrast, patients with SAD avoid contact with physicians and believe their fear is normal. Generally speaking, lack of information and stigma remain popular issues (Schofield et al., 2020). Finally, financial aspects and the limited availability of correct treatment may explain this gap. Psychiatrists are still a rare human source; globally, there is less than one psychiatrist for 10,000 people. This effect is even more substantial in low- than in high income countries, with around 120 times more psychiatrists in in the latter (World-Health-Organization, 2018). Baldwin et al. (2012) highlight this issue, showing that a total of 45% of the patients with GAD show symptoms for around two years before they were diagnosed and treated.

Medications and psychological interventions, e.g., cognitive-behavioral therapy (CBT), are the two major treatment options for anxiety disorders. Both monotherapies are effective; however, medical therapy's beneficial effect is superior to psychological interventions. Still, a combination of both therapies is state of the art (Bandelow et al., 2015; Ströhle

et al., 2018). Since the current study focuses on pharmacological therapy, only pharmaceutical targets will be discussed in more detail.

Several medications are used for anxiety disorders. The most common class of drugs are antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs) and selective serotonin and noradrenaline inhibitors (SSNRI) (Bandelow et al., 2012). By interfering with serotonin and noradrenaline, these drugs target the disrupted neurotransmitter system in anxiety disorders. The drugs prevent the selective reuptake of both neurotransmitters from the synaptic cleft and provide an anxiety-relieving effect (Dell'Osso et al., 2010; Baldwin et al., 2010). SSRI and SSNRI are first-line treatment options for panic disorder, SAD, and GAD (Bandelow, 2014).

Further effective treatment of anxiety disorders are benzodiazepines. By allosteric activating specific $GABA_A$ receptor sites, benzodiazepines increase the brain's inhibitory neurotransmission, promoting the anxiolytic effect. The $GABA_A$ receptor has several subunits (α_1, α_2, α_3, ...). Different receptors regulate different activities with different subunits. The α_1 subunit, for instance, is involved in sedative-hypnotic activity, whereas mainly the α_2 and the α_3 subunit plays a key role as a target for anxiolytics (Löw et al., 2000; Möhler, 2006). Most benzodiazepines bind to all subunits, causing them to produce many effects (Balon & Starcevic, 2020). Benzodiazepines are effective with a rapid onset of action in treating GAD, SAD, and panic disorder but have limited impact in other anxiety disorders (Baldwin et al., 2010). Since 1960, benzodiazepines have become a widely prescribed class, Olfson et al. (2015) show that in 2008 5,4% of American aged 18 to 80 years used benzodiazepines. However, benzodiazepines show severe side effects like central nervous system (CNS) depression, impairment of cognitive functions, and drug tolerance (Balon & Starcevic, 2020). Prolonged use of the drug, approximately 4-6 months, is associated with drug dependence (Shader & Greenblatt, 2010). Due to the troublesome side effects, benzodiazepines should only be prescribed after careful evaluation (Bandelow, 2014; Garakani et al., 2020). Benzodiazepines do not treat depression, a common comorbidity of anxiety disorders, in comparison to SSRIs and SSNRIs (Bandelow, 2020).

1.3 Depression

1.3.1 Definition

According to the WHO, one person dies due to suicide every 40 seconds, so suicide is among the top twenty most frequent causes of death worldwide. Most of these suicide numbers are assigned to depression (World-Health-Organization, 2019b). Depressive disorders are characterized by sad, irritable, or empty moods for at least two weeks. In addition, the patient's ability to function is affected as somatic and cognitive changes occur. Sleep disturbances, poor concentration, and reduced appetite are common symptoms (Otte et al., 2016). The term depressive disorder includes several disorders such as disruptive mood disorder, mood dysregulation disorder, major depressive disorder, persistent depressive disorder, premenstrual dysphoric disorder, substance-induced depressive disorder, depressive disorder due to another medical condition or another specific or unspecific depressive disorder (American-Psychiatric-Association, 2013). Major depression is highly comorbid with anxiety disorders (Andrade et al., 2003).

1.3.2 Epidemiology

According to the WHO, over 300 million people worldwide suffer from depression. This number equals 4% of the world's population (World-Health-Organization, 2017). In Germany, the lifetime prevalence of a diagnosed depression was 11,6% and is highest among persons between 60 and 69 years (Busch et al., 2013). Several studies found an association between gender, marital status, and depression. Depression is about two times more prevalent among women than men (Seedat et al., 2009; Van de Velde et al., 2010). The course of the disease is variable, with differences in chronicity and remission. A population survey in the Netherlands estimated that the mean duration of the disease is around six weeks (ten Have et al., 2017). However, compared to depressive disorders, anxiety disorders still seem to have a longer course of disease (Penninx et al., 2011). Childhood trauma, higher severity of symptoms, and comorbidity are associated with a less favorable course of illness (Hovens et al., 2012; Penninx et al., 2011). After the remission

of depression, functional impairment and residual symptoms often remain (Ormel et al., 2004). Despite the relatively short mean course of the disease, it is assumed that 90% of patients affected with a depressive disorder experience recurrence once in their life (Vos et al., 2004).

1.3.3 Etiology and risk factors

The pathophysiology of depressive disorders is complex and, until now, not completely understood (Otte et al., 2016; Malhi & Mann, 2018). Yet, neurotransmitters have long been considered a key role in understanding the development of depressive disorders. Especially the depletion of monoamines (serotonin, noradrenaline, and dopamine) seems to reflect a common pathophysiology (Deakin & Graeff, 1991; Malhi & Mann, 2018). Similar to the development of anxiety disorders, patients with MDD present disrupted neurotransmitter systems (Luscher et al., 2011; Dell'Osso et al., 2010). This hypothesis is further endured as modulation of the neurotransmitter system leads to sufficient antidepressant treatment (Hill et al., 2012; Kraus et al., 2017).

Genetics plays an important role in the development of the disease. First-degree relatives have a strongly increased risk of depression with a heritability of the disorder of around 35% (Geschwind & Flint, 2015). However, no significant genetic associations are found for depressive disorder (Bosker et al., 2011). This might be explained as depression is a polygenic disorder with a wide range of phenotypes (Hyman, 2014). Several genome-wide studies try to find correlating genetic loci, hoping that there will be further discoveries in this area in the future (Bosker et al., 2011). In genetically manipulated animal models, similar behaviors and core symptoms of depressed humans are observed. Animals show increased immobility in the forced swim test, an index for depressive-like behavior, reduced active social interaction and altered maternal behavior and altered maternal behavior (Overstreet et al., 1992; Overstreet & Griebel, 2004; Friedman et al., 2006). Antidepressant treatment could partially reverse this depression-like phenotype (Overstreet & Griebel, 2004).

Further, several environmental factors are associated with the appearance of depres-

sive disorders. Separated or divorced people have higher rates of depression than married people (Weissman et al., 1996; Andrade et al., 2003). Higher socioeconomic status is associated with a lower prevalence of depression (Busch et al., 2013). The age of onset seems to peak in older adulthood between 55-74 years. Depression also occurs in childhood, but with a lower prevalence than adulthood (World-Health-Organization, 2017; Busch et al., 2013). The events can either be temporally linked to the onset of the disease or early life-threatening events. Loss of employment, financial instability, or violence exposure are temporally related events. Physical neglect, abuse, and exposure to domestic violence are often early lifetime events (Juruena et al., 2009). With several different environmental manipulations, such as early life stress, learned helplessness, and chronic unpredictable stress, environmental animal models of anxiety and depression are created (Ménard et al., 2016).

Early life stress events and, thus, a disrupted HPA axis can trigger depressive disorders (Juruena et al., 2017). Patients with depressive disorders display higher cortisol levels (Goodyer et al., 2000). Environmental animal models show this correlation between stress and depressive-like behavior (Willner, 2016). Further, morphological and neurochemical changes, such as reduced hippocampal volume, were similar to observations in depressed patients (Bhutani et al., 2009; Hill et al., 2012; Papp et al., 2002). Individuals who have experienced traumatic events in childhood, such as violence or abuse, show increased activity of the HPA axis in adulthood when exposed to standardized stressors (Boschloo et al., 2014).

1.3.4 Treatment

Treatment options for depressive disorders include psychological interventions and pharmacological treatments. Pharmacological treatment options have many targets, such as the disrupted neurotransmitter systems (Malhi & Mann, 2018). The antidepressants act on synaptic receptors and enhance the concentration of neurotransmitters, thereby enhancing signal transduction (Willner et al., 2013). Despite the role of the HPA axis in depressive disorders, promising treatment options regarding the HPA axis were not found

yet (Stetler & Miller, 2011). As mentioned in 1.2.5, an enormous treatment gap for mental disorders exist (Kohn et al., 2004). Therefore further investigations and studies for depressive disorders are of utmost importance.

1.4 Translocator Protein

1.4.1 Structure and Function

The Translocator Protein (TSPO), with a molecular mass of 18 kDa, is a transmembrane protein located in the outer mitochondrial membrane (Rupprecht et al., 2010). Discovered in 1977, TSPO, previously known as the peripheral-type benzodiazepine receptor, has been a subject of scientific exploration (Braestrup & Squires, 1977). Throughout evolution, the sequence of TSPO is very well conserved, and knock out of TSPO leads to a lethal phenotype in mice (Lacapère & Papadopoulos, 2003; Papadopoulos et al., 1997).

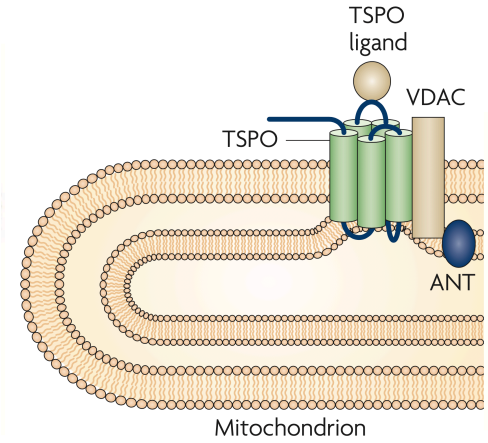

Figure 1.4.1: Structure of TSPO with the associated VDAC and ANT (Rupprecht et al., 2010).

Although TSPO is found in almost all body cells, the expression of the protein is particularly high in tissues that contain steroid-synthesising-cells like adrenal, gonad, and brain cells (Papadopoulos et al., 2006). The protein is arranged as a five-membrane helix structure, consisting of a 169 amino acid sequence, and exists as a monomer, dimer, or polymer (Liauzun et al., 1998). The functional TSPO, however, is a dimer (Caballero et al., 2013). TSPO forms complexes with proteins associated with the inner and outer mitochondrial membrane. TSPO interacts directly with the voltage-dependent anion channel

(VDAC) and several other mitochondria proteins, forming a super-complex. This super-complex impacts several biochemical processes, especially related to the mitochondrion (Papadopoulos et al., 2006). VDAC is associated with the adenine nucleotide translocator (ANT) and the steroidogenic acute regulatory protein (STAR), forming the mitochondrial permeability transition pore (MPTP), a major part of the outer membrane, functioning as a channel for ions, metabolites, and ADP/ATP and playing an important role in cell-apoptosis (Shoshan-Barmatz et al., 2006).

TSPO is a multifunctional protein. As part of the outer membrane of the mitochondria, TSPO mediates several mitochondrial functions, mainly cholesterol transport and steroid hormone synthesis. Further, TSPO is essential for mitochondrial respiration (Hirsch et al., 1989) and permeability, generation of reactive oxygen species, as well as cell proliferation (Corsi et al., 2008) and cell death (Veenman et al., 2007). However, the complete structure, function, and role in cellular processes of TSPO needs to be further investigated.

1.4.2 TSPO and neurosteroids

TSPO is rate-limiting in neurosteroidogenesis as it transports cholesterol into the mitochondria. Neurosteroids are endogenous steroids produced in either the CNS or peripheral sources (Robel & Baulieu, 1994; Rupprecht & Holsboer, 1999). The steroid synthesis begins with the transfer of cholesterol into the mitochondria. As cholesterol is a high lipid compound, it can not freely diffuse into the mitochondria. Enzymes then metabolize cholesterol in the mitochondria and endoplasmic reticulum of steroid-forming cells. The observation that TSPO ligands notably elevate the rate of steroid synthesis serves as evidence for the rate-limiting role of TSPO (Papadopoulos et al., 2018).

The effects of steroids on CNS neurotransmitter functions involve both genomic and nongenomic actions (McEwen, 1991). Steroid hormones can regulate the transcription of DNA. They bind associated intracellular receptors and regulate gene expression, acting as transcription factors (Evans, 1988). Further, neurosteroids are assumed to indirectly work as allosteric modulators at the cellular receptors in the brain and as modulators of the release of neurotransmitters such as GABA, glutamate, and acetylcholine (Zheng,

2009).

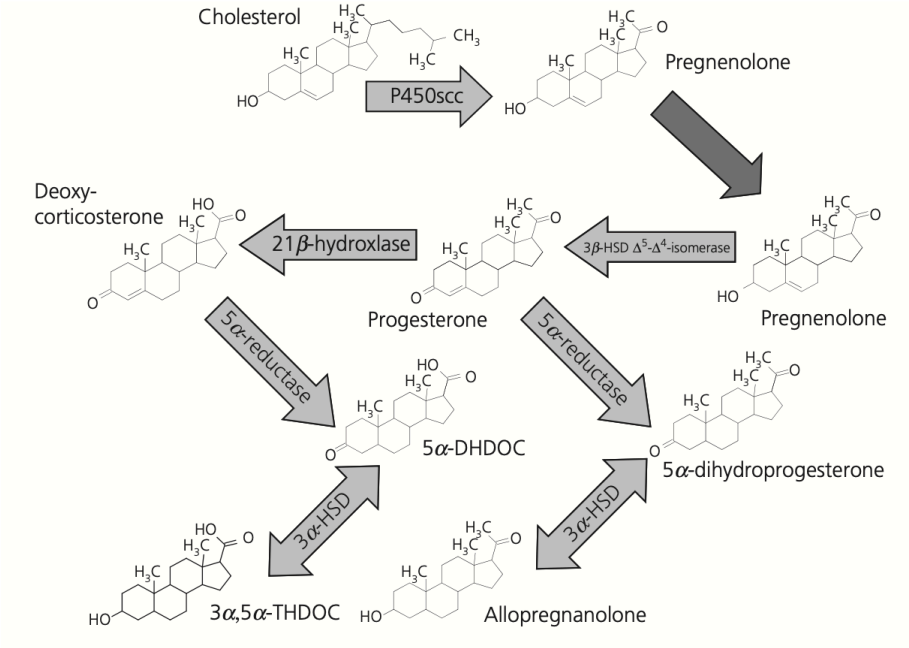

Figure 1.4.2: Neurosteroid synthesis (Rupprecht & Holsboer, 1999; Nothdurfter et al., 2012).

Besides their action on gene expression, neurosteroids can alter neuronal excitability through interaction with specific neurotransmitter receptors such as $GABA_A$, N-methyl-D-aspartate (NMDA), and glutamate receptors (Eser et al., 2008; Rupprecht, 2003; Rupprecht & Holsboer, 1999). As previously mentioned, $GABA_A$ receptors are chloride channels, mediating inhibitory neurotransmission (Olsen & Sieghart, 2009). Among these steroids, allopregnanolone, allotetrahydrodeoxy corticosterone, and pregnanolone are positive allosteric modulators of the $GABA_A$ receptors, potentiating their effects (Bitran et al., 1991; Brot et al., 1997). Whereas 3-α-5-β-tetrahydro-progesterone is a negative modulator of $GABA_A$ (Eser et al., 2008; Robel & Baulieu, 1994). The frequency, as well as the duration of opening of the $GABA_A$, gated Cl- channel, is modified by neurosteroids in low nanomolar concentrations, resulting in membrane hyperpolarization and reduced neuronal excitability (Lambert et al., 1995; Paul & Purdy, 1992). Neither the amplitude nor the rise time of the postsynaptic current is changed (Lambert et al., 2003; Puia et al., 1990). The interaction between neurosteroids and $GABA_A$ receptors is highly selective and different between neurons and brain regions (Belelli & Lambert, 2005).

Neurosteroid levels alter in different physiological and pathophysiological conditions,

such as an increase in acute stress (Purdy et al., 1991) and pregnancy (Verbe et al., 2019), and a decrease in aging (Schumacher et al., 2003). Some of the behavioral effects of neurosteroids are shortening sleep time (Majewska, 1992) and reducing aggressive behavior (Haug et al., 1988).

In fact, neurosteroids play an important role in anxiety disorders, as suggested to be important modulators of anxiety and depression (Romeo et al., 1998; Ströhle et al., 2003). For example, patients with panic disorder show decreased neurosteroids during panic attacks (Ströhle et al., 2003). The baseline concentrations of neurosteroids in patients with depression are decreased, whereas those in patients with panic disorder were increased (Ströhle et al., 2002). The disequilibrium of neurosteroids in depressed patients is balanced with successful antidepressant therapy (Romeo et al., 1998). In female patients with panic disorder, elevated plasma concentrations of allopregnanolone are found in all phases of the menstrual cycle (Brambilla et al., 2003).

The neurosteroids allopregnanolone (ALLO), allotetrahydrodeoxycorticosterone (THDOC), and, to a minor extent, pregnenolone (PREG) act anxiolytic by increasing the chloride current at the $GABA_A$ receptor (Bitran et al., 1991; Patchev et al., 1994). The benzodiazepine antagonist Flumazenil does not block the anxiolytic effect of allopregnanolone, suggesting that neurosteroids do not directly bind the benzodiazepine site (Brot et al., 1997). However, in patients with GAD, SAD, or mixed anxiety-depressive disorders, no alterations of allopregnanolone levels are revealed (Bicíková et al., 2000; Heydari & Le Mellédo, 2002; Semeniuk et al., 2001). Nevertheless, Khisti et al. (2000) show a reduced immobility time in the FST due to administration of allopregnanolone, indicating reduced depressive-like behavior.

1.4.3 TSPO in psychiatric disorders

Several pathophysiological conditions are associated with alternations and functional changes in TSPO. Interestingly, anxiety disorder and depression are associated with reduced TSPO levels (Nudmamud et al., 2000). Abnormal concentrations are linked to cancer, neurodegenerative and inflammatory diseases, such as Parkinson's and Alzheimer's,

primary hypogonadism, and neuropsychiatric disorders (Rupprecht et al., 2010).

TSPO deficiency is associated with the regulation of anxiety and depression related behavior, as shown in TSPO knockout experiments (Barron et al., 2021). In order to gain a deeper understanding of TSPO's role in psychiatric disorders, mRNA expression and TSPO ligand binding capacities were studied. Significant reduction of TSPO mRNA in platelets is shown in anxious patients (Nudmamud et al., 2000). Reduced TSPO mRNA expression in general anxiety disorder (Rocca et al., 1998), lower TSPO density in patients with panic disorder and adult separation disorder (Pini et al., 2005), as well as SAD (Johnson et al., 1998) are discovered. Additionally, comorbidity of depression or bipolar disorder, such as suicidality (Soreni et al., 1999) and adult separation anxiety disorder (Chelli et al., 2008; Abelli et al., 2010) are associated with reduced TSPO expression. However, due to large fluctuations of the TSPO mRNA, its measurement does not seem to be suitable for the diagnosis of anxiety disorders (Nudmamud et al., 2000). A high frequency of the single nucleotide polymorphism A147Th is found in patients with depression and anxiety disorder. The polymorphism is associated with the previously mentioned increased anxiety behavior (Costa et al., 2009b), reduced TSPO drug ligand binding (Owen et al., 2012), and reduced pregnenolone production (Costa et al., 2009a).

1.4.4 TSPO ligands

Besides endogenous TSPO ligands such as cholesterol and porphyrins (Verma et al., 1987; Li et al., 2001), several synthetic TSPO ligands are described. Some of the synthetic ligands are shown to be anxiolytic in rodents (Serra et al., 1999; Kita & Furukawa, 2008; Okuyama et al., 1999; Costa et al., 2011). Supposedly, this anxiolytic effect is due to increased production of neurosteroids, then acting as positive modulators of the GABA receptor (Rupprecht, 2003). Administration of TSPO ligands increases the levels of neurosteroids independent of peripheral sources. The amygdala mediates the observed anxiolytic effect. (Serra et al., 1999; Verleye et al., 2005; Romeo et al., 1993). Akwa et al. (1999) and Engin & Treit (2007) record a correlation of reduced anxiety-related behavior in rodents after a direct infusion of allopregnanolone into the amygdala.

An example of a synthetic TSPO ligand is etifoxine hydrochloride (6-chloro-2-ethylamino-4-methyl-4-phenyl-4H-3, 1-benzoxazine hydrochloride / etifoxine). Etifoxine is a non-benzodiazepine anxiolytic and anticonvulsant drug in the benzodiazepine class (Sigma-Aldrich, 2021). Further, etifoxine acts neuroprotective (Nuss et al., 2019; Shehadeh et al., 2019), modulates inflammatory response (Girard et al., 2012), and helps to reduce neuropathic pain (Aouad et al., 2009). Etifoxine has two different ways of action. One of them is a direct positive modulation of the $GABA_A$ receptor at a separate site than the benzodiazepines, where etifoxine binds the β_2 and β_3 subunits of the $GABA_A$ receptor (Hamon et al., 2003). The other mechanism is an indirect effect of etifoxine, involving the stimulation of TSPO and presumably including neurosteroid synthesis. (Schlichter et al., 2000).

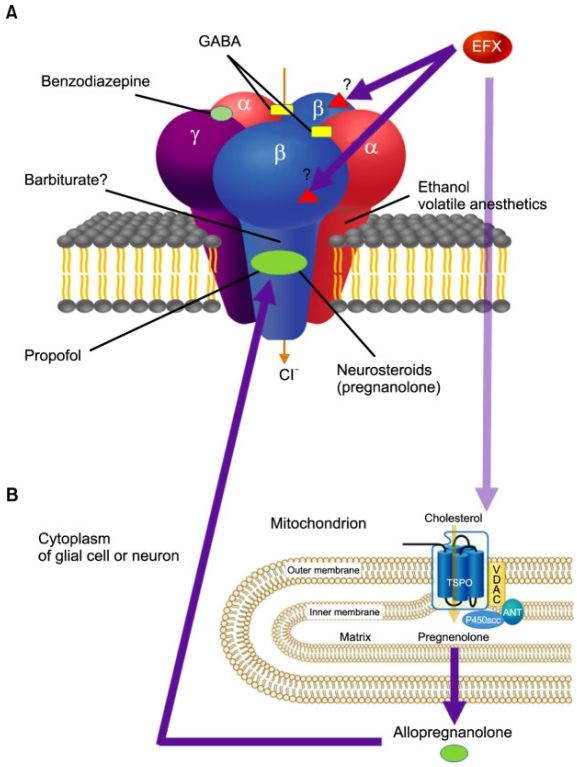

Figure 1.4.3: (A) Hypothetical model of the $GABA_A$ receptor with five subunits arranged around a central chloride-selective pore. Various compounds can allosterically modulate the receptors at different binding sites. Etifoxine (EFX) can directly bind the $GABA_A$ receptor, presumably via β_2 and β_3 subunits, mediating anxiolytic action. (B) Through binding the Translocator Protein (TSPO), etifoxine enhances neurosteroid synthesis. These neurosteroids (allopregnanolone in the figure) bind the $GABA_A$ receptor. Adapted from Choi & Kim (2015); Rupprecht et al. (2010).

The TSPO ligands are positive modulators of the previously described neurosteroid synthesis in the brain. The neurosteroids enhance the function of the $GABA_A$ receptor,

thus reducing anxious behavior (Verleye et al., 2005). do Rego et al. (2015) demonstrate that etifoxine increases the neurosteroid synthesis using a frog hypothalamus model. Neither $GABA_A$-antagonist nor TSPO antagonist PK11195 reverse this enhancing effect for the neurosteroid synthesis. Inhibition of the 5-α-reductase, an enzyme of the neurosteroid synthesis, does not entirely reduce the anxiolytic effect of etifoxine, supporting the hypothesis of etifoxine's direct mechanism of action on the $GABA_A$ receptor (Schlichter et al., 2000). A double-blind control study with etifoxine and buspirone (a serotonergic and noradrenergic anxiolytic) in patients with adjustment disorder with anxiety (ADWA) reported a significantly higher treatment effect for the patients treated with etifoxine (Servant et al., 1998). The same improvement in favor of etifoxine is within patients with ADWA treated either with etifoxine or the benzodiazepines lorazepam or alprazolam (Nguyen et al., 2006; Stein, 2015). Etifoxine was originally released in France and has since been prescribed as an anxiolytic in 40 different countries. Even though etifoxine shows fewer side effects such as sedation and anterograde amnesia, as well as less rebound of anxiety symptoms after stopping the treatment in comparison with benzodiazepines, it has not yet been able to assert itself against benzodiazepines (Micallef et al., 2001; Nguyen et al., 2006). Even in older adults, etifoxine is associated with fewer side effects (Deplanque et al., 2018). However, etifoxine comes with side effects such as initial drowsiness, and the treatment with etifoxine correlates with acute liver damage in some cases, yet with unknown overall incidence (Moch et al., 2012).

1.5 Aims and Objectives

Mood disorders are highly prevalent and a tremendous burden in our society. There is still massive uncertainty about psychopathology and, therefore, the correct treatment of these disorders. The present study aims to create a new understanding and more in-depth insights into TSPO and its function in anxiety disorders and depression. Therefore, we studied the effect of the TSPO ligand etifoxine on various levels, from emotionality and stress reactivity to the brain OXT system, using the rodent model for psychopathologies, namely HAB and LAB rats. Furthermore, to include potenial differences between the sexes, all expermeints were performed in males and females and NAB rats served as controls. This study will provide valuable insights into the function and effect of TSPO in mood disorders, thereby adding more knowledge to the development of new therapeutic agents.

Chapter 2

Material and Methods

2.1 Animals and housing conditions

All rats used in the experiments were either bred in the animal facility of the University of Regensburg (HAB and LAB) or purchased from Charles River Laboratories, Sulzfeld (NAB rats). Animals were housed in groups of two to four per cage, maintained on a 12h light-dark cycle with light between 7 a.m. and 7 p.m. The temperature was controlled to 22–24 C with 55% humidity. The bedding was changed once a week. Water and food (standard rat chow) were given to the animals *ad libitum*. The weight of the animals at the time of testing was between 200g and 400g, depending on age and gender. Animals were tested at the age of 12-15 weeks. Animals were handled daily until the first day of the injection to decrease non-specific stress during the testing. The experiments were performed in the morning between 9 a.m. and 12 p.m.

All procedures were approved by the Committee on Animal Health and Care of the local government of the Oberpfalz. The experiments were performed according to the Care and Use of Laboratory Animals guidelines by the National Institute of Health. All efforts were made to reduce, refine, and replace animals used in animal testing.

2.2 Behavioral tests

All animals were brought into the experimental room for habituation one day before testing.

2.2.1 Light-Dark-Box

Anxiety-related behavior was tested in the Light-Dark-Box (LDB). This test was developed as a paradigm to determine anxiety-related behavior in rodents. The test creates a conflict between the rodent's natural aversion to bright-lit areas and its exploratory behavior (Crawley & Goodwin, 1980). We used an adopted experimental design for rats, as previously described (Chaouloff et al., 1997; Henniger et al., 2000). The LDB is a two-chambered box; the lit compartment was 40cm x 50cm (100 Lux), and the dark chamber was 40cm x 30cm (0 Lux). Before each trial, the LDB was cleaned with water and detergent and dried with a paper towel. Each rat was placed in the lit area, and the behavior was recorded with an overhead camera. During the 5-minute test sessions, the animals could freely move between the two compartments. The time spent in the lit compartment, as an indicator for reduced anxiety-related behavior, and the distance traveled, as an indicator of locomotion, were assessed. The threshold for the evaluation with NOLDUS (EthoVision XT, version 12, Netherlands) was at 5%.

2.2.2 Forced-Swim-Test

A paradigm for the assessment of depressive-like behavior is the Forced Swim Test (FST). The test is based on the observation that rats exposed to water initially show intense escape behavior, such as struggling and swimming. Struggling is defined as the behavior when all paws are above the water's surface; swimming is an active movement in the water. Later, the animals stop the active behavior and show passive, immobile behavior. This behavior seems to reflect either a failure to maintain escape-directed behavior after stress or the inability of the animal to perform active stress-coping behavior (Cryan et al., 2002; Slattery & Cryan, 2012). Several antidepressants increase active behavior in the FST (Cryan et al., 2005). Animals were placed individually in a cylinder tank (21 x 46cm) with water (22-24 °C) to a depth of 30cm. The water in the tank was changed after each rat. For 10 minutes, a camera placed in front of the cylinder recorded the behavior of the animals, which was further analysed with JWatcher (Version 1.0).

2.3 Etifoxine Suspension

For the etifoxine suspension, a stock solution of 100mg/ml etifoxine in 0.9% NaCl(B. Braun, Melsungen, Germany) and 10% Tween 80 (Sigma Aldrich, Darmstadt) was prepared and then stored at -20 °C degrees. Etifoxine powder was mixed with 900μl NaCl and 100μl Tween. One stock (100μl) was filled with 900μl NaCl to create a concentration of 1% Tween concentration. The suspension was freshly made every day from the stock. The suspension was gently mixed with the pipette until a homogeneous solution was obtained. The filled syringes were permanently kept in motion (Liere et al., 2017; Shehadeh et al., 2019; Verleye et al., 2005).

2.4 Experimental design

Female and male animals (HAB - LAB - NAB) were included in the experiment (for details, see 1.2.4). The animals were assigned to either a treatment or a control group, resulting in a group size of 8-10 animals. The rats received one intraperitoneal (ip) injection daily at 9 a.m. over a total of nine days containing either etifoxine at a dose of 50 mg/kg/5ml dissolved in Saline with 1% Tween 80 (Sigma Aldrich) or the same amount of only 1% Tween 80 in vehicle.

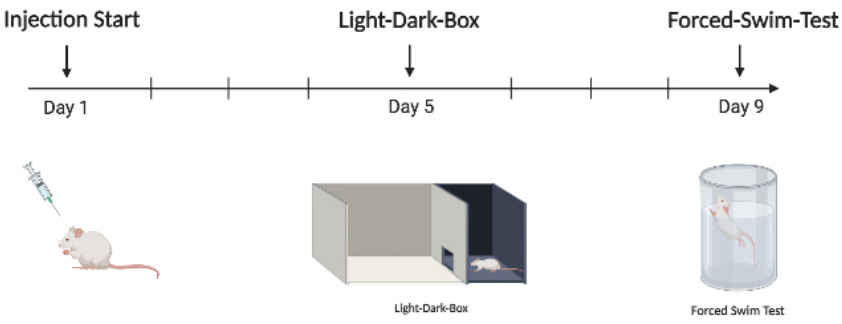

Figure 2.4.1: Animals receive treatment for nine days. behavioral testings after the 5th and after the ninth injection day (Created with BioRender).

2.4.1 Experiment 1: Effect of TSPO ligand etifoxine on innate emotionality and stress reactivity

2.4.1.1 Behavioral Observations

On the fifth day, each rat was tested in the LDB, and on the 9th day, in the FST. Five minutes after the FST, the animals were decapitated, and trunk blood and brains were collected and stored at -20°C for later analysis. To control the influence of the oestrus cycle on female behavior, vaginal smears were taken daily during the experiments to control the state of the oestrus cycle. Females were included in the experiment once they were in metestrus or diestrus. In this phase of the estrus cycle, the animals showed the highest anxiety-related behavior (D'Souza & Sadananda, 2017).

2.4.1.2 Stress reactivity

The enzyme-linked immunosorbent assay (ELISA) quantifies the presence of a ligand or antibody. Enzyme-linked antibodies are used to bind a substrate and convert a colorless molecule into a luminescent product for detection. Trunk blood was collected and centrifuged (5000 rpm, 10 min, four °C). The plasma was collected and stored at – 20 °C until further processing. Plasma CORT (sensitivity < 0.56 ng/ml; intra-assay and inter-assay coefficients of variation $< 6.35\%$) was measured using a commercially available ELISA kit (IBL International GmbH, Hamburg, Germany). The complete ELISA kit consits of 96 well plates are precoated with antigen/antibody. It is essential to assign a standard to which the respective measured values are then plotted. The enzyme conjugate (antibody) is mixed with the diluent. 20μl of the sample (CORT) is transferred to the well plate. The sample is usually double-determined, and the mean value of the two is used for statistics. In the next step, the enzyme conjugate is pipetted onto the samples. The wells are covered with foil and incubated on a shaker (300/min) for one hour. Meanwhile, the wash buffer (1200ml water + buffer from the kit) is prepared. After incubation, three wash steps are conducted. Then, the color developer is pipetted, and a slight color change indicates the developer's function. As the color is light-sensitive, 15min incubations in

the dark follow. The Elisa reader and the distribution of the wells are set up. After the incubation, the stop solution inhibits further color development. The Elisareadaer can measure the dilution factor and provide the results. A linear regression curve shows the relation to the standard. The optical density and its mean value are used to compare the values (Beiderbeck et al., 2012; Lassé et al., 2021).

2.4.2 Experiment 2: Impact of peripheral TSPO ligand application on oxytocin receptor binding in selected brain regions

The receptorautoradiography for the oxytocin receptor binding was conducted after an established protocol in our laboratory (Bosch & Neumann, 2008, 2010). The collected brains from Experiment 1 (2.4.1) were cut with the cryostat (Leica, Wetzlar) into 16µm coronal slices. The slices containing the prefrontal cortex, lateral septum, nucleus ventromedialis hypothalami, and central amygdala were stored at -20 °C degrees. On the autoradiography day, the slides were thawed for 30 minutes at 4 °C, dried for 30 minutes at room temperature and fixed for two minutes in 0.1% paraformaldehyde. The slides were washed twice for ten minutes in Tris buffer (NO MgCl2) (50 mM Tris, pH 7.4; Sigma Aldrich, Darmstadt, Germany). Afterwards, the slides were incubated 50 mM (7.4) tris buffer with 10mM magnesium chloride (MgCl2), 0.1% bovine serum albumin and 50 pM of the radioactive tracer (2000cp,/10l) for 60 minutes. 125I-ornithine vasotocin analog (125I-d(CH2)5(Tyr(Me)2, Thr4, Orn8)(125I)Tyr9-NH2); Perkin Elmer, Überlingen, Germany) was used for the oxytocin receptor autoradiography. Then, the slides were washed three times for seven minutes in Tris, MgCl2 buffer. After a final rinse for 30 min in spinning Tris, MgCl2 buffer, the slides were dipped into distilled water before air-drying overnight. In a final step, the slides were exposed to a Kodax BioMaxMR film (Kodak, Rochester, NY, USA) for three days, and the autoradiographs were then developed (Kodak D19). The slides from the female and male groups were processed simultaneously. The oxytocin receptor binding is quantified, as grey density, with an NIH Image program (Fiji, ImageJ V. 2.1.0, National Institutes of Health). Bilateral measurements from six sections per rat were taken for each brain region, background activity was subtracted, and

the mean density was calculated. The three highest values were used to calculate a mean to establish better comparability between the animals. Brain regions were identified with the stereotaxic rat brain atlas of Paxinos and Watson (6th edition, 2007).

2.4.3 Experiment 3: Effect of central infusion of TSPO ligand etifoxine on innate emotionality and stress reactivity

Some animals underwent stereotactic surgery with an intracerebroventricular cannula under semi-sterile conditions. Therefore the rats were deeply anesthetized with the inhalation anesthesia isoflurane (Baxter GmbH, Unterschleißheim, Germany) and placed onto a stereotactic apparatus and a thermal pad (32°C) to avoid hypothermia. The skull was shaved, and a midline incision of 2 cm with a scalpel was made from between the eyes until the beginning of the ears. Bregma was identified as a reference point for the implantation of the cannulas. Two stainless steel screws were inserted into the skull to improve implant adhesion. A 21 G stainless steel guide cannula targeting the right lateral ventricle (+ 1.0 mm caudal, +1.6mm lateral, and + 2.1 mm ventral to bregma (Paxinos, Watson, 6th edition, 2007)) was implanted. The cannula was secured with a light-hardening dual-curing self-adhesive resin cement (Heraeus Kulzer GmbH, Hanau, Germany). The cannula was closed after surgery using stainless steel stylets that were removed and disinfected daily. The animals were treated with either 5μl etifoxine solution (see 2.3) or 5 μl vehicle. Surprisingly, the animals showed severe adverse effects independent of the vehicle or etifoxine treatment. The animals showed barrel rotations, and strong and unusual motor disturbance with rotation around the longitudinal axis. Barrel rotations were also seen in rats after ICV applications with neuropeptides (Diamant et al., 1994; Perry, 1987). However, so far, no data regarding tween administration and an occurrence of barrel rotations have been reported. It remains unclear if the application of tween has any influence on the development of barrel rotations in a similar way as described, for example, vasopressin (Marrannes & Wauquier, 1988). Therefore, the complete understanding of the occurrence of barrel rotations in our series of experiments is still unclear. At the same time, we had already carried out local surgical procedures with a cannula into the amygdala. De-

spite the observation that a local administration of 5μl of the vehicle did not generate barrel rotations, we decided, as of technical and ethical concerns, against continuing the experiment.

2.5 Statistical Analysis

The behavioral test data (Experiment 1) is presented as group means in each behavioral category with the standard error of the mean (SEM). The CORT Levels (Experiment 1) are shown as group means +SEM. The receptor autoradiography studies (Experiment 2) results are presented as group means of gray density +SEM. Statistical analysis was performed using SPSS for MacOS (Version 25-29, SPSS Inc, Chicago, IL, USA). Differences between the groups were statistically tested by two-way analysis of variance (ANOVA) (treatment;breeding) or, if data were not normally distributed, using the non-parametric Kruskal Wallis test. If overall significance was preserved, *post hoc* tests were carried out using either Bonferroni, Fisher's LSD test or Mann–Whitney U test. For all statistical tests, the significance level was set at $p \leq 0.05$; p-values between 0.05 and 0.1 were noted as a trend towards significance. The graphs were plotted with Graph Pad Prism8 for MacOS (Version 8.2.1, San Diego, USA).

Chapter 3

Results

3.1 Experiment 1: Effect of the TSPO ligand etifoxine on innate emotionality and stress reactivity

3.1.1 Behavioral observations

Anxiety-related behavior

To study anxiety-related behavior, female and male HAB, LAB, and NAB rats were tested in a five-minute session in the Light-Dark-Box (LDB) 30 minutes after etifoxine treatment. Significant differences in breeding line and treatment (two-way ANOVA; dF = 3.713 ; p = 0.031) were detected in females (Fig. 3.1.1a). Bonferroni correction in post-hoc test showed significant differences between NAB and LAB (p = 0.000), as well as NAB and HAB (p = 0.000) rats. No significant difference regarding the breeding line in females between LAB and HAB rats was detected. However, separate statistics (independent t-test, p = 0.000) revealed a significant difference between the female control LAB and HAB rats (Fig. 3.1.2). The treatment with etifoxine led to a significantly increased time etifoxine-treated HAB females spent in the light box (dF = 7.594 ; p = 0.008). Two-way ANOVA analysis (dF = 5.316; p = 0.008) revealed significant differences in males (Fig.3.1.1b) regarding the breeding lines of the rats. Post-hoc tests with Bonferroni correction indicate that LAB (p = 0.043) and NAB (p = 0.008) rats spent significantly more time in the light box than HAB rats.

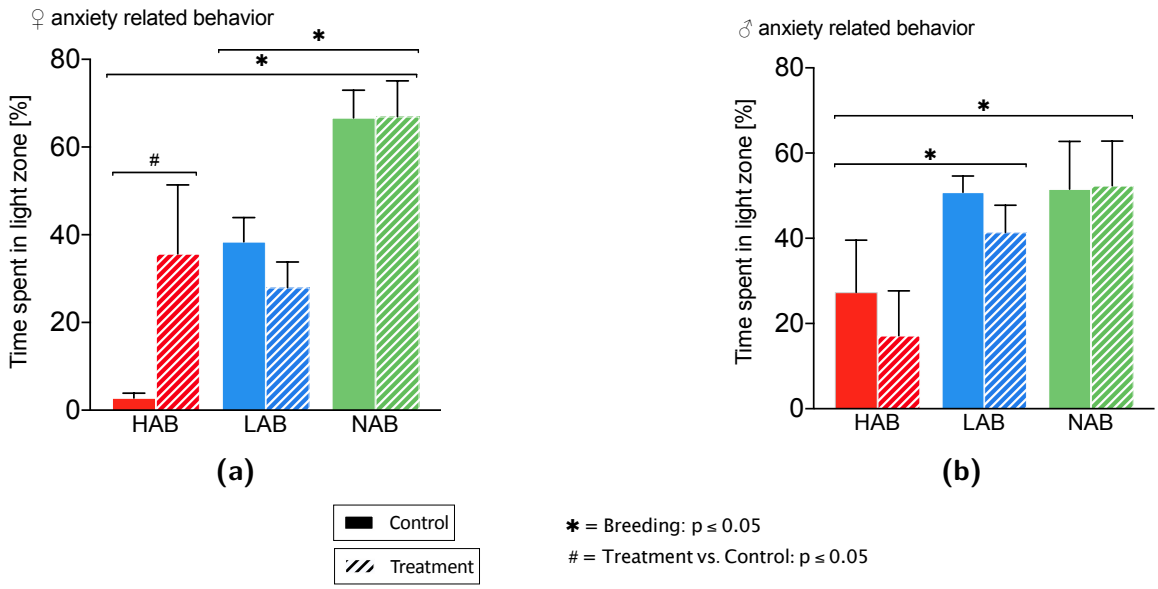

Figure 3.1.1: Effects of the etifoxine treatment on anxiety-related behavior. Time spent in the light compartment of the LDB of female **(a)** and male **(b)** HAB, LAB, and NAB rats was scored during the five-minute trial. Rats received ip. injections of either vehicle (1% Tween 80 in 0,9% NaCl) or treatment (50/mg/kg etifoxine in 1% Tween 80 in 0,9% NaCl) ref.: 2.4. Data is presented as mean + SEM, n = 8-10 per group; two-way ANOVA, factors: breeding x treatment.

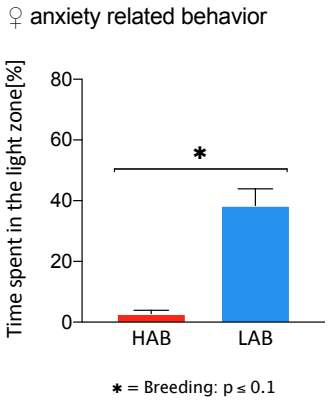

Figure 3.1.2: Effect of the TSPO ligand etifoxine on anxiety-related behavior of female control rats. Rats received ip injections of vehicle (for treatment see chapter 2.4). Data is presented as mean + SEM, n = 9-10 per group; t-test: Mann-Whitney test.

Further analysis of the locomotion of the rats is shown in Figure 3.1.3. Females exhibited significant treatment (dF = 7.605; p = 0.008) and breeding line (dF = 29.433; p = 0.000) differences regarding the total distance traveled (Fig. 3.1.3a). Bonferroni post-hoc comparisons were significant between all breeding lines. However, only HAB females in

the treatment group travelled significantly less, than vehicle HAB females (p = 0.037). The two-way ANOVA analysis for overall velocity in females (Fig. 3.1.3c) revealed a significant difference in treatment (dF = 7.647; p = 0.008) and breeding line (dF = 28.130; p = 0.000). Post-hoc tests with Bonferroni corrections showed significant breeding line differences between all three groups (p $p \leq 0.01$). Etifoxine-treated HAB females had significantly lower velocity overall than their controls (p = 0.036).

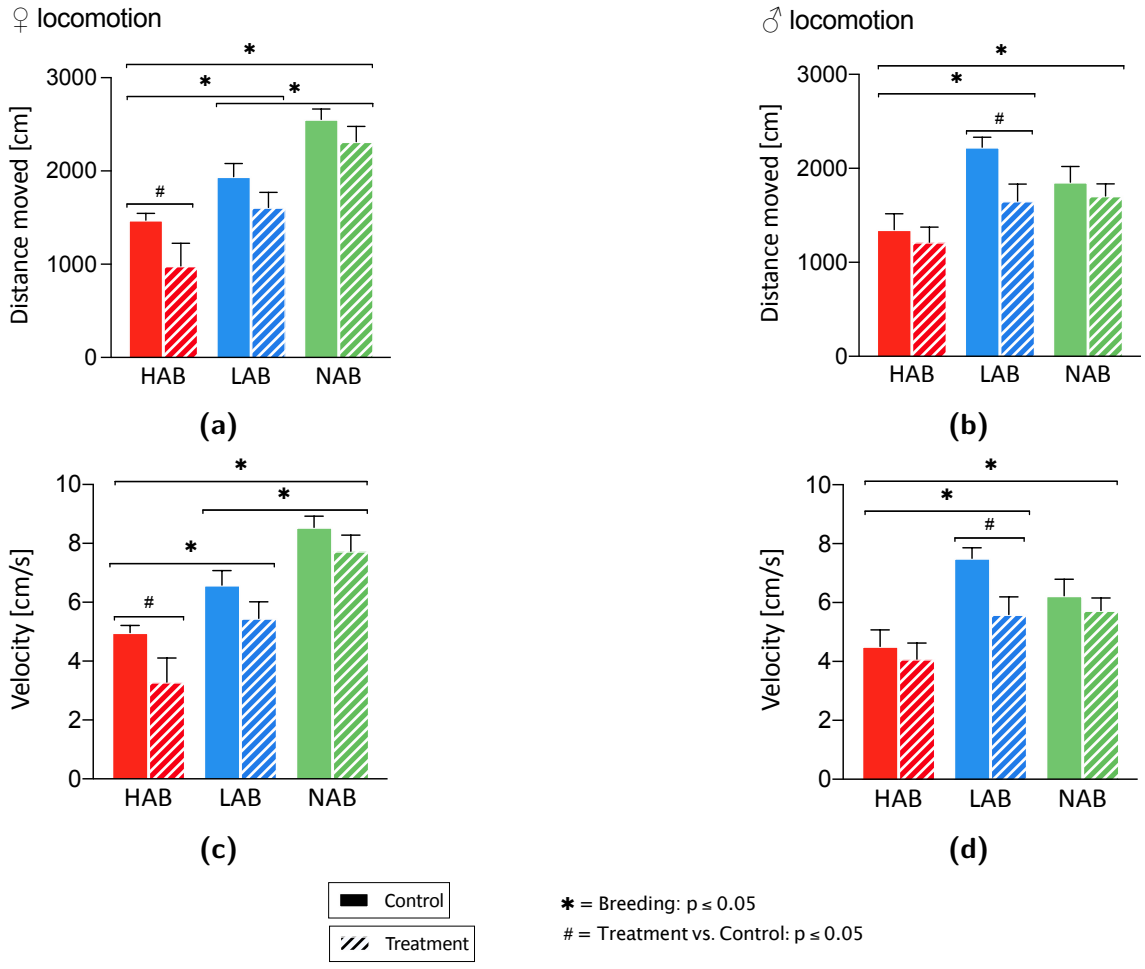

Figure 3.1.3: Effect of the TSPO ligand etifoxine on locomotion in the LDB. Distance moved [female:**(a)** and male:**(b)** HAB, LAB and NAB rats] and total velocity [female:**(c)** and male:**(d)** HAB, LAB and NAB rats] observed in the LDB are shown in this graph. For details on treatment, see chapter 2.4. Data is presented as mean + SEM, n = 8-10 per group; two-way ANOVA, factors: breeding x treatment.

The observation of the total distance traveled in male rats (Fig. 3.1.3b) revealed differences in breeding lines (dF = 9.461; p = 0.000) and treatment (dF = 5.099; p = 0.028). HAB males moved significantly less than LAB (Bonferroni: p = 0.000) and NAB

(Bonferroni: p = 0.007) males. In addition, a treatment effect after the post-hoc test is only shown in LAB males (p = 0.009). Etifoxine-treated LAB rats moved less than their controls. The same effect was observed regarding the total velocity of the males (Fig. 3.1.3d) with breeding line (dF = 9.815 ; p = 0.000) and treatment (dF = 5.050 ; p = 0.029) differences. HAB males had an overall lower velocity than LAB (Bonferroni: p = 0.000) and NAB (Bonferroni: p = 0.006) males. Etifoxine-treated LAB males had reduced velocity compared to the rest of the LAB group (p = 0.010).

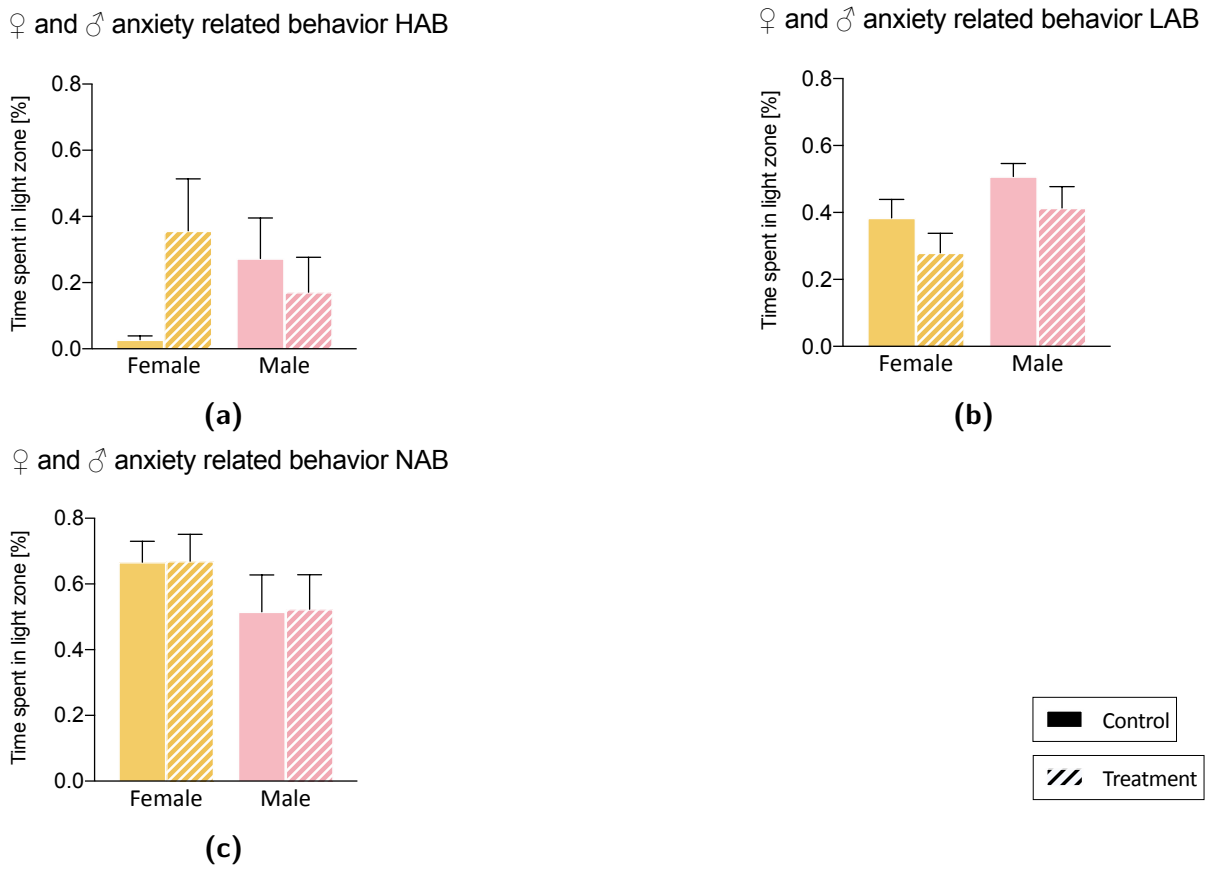

Figure 3.1.4: Effect of the TSPO ligand etifoxine on anxiety-related behavior in the LDB Time spent in the light compartment of the LDB: HAB **(a)**, LAB **(b)**, and NAB **(c)** rats. For details on treatment, see chapter 2.4. Data is presented as mean + SEM, n = 9-10 per group; two-way ANOVA, factors: gender x treatment

The two-way ANOVA analysis for sex differences revealed significant sex differences in LAB (dF = 5.546; p = 0.024). However, in the post-hoc test Bonferroni correction, only a tendency was found (p = 0.055). No significant differences were for either NAB or HAB.

Depressive-like behavior

Regarding depressive-like behavior, LAB, NAB, and HAB were tested in the 10-minute FST. In females, the two-way ANOVA analysis of the floating behavior revealed significant differences regarding the breeding line (dF = 18.828; p = 0.000). Bonferroni corrections show that LAB females have significantly less floating behavior than NAB (p = 0.000) and HAB (p = 0.002) rats. However, Fisher´s LSD corrections show that HAB females float significantly less than NAB females (p = 0.025). Treatment did not resolve significant differences.

A significant difference in the total time floating was found in males regarding the breeding lines (two-way ANOVA; dF = 25.526; p = 0.000). Post-hoc test with Bonferroni correction revealed that LAB males show less floating behavior than NAB (p = 0.000) and less than HAB (p = 0.000) males. Yet, with Fisher´s LSD corrections, HAB males float significantly less than NAB males (p = 0.048). No differences regarding the treatment were observed.

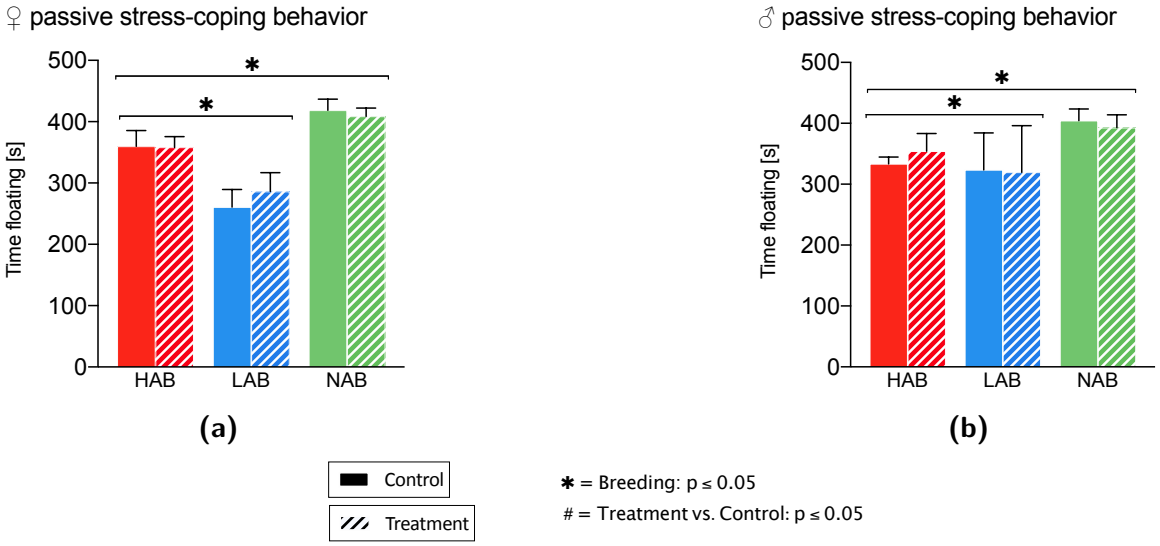

Figure 3.1.5: Effect of the TSPO ligand etifoxine on passive stress-coping behavior in the FST. Passive, floating behavior of females **(a)** and males **(b)** HAB, LAB and NAB rats in the FST. Rats received treatment as described in chapter 2.4. Data is presented as mean + SEM, n = 8-10 per group; two-way ANOVA, factors: breeding x treatment.

Observing the overall time struggling, breeding line differences were found regarding the behavior of females (Fig. 3.1.6a) (two-way ANOVA; dF = 21.479; p = 0.000). Female

LAB rats show significantly more struggling behavior than NAB (p = 0.000) and HAB (p = 0.000) females (post-hoc; Bonferroni). Significant treatment effects were not observed.

Significant differences within the breeding lines were found in males (Fig. 3.1.6b) (two-way ANOVA; dF = 4.278; p = 0.019). Bonferroni corrections in the post-hoc test showed that these differences are not consistent over all groups. Only HAB males struggled significantly less than LAB males (p = 0.018). Fisher´s LSD corrections, however, were also significant for the difference of NAB and HAB males (p = 0.035). HAB males struggled less than LAB and NAB. No treatment differences were observed in any group.

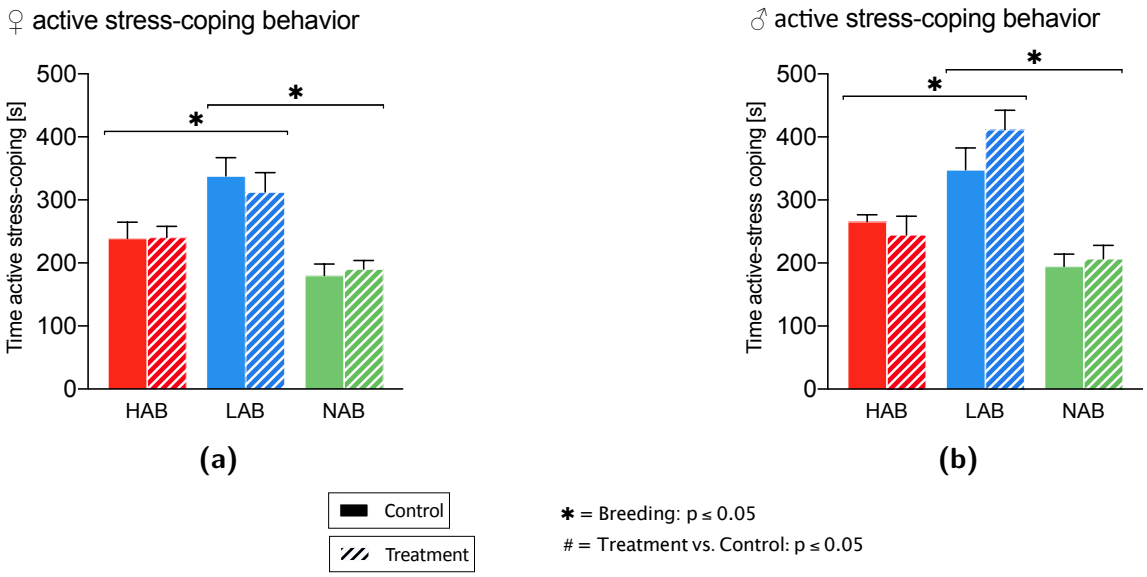

Figure 3.1.6: Effect of the TSPO ligand etifoxine on active stress-coping behavior in the FST. The struggling and swimming behavior, seen as active stress-coping behavior of females **(a)** and males **(b)** HAB, LAB, and NAB rats, were scored during the 10-minute trial. Rats received ip injections of either vehicle or treatment (for treatment see chapter 2.4. Data is presented as mean + SEM, n = 8-10 per group; two-way ANOVA, factors: breeding x treatment.

3.1.2 Stress reactivity

To assess the stress reactivity depending on etifoxine treatment in female and male HAB, LAB, and NAB rats, plasma CORT levels were measured 5 minutes after ending the FST. Significant differences regarding a treatment effect on CORT levels were found in both NAB female (Fig. 3.1.7a) (two-way ANOVA; dF = 4.625; p = 0.014) and male (Fig. 3.1.7b) (two-way ANOVA; dF = 8.575; p = 0.005). In both female and male NAB rats, etifoxine-treated rats showed greater CORT levels after the FST than their controls. Bonferroni-adjusted post-hoc analysis revealed a significant difference ($p < 0.05$) regarding the breeding lines in females. CORT levels in NAB rats were higher than in HAB and LAB rats. In males, no breeding line difference in CORT levels was present.

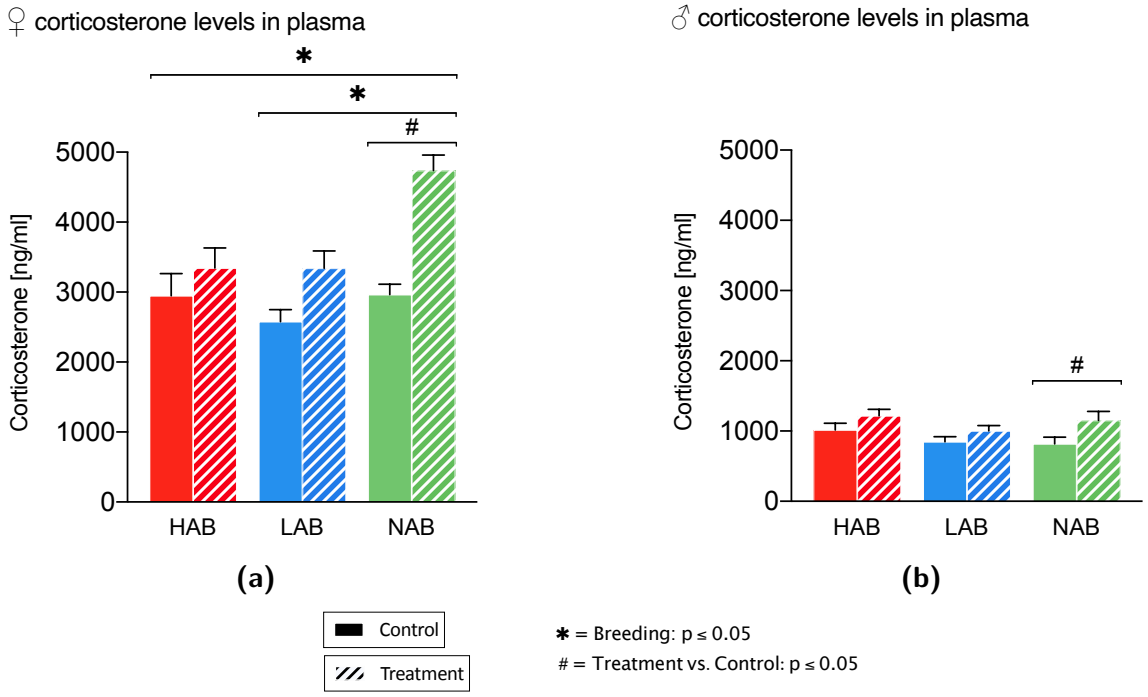

Figure 3.1.7: Effect of the TSPO ligand etifoxine on stress reactivity in the plasma CORT levels of females **(a)** and males **(b)** HAB, LAB and NAB rats. For treatment, see chapter 2.4.

3.2 Experiment 2: Impact of peripheral TSPO ligand application on OXT receptor´s expression in a selected brain region

OXT receptor binding in the central amygdala was measured using receptor autoradiography. In female rats, a significant difference was detected (two-way ANOVA; dF = 4.902; p = 0.011). Post hoc tests with Bonferroni corrections revealed that HAB females etifoxine-treated with etifoxine expressed higher levels of OXT receptor in the right central amygdala than the vehicle control group (p = 0.018). However, no breeding line differences were observed on either side.

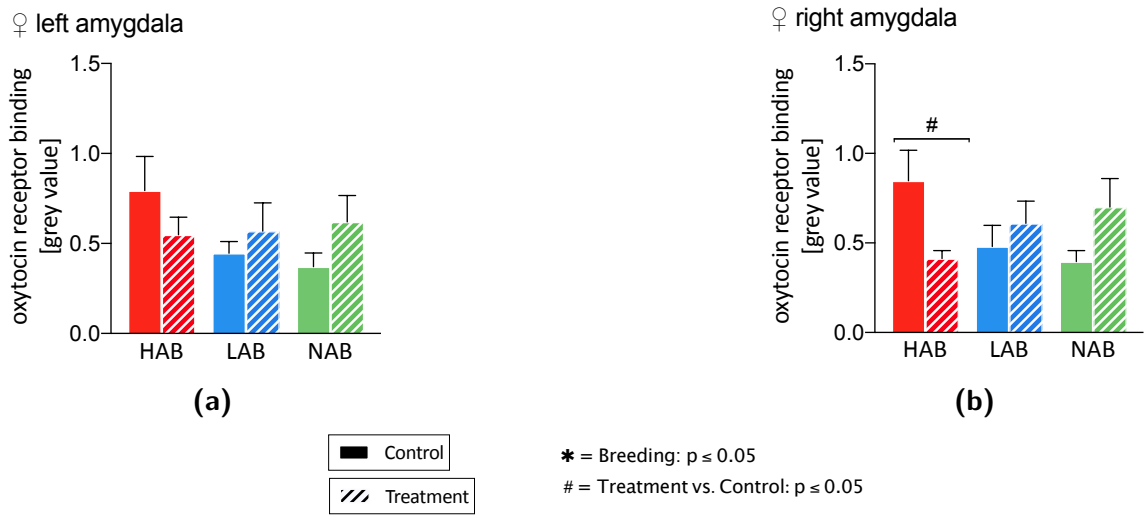

Figure 3.2.1: Effect of the TSPO ligand etifoxine on OXT receptor binding in the central amygdala OXT receptor binding in the left **(a)** and right **(b)** central amygdala of female HAB, LAB and NAB rats. For treatment, see chapter 2.4.

In male rats, OXT receptor binding in the central amygdala has significant breeding difference on both sides (left: two-way ANOVA; dF = 9.439; p < 0.001) (right: two-way ANOVA; dF = 11.001; p < 0,001). Post hoc tests with Bonferroni corrections revealed that NAB males express lower levels of OXT receptors in the right and left central amygdala than HAB and LAB animals (p <0.001). However, no treatment differences were observed on either side.

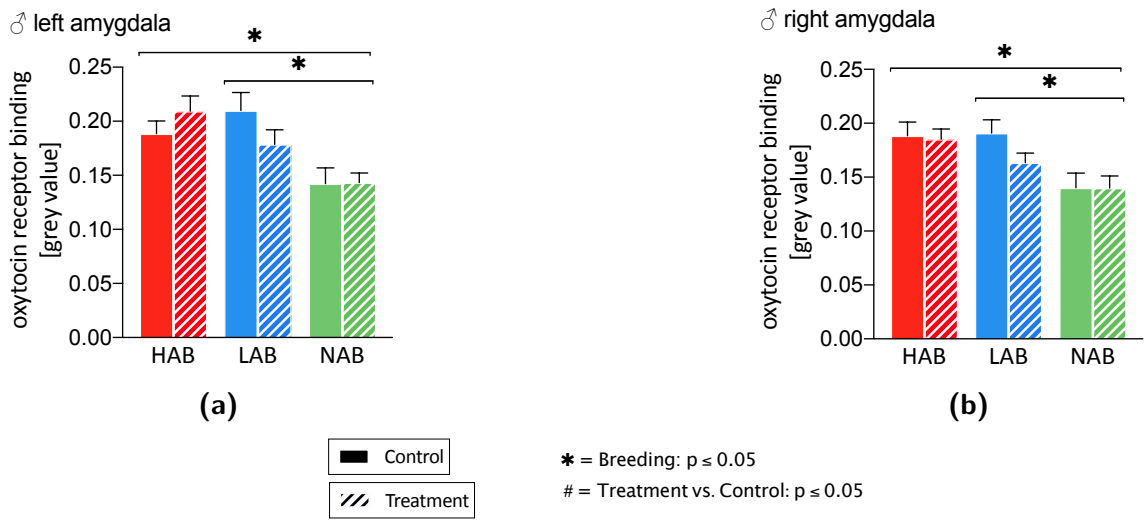

Figure 3.2.2: Effect of the TSPO ligand etifoxine on OXT receptor binding in the central amygdala OXT receptor binding in the left **(a)** and right **(b)** central amygdala of male HAB, LAB and NAB rats. For treatment, see chapter 2.4.

Chapter 4

Discussion

4.1 Summary of results

Anxiety and depression are among the most frequently diagnosed diseases worldwide and the leading cause for a high amount of DAYLY (World-Health-Organization, 2017). A better understanding of these life-threatening diseases is of utmost importance. In treating anxiety disorders, benzodiazepines are emergency drugs because of their rapid onset of action (Olfson et al., 2015). However, these drugs can lead to tolerance and abuse, associated with withdrawal symptoms, and can have sedative side effects (Balon & Starcevic, 2020; Shader & Greenblatt, 2010). Initial long-term treatment of anxiety disorders can be started with antidepressants. However, the onset of action of these antidepressants can be delayed up to weeks (Dell'Osso et al., 2010). Thus, there is a demand for innovative pharmacological interventions that concurrently offer rapid anxiolytic effects while minimizing issues such as the induction of tolerance and susceptibility to abuse (Bandelow, 2014; Nothdurfter et al., 2012).

TSPO has gained significant attention regarding the pathophysiology and treatment options of anxiety disorders (Nguyen et al., 2006; Micallef et al., 2001; Rupprecht et al., 2010; Servant et al., 1998). This thesis evaluated the effect of etifoxine on anxiety-related behavior in a psychopathological animal model for anxiety and depression (Gryksa et al., 2023). Therefore, etifoxine and vehicle were administered once daily over nine days. On day five, the anxiety-related behavior of both female and male HAB, LAB, and NAB rats

was tested, followed by the depressive-like behavior test on day nine. The stress reactivity and OXT receptor binding in the central amygdala were further evaluated. The present study confirms the anxiolytic effect of etifoxine, but only in female HAB rats, thereby revealing a sex-specific treatment effect. Further, it was discovered that etifoxine treatment influenced the binding properties of OXT in a similar pattern. However, etifoxine did not affect depressive-like behavior. Interestingly, it was observed that etifoxine altered the stress reactivity only in NAB rats, i.e., in rats not selected for anxiety-related behavior. With the present findings, we extend our knowledge about TSPO and its ligands (Ugale et al., 2007; Verleye et al., 2005; Verleye & Gillardin, 2004), and propose to consider the sex difference in treatment strategies for anxiety disorders. The study verifies the HAB breeding line as a model for innate anxiety-related behavior with etifoxine as a potential treatment. Hence, the HAB-LAB model for anxiety and depression provides a unique model for a better understanding of the mechanisms and treatment options of anxiety and depressive disorders, especially with a focus on sex differences.

4.2 Effects of etifoxine treatment on innate emotionality and stress reactivity

Etifoxine was the initial TSPO ligand shown to be anxiolytic in a clinical trial (Nguyen et al., 2006). TSPO is involved in the rate-limiting step of the neurosteroid synthesis (Papadopoulos et al., 1997). Thus, the observed anxiolytic effect of etifoxine is probably due to enhanced neurosteroid synthesis, particularly allopregnanolone. Etifoxine also elevates plasma and brain levels of pregnenolone, 5-α-dihydroprogesterone and progesterone (Okuyama et al., 1999; Serra et al., 1999; Verleye et al., 2005). Within hypothalamic neurons, etifoxine amplifies tonic inhibition, a process mediated by extrasynaptic $GABA_A$ receptors (Romeo et al., 1993; Serra et al., 1999). Neurosteroids seem to alternate the release of GABA neurotransmitter through binding GABA-receptor subunits (Belelli et al., 2006; Mellon & Griffin, 2002). This observation is supported by the fact that the anxiolytic effect of etifoxine is reversed by the steroid synthesis inhibitor finasteride (Schlichter et al., 2000). The release of neurosteroids is brain-specific, as the increase is independent

of peripheral sources (Verleye et al., 2005). The mentioned anxiolytic effects are mainly mediated by the amygdala (Akwa et al., 1999). Etifoxine is also shown to bind $GABA_A$ receptors at β_2 and β_3, thus further facilitating GABAergic transmission (Hamon et al., 2003). This allosteric site is different from that of neurosteroids and benzodiazepines (Verleye et al., 1999, 2001; Schlichter et al., 2000). As etifoxine also functions as a weak direct $GABA_A$ receptor enhancer (Schlichter et al., 2000), the extent to which the neurosteroidogenesis is related to the anxiolytic properties of etifoxine is not completely clarified (Rupprecht et al., 2010).

Especially in recent years, sex differences in psychiatric diseases have become increasingly apparent. Women tend to suffer from more mood disorders than male patients (Craske et al., 2017; Seedat et al., 2009; Seney & Sibille, 2014). Female patients have a two times higher risk of developing an anxiety disorder than men (Bandelow & Michaelis, 2015). Additionally, women are more likely to have a disease with a chronic course, more comorbidity, and vaster functional impairment (Jalnapurkar et al., 2018). Various factors have drawn attention regarding the different sex-dependant prevalence. Besides psychological, environmental, and cultural factors, biological features may explain these differences (Bangasser & Cuarenta, 2021; Farhane-Medina et al., 2022). Twin studies declare genetic factors responsible for the sex difference in anxiety disorders (Ask et al., 2014; Merikangas & Almasy, 2020). However, the occurrence of anxiety disorders cannot be explained by one specific genetic difference, as several factors contribute to the development of anxiety and, therefore, also to the different manifestations of both genders (Gottschalk & Domschke, 2017). Rather apparent is the gender difference within changes in hormones. Females and males display different levels and functions of various hormones, such as estrogen or testosterone (Domonkos et al., 2017). Especially the hormones within the menstrual cycle play a vital role in the sex difference in anxiety disorders. It is suggested that estrogen and progesterone increase the vulnerability of females to anxiety disorder, as well as facilitate the maintenance of symptoms after the development of the disorder (Li & Graham, 2017; Nillni et al., 2021). In preclinical studies, sex-dependent implications on fear memory and extinction are observed (Dalla &

Shors, 2009; Gupta et al., 2001; Marcondes et al., 2002). Growing evidence suggests additionally the difference in brain circuits mediating anxiety- and depressive-like behavior for either females or males (Bangasser & Cuarenta, 2021). For example, Labonté et al. (2017) found sex differences regarding stress susceptibility in MDD patients on a transcriptional level. Females exhibit greater vulnerability to negative emotions, with higher activation of amygdala-associated brain circuits (Gardener et al., 2013; Lungu et al., 2015). Further, in preclinical studies, sex as a biological variable remains underrated (Beery & Zucker, 2011; Kokras & Dalla, 2017). Especially in neuroscientific research, a sex-bias is reported, with many male animals included in preclinical studies (Beery & Zucker, 2011; Will et al., 2017). Mentioned disproportion regarding sex can negatively affect the development of sufficient drug treatments (Check Hayden, 2010).

Interestingly, the present results demonstrate an anxiolytic effect of etifoxine only in female rats with high innate anxiety, as those spent more time in the illuminated compartment of the LDB, indicating reduced anxiety-related behavior (Henniger et al., 2000). Therefore, there is a sex-specific effect of etifoxine. However, comparable results of both sexes still need to be included in most pre-clinical etifoxine studies. (Ugale et al., 2007; Verleye et al., 2005; Verleye & Gillardin, 2004). Consequently, studying these sex-dependent results in animals and humans is crucial, as this would influence the clinical use of etifoxine. A possible hypothesis for the present sex differences might be linked to the role of neurosteroids. Etifoxine acts anxiolytic through enhanced neurosteroid synthesis (Schlichter et al., 2000; Verleye et al., 2005). The neurosteroid allopregnanolone was found to be anxiolytic, especially in female rats (Bitran et al., 1991; Brot et al., 1997). Moreover, females show a greater sensitivity towards neurosteroids. In a postnatal stress model, allopregnanolone reduces anxiety-like behavior only in females, and female mice have a greater sensitivity to neurosteroid treatment in epileptic seizures (Brunton et al., 2015; Reddy et al., 2019). In addition, studies showed a sex-dependent effect of allopregnanolone, as in prenatally stressed rats, neuroactive steroids were shown to reduce the activity of the HPA axis in females only (Zimmerberg et al., 1999). Furthermore, recent results show that anxiolytic neurosteroids have basal sex differences in various brain re-

gions (Giatti et al., 2020). For example, allopregnanolone levels in the hippocampus and cerebral cortex are higher in female rats. Those sex differences might be due to differences in the machinery of the neurosteroid synthesis, as the gene expression of the steroidogenic acute regulatory protein (StAR) also exhibits sex differences (Giatti et al., 2019; Lavaque et al., 2006). Therefore, the sex difference may be due to a complex interplay between neurosteroid level and sensitivity to neurosteroids, as downregulation and imbalance of neurosteroids contribute to the development of anxiety disorders (Schüle et al., 2014; Nothdurfter et al., 2012; Rupprecht et al., 2010). In favor of the mentioned hypothesis, Bahr et al. (2021) propose that the therapeutic effect of etifoxine is limited to patients suffering from psychiatric diseases with disrupted neurosteroid systems. Treatment options targeting the neurosteroid system may need to be carefully adapted to the sexes. To shed more light on this, it would be interesting to determine neurosteroid levels in HAB rats.

Another explanation for the sex-dependent effect of etifoxine could be hormonal. It is well known that the occurrence and onset of anxiety disorders are dependent on fluctuations of the estrous cycle, as well as their course and severity (Basso et al., 2011; Domonkos et al., 2017). Regarding therapeutic studies in animals and humans, it remains inevitable to consider that previous studies did not always test the efficacy of anxiolytics in both sexes. This is in part due to females being considered more variable and more difficult to investigate (Beery & Zucker, 2011). However, observations regarding neurosteroids and the estrous cycle remain ambiguous (Lovick & Zangrossi, 2021). Considering neurosteroid levels, a change in allopregnanolone is observed during the estrus cycle. Especially during proestrus, the levels of allopregnanolone were high (Palumbo et al., 1995; Schmidt et al., 1994) and females exhibited reduced anxiety-related behaviors (D'Souza & Sadananda, 2017). To reduce the variability of neurosteroids and their influence on behavior, only female rats in the LDB and FST were included in the metestrus-diestrus tests. Nevertheless, it would be interesting to compare the effect of etifoxine on behavior in female rats in proestrus-estrus to detect if the anxiolytic effect of etifoxine is affected. In humans, allopregnanolone was associated with improvement in patients with premenstrual

syndromes during one estrus cycle (Wang et al., 1996). Furthermore, women are also vulnerable to developing mood and anxiety disorders when endogenous estradiol levels are low during menopausal and postpartum periods (Altshuler et al., 1998; Schnatz et al., 2010). During pregnancy, allopregnanolone serum levels are increased, and postpartum mood alterations are due to the withdrawal of neurosteroids after birth (Majewska, 1992). These fluctuations in females and, thus, a possible enhancement of the neurosteroid levels could also explain why an effect of neurosteroids was found in females.

On the other hand, obtained sex differences and the lack of a treatment effect on males contrast earlier studies that showed an anxiolytic effect in male rats. However, these studies have either used different experimental patterns or been conducted in healthy animals without innate anxiety (Ugale et al., 2007; Verleye et al., 2005; Schlichter et al., 2000). Thus, etifoxine may act different in psychopathological conditions, as proposed by Bahr et al. (2021). Regarding etifoxine effects on anxiety, previous anxiolytic properties of etifoxine are present in healthy male rats after exposure to short-time stressors (Ugale et al., 2007; Verleye et al., 2005; Schlichter et al., 2000). However, the present study used rats with a high innate anxiety phenotype (Landgraf & Wigger, 2002). This difference is particularly relevant given the high genetic component of anxiety disorders in humans, which is represented by the HAB rats (Craske et al., 2017; Gottschalk & Domschke, 2017; Shimada-Sugimoto et al., 2015). Different underlying pathophysiology of the anxiety circuits and further tools of assessing anxiety-related behavior, might explain the sex difference of the present study. An example is the use of the Vogel's conflict test (VGT) (Schlichter et al., 2000; Verleye et al., 2005), where water-deprived animals receive a mild shock when they drink water. Conflict is created between the animal's instinctive behavior and fear-associated behavior (Vogel et al., 1971). Anxiety and fear can be distinguished. Fear emerges when a specific threat or clear signals indicate danger, yet anxiety arises in situations characterized by uncertainty and unpredictability, where potential dangers are not indicated (Davis et al., 2010). This can be seen when rodents are exposed to unfamiliar open spaces, creating a feeling of danger. It remains essential to consider the nature of the anxiety or fear-inducing stimuli (Armario & Nadal, 2013). Thus, the

behavior assessed in the LDB measures more preciously innate and sustained fear, whereas the behavior observed in the VGT is more suitable to detect phasic and learned fear (Davis et al., 2010; Lisboa et al., 2010; Millan, 2003). Etifoxine shows an effect on short-term punishment of the VGT in males but no impact on the traits of anxiety in the HAB animal model. Regarding its importance in estimating the level of anxiety with several different tests (Sudakov et al., 2013), it is crucial to consider the contrasting results of males of the VGT and the LDB might add up together to a more precise understanding of the effect of etifoxine on anxiety. Nevertheless, to prove this hypothesis and suggest sex differences, it would be important to analyze the effect of etifoxine in females exposed to a short-time stressor. Together, the findings indicate that the anxiolytic effect of etifoxine observed in males based on learned fear differs from those of animal models involving an innate fear of animals. The reasons for the different ways of action of etifoxine are unknown.

Overall, the study highlights the importance of the HAB animal model in studying sex differences. The animal model is essential as it allows a better mimic of anxiety in humans, combining genetic alterations and stress exposure (Gryksa et al., 2023; Neumann et al., 2011). So far, no sex-specific differences are present in this animal model (Landgraf & Wigger, 2002). Thus, HAB rats provide an interesting model for studying sex-specific differences in treating psychiatric disorders. Interestingly, Schmidtner et al. (2019) reveal a sex- and trait-dependent difference in treating depressive-like behavior in HAB males. Thereby, again highlighting the importance of including female animals in neurobiological research.

With regard to human research, etifoxine was found to be anxiolytic in clinical trials. However, a closer look reveals that more women (2/3) were included in the study, yet a more detailed breakdown of the effectiveness of etifoxine in both sexes is missing from the study. Nevertheless, the study design could already provide an indication of a possible sex difference (Nguyen et al., 2006). Nuss et al. (2019) suspect a possible sex difference regarding etifoxine treatment in humans. The conclusion is also drawn in regards to the greater rate of women included in ineffective etifoxine trials (Alexandrovsky et al., 2010; Stein, 2015; Servant et al., 1998), which needs to be interpreted carefully. Finally,

regarding the target of etifoxine, a recent publication provides further evidence for sex differences within TSPO. Higher TSPO distribution in the brain was present in women, using the radioligand [11C]PBR28 (Tuisku et al., 2019). In light of these considerations, the present study suggests that women show greater susceptibility to treatment options with TSPO ligands, most likely resulting in stronger alterations of neurosteroid synthesis. Hence, the management of TSPO targets should be considered precisely in clinical settings. However, more evidence is required to prove the presumed sex difference between TSPO and etifoxine, particularly in regard to anxiety disorders.

Anxiety and depression are highly co-morbid (Craske et al., 2017). Therefore, the HAB-LAB animal model provides an important tool to look closely at depressive-like behavior. Concomitant with the high-anxiety phenotype, the behavior observed in the LDB and the FST confirmed the same breeding line difference in anxiety-related and depressive-like behavior (Landgraf & Wigger, 2002; Ohl et al., 2001a). HAB rats of both sexes were more anxious than LAB rats. In addition, the HAB rats showed less active stress-coping behavior. However, the current analysis revealed no significant differences due to etifoxine treatment on depressive-like behavior. Despite the observation that allopregnanolone reduces depressive-like behavior in the FST (Khisti et al., 2000), no effect of etifoxine was seen in this experiment. Nevertheless, recent research highlights the relationship between depression and TSPO (Rupprecht et al., 2023). The role of TSPO in depressive disorder might be linked to microglial activation and synaptic plasticity and, therefore, not possible to detect in a short-term etifoxine treatment (Schmidtner et al., 2019; Setiawan et al., 2015).

Benzodiazepines are very effective in the treatment of anxiety disorders and are widely prescribed for rapid onset treatment (Olfson et al., 2015), although the treatment is associated with several severe side effects, such as CNS depression, drug dependence, or intense symptoms of withdrawal (Balon & Starcevic, 2020; Schweizer & Rickels, 1998; Shader & Greenblatt, 2010). Therefore, it is indispensable to rethink the use of benzodiazepines in anxiety disorders and develop new forms of therapy (Nothdurfter et al., 2012;

Rupprecht et al., 2010). For instance, Micallef et al. (2001) compared the psychomotor and memory effects of etifoxine and the benzodiazepine lorazepam in healthy participants. The double-blind, placebo-controlled study investigated psychomotor performance, attention, and memory. In comparison to the lorazepam group, the etifoxine group exhibited a significantly quicker reaction time. Additionally, participants administered lorazepam reported significantly higher levels of drowsiness, fatigue, and somnolence, while also expressing reduced feelings of energy. An additional study provides further information on alertness and cognitive impairment of etifoxine in comparison to lorazepam with healthy older volunteers included in the study. Etifoxine administration had no harmful effects on alertness, where it was significantly reduced with lorazepam. Moreover, no effects on attention, information processing, and memory were observed due to the etifoxine treatment (Deplanque et al., 2018). Interestingly, the present study revealed different observations. The locomotion of etifoxine-treated HAB females that showed less anxiety-related behavior was reduced in comparison to the control group. However, in terms of analyzing the effect of the locomotion on anxiety-related behavior, those results are important, as they show that reduced anxiety-related behavior was independent of the arousal state of the animal. This is important, as high arousal states can increase locomotor activity, which might falsely be interpreted as reduced anxiety-related behavior (Ohl et al., 2001b; Rex et al., 2005). Yet, the animal model and observed locomotion might not be suitable to quantify and compare the side effects of etifoxine in humans.

Regarding stress reactivity, the obtained CORT levels revealed some interesting findings, as plasma CORT levels were only elevated in NAB rats treated with etifoxine but not in HAB or LAB rats. Interestingly, while the baseline CORT levels of HAB and LAB rats are indifferent, the stress-induced CORT response is significantly higher in HAB versus LAB rats (Landgraf et al., 1999; Liebsch et al., 1998). However, these differences were not seen in the present experiment. This might be due to a reduced stress response of the HAB and LAB rats following etifoxine treatment, as previously shown in non-selected rats (Verleye & Gillardin, 2004). However, results from NAB rats, where etifoxine increased stress-induced plasma CORT levels, are in contrast with the observation that etifoxine

does not affect the stress-induced activity of the HPA axis in healthy humans, measured by salivary CORT levels (Bahr et al., 2021). However, detailed information on changes in the steroid system of HAB, LAB, and NAB rats is missing. The observed alteration in plasma CORT levels was restricted to animals without altered behavioral phenotypes. Psychopathological conditions, such as anxiety and depression, are often associated with a disrupted HPA axis (Kinlein et al., 2019). This disrupted system of the HPA due to the altered phenotype of the animals may explain why no effect of etifoxine was seen in HAB and LAB rats (Schüle et al., 2014). Interestingly, plasma CORT alterations were present in females and males who did not display behavioral alterations. The obtained results reject the assumption that etifoxine has a more significant influence on the stress response in pathological conditions in humans (Bahr et al., 2021). In line with this finding, research goes even further by questioning the role of TSPO in steroid synthesis (Morohaku et al., 2014; Banati et al., 2014). Additional experiments with TSPO-knockout revealed an influence of TSPO on steroid synthesis but not on CORT levels (Fan et al., 2020). However, Papadopoulos et al. (2018) failed to reproduce the experiments that challenged the importance of TSPO in steroid synthesis and further highlight their role of action (Owen et al., 2017). The measurement of plasma CORT might not contribute to understanding the treatment effects of etifoxine in psychiatric conditions. Therefore, further studying TSPO´s mechanism of action in psychiatric disorders and linked therapy is inevitable (Rupprecht et al., 2022).

4.3 Impact of peripheral TSPO ligand application on OXT receptor's density in a selected brain region

One of the most critical brain regions regulating anxiety is the amygdala (Craske et al., 2017). Several mechanisms are essential to understand the pathophysiology of psychiatric disorders. One important mediator is the neuropeptid OXT (Yoon & Kim, 2020). Especially in the amygdala, OXT is associated with an anxiolytic effect (Bale et al., 2001; Viviani et al., 2011). OXT regulates fear negatively through increased excitability of OXT receptors in the amygdala and inhibition of the fear response through GABA

(Huber et al., 2005; Viviani et al., 2011).

In order to study the potential effects of etifoxine treatment on anxiety via modulation of the OXT system, the binding of OXT receptors in the central amygdala was examined. In fact, etifoxine had a significant effect on OXT receptor binding in HAB female rats only. Etifoxine-treated HAB females had reduced OXT receptor binding in the right central amygdala. Interestingly, this effect was found in the same breeding line that showed behavioral changes after etifoxine treatment, i.e., reduced anxiety-related behavior in the light-dark box. Hence, activation of TSPO might be associated - directly or rather indirectly - with OXT receptor binding. Indeed, OXT administration has anxiolytic properties (Neumann et al., 2000). Therefore, treatment with etifoxine and activation of TSPO might be associated with an increase in OXT levels, which in turn could lead to a downregulation of OXT receptors in the amygdala. Increased OXT levels due to chronic OXT administration over 14 days lead to decreased levels of OXT receptors. However, in contrast to this result, the downregulation of OXT receptors was associated with increased anxiety (Peters et al., 2014; Huang et al., 2014). It is essential to consider that the observed reduction in anxiety-related behavior was on the fifth day of the administration and the evaluation of the OXT receptor levels after the ninth day of the administration. For more comparable results, it would be interesting to repeat the test for anxiety-related behavior after a more extended period of etifoxine treatment or evaluate the OXT receptor binding after five days of etifoxine treatment.

So far, differences in the basal level of OXT receptor binding in HAB and LAB females and males have not been reported (Gryksa et al., 2023). Interestingly, in the HAB animal model, an anxiolytic effect of OXT was only apparent in female, but not male HAB rats (Landgraf & Wigger, 2003). Yet, within the OXT system, there could be a link to the observed sex differences. Furthermore, OXT receptor expression could also be regulated through enhanced levels of neurosteroids induced by TSPO activation. An example is the steroid estrogen, known to influence the induction of OXT receptor expression (Choleris et al., 2003; Young et al., 1998). Further observations might help to understand underlying neurobiological mechanisms.

Overall, the current behavioral investigations urge the importance of considering sex differences in psychiatric disorders. Nevertheless, further and more comprehensive prospective studies are needed to validate these results and consolidate the potential role of TSPO in psychiatric disorders.

4.4 Limitations and Restrictions

Although animal behavior is reproducible, reliable, and well-studied, some limitations remain. One of them is the difficulty of modeling diseases using an animal model; despite promising research and reliable animal models, there are still factors that cannot be sufficiently modeled in animals, especially when it comes to psychiatric diseases. These include, for example, the nature and complexity of many symptoms and the lack of objective diagnostic tools (Nestler & Hyman, 2010). Another main factor of limitation in the analysis might be the small sample size, which may have influenced results and, therefore, should be considered when interpreting results. Regarding the laboratory conditions, some limitations might also occur, such as the influence of the human person conducting and evaluating the experiment. However, these errors were kept to a minimum through established laboratory standards.

4.5 Future Research

Considering the role of TSPO in psychiatric diseases, future research should reach out in attempting to understand and describe the underlying mechanism. Moreover, forthcoming research should be conducted to understand the sex differences in psychiatric diseases, focusing on the treatment options and difficulties. This is particularly important in order to establish better treatment regimes for women. To further cement the reliability and validity of present findings, the author encourages reevaluating the results in further experiments.

Chapter 5

Summary

The overall goal of this study was to investigate TSPO 18 kDa in the HAB-LAB animal model of psychopathologies and gain deeper insights into TSPO as a therapeutic target in psychiatric disorders. TSPO is a channel protein located in the outer mitochondrial membrane that is essential for neurosteroid synthesis with rate-limiting features. Neurosteroids facilitate neurotransmission at the $GABA_A$-receptor, thereby mediating anxiolytic properties. The TSPO ligand etifoxine acts through enhanced neurosteroid synthesis as an anxiolytic compound. The present study sought further insights into the functioning of etifoxine, especially under pathological conditions. The present study confirms the anxiolytic effect of etifoxine but also unravels a sex difference, as etifoxine acted anxiolytic in HAB females, only. In males of the HAB breeding line, etifoxine did not lead to a reduction of anxiety-related behavior. These results are significant concerning the higher prevalence of psychiatric disorders, especially anxiety disorders in females. The study extends previous observations that the therapeutic effect of etifoxine might be limited to pathological conditions with a disrupted neurosteroid system. Other sex-related differences need to be considered in the use of TSPO. The study further confirms that the TSPO ligand etifoxine has no ameliorative effect on depressive behavior in rats bred for high and low anxiety-related behavior.

Consistent with the current state of the relevant literature, etifoxine had no effect on stress reactivity in animals with pathological conditions. However, treatment with etifoxine resulted in an increased corticosterone response in animals that were not selected

for anxiety-related behavior. These results are in contrast with earlier findings. The results highlight the importance of further investigating the relationship between TSPO and stress response in pathological conditions, particularly in the context of anxiety disorders.

In addition, etifoxine treatment revealed a reduction of OXT receptor binding in the central amygdala of HAB females. This could be due to increased OXT signaling in the brain, but it requires further proof. So far, limited research is available regarding the relationship between TSPO and OXT. Importantly, those findings reinforce the significance of considering neuroendocrine systems in anxiety disorders' onset.

The present work proved the potential of TSPO as a target for the treatment of anxiety disorders and showed the pivotal importance of including female subjects in basal research, especially in the neurobiological fields.

References

Abelli, M., Chelli, B., Costa, B., Lari, L., Cardini, A., Gesi, C., Muti, M., Lucacchini, A., Martini, C., Cassano, G. B., & Pini, S. (2010). Reductions in platelet 18-kDa translocator protein density are associated with adult separation anxiety in patients with bipolar disorder. *Neuropsychobiology*, 62(2), 98–103.

Adolphs, R. (2008). Fear, faces, and the human amygdala. *Current Opinion in Neurobiology*, 18(2), 166–172.

Akwa, Y., Purdy, R. H., Koob, G. F., & Britton, K. T. (1999). The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. *Behavioural Brain Research*, 106(1), 119–125.

Alexandrovsky, Y., Krasnov, V., Neznanov, N., & Romasenko, L. (2010). Efficacy of etifoxine in comparison with phenazepam in the treatment of patients with adjustment disorders (an open randomized controlled study). *Russian J Psychiatry*, (pp. 74–78).

Alonso, J., Ferrer, M., Romera, B., Vilagut, G., Angermeyer, M., Bernert, S., Brugha, T. S., Taub, N., McColgen, Z., de Girolamo, G., Polidori, G., Mazzi, F., De Graaf, R., Vollebergh, W. a. M., Buist-Bowman, M. A., Demyttenaere, K., Gasquet, I., Haro, J. M., Palacín, C., Autonell, J., Katz, S. J., Kessler, R. C., Kovess, V., Lépine, J. P., Arbabzadeh-Bouchez, S., Ormel, J., & Bruffaerts, R. (2002). The European Study of the Epidemiology of Mental Disorders (ESEMeD/MHEDEA 2000) project: Rationale and methods. *International Journal of Methods in Psychiatric Research*, 11(2), 55–67.

Alonso, J., Lépine, J.-P., & ESEMeD/MHEDEA 2000 Scientific Committee (2007).

Overview of key data from the European Study of the Epidemiology of Mental Disorders (ESEMeD). *The Journal of Clinical Psychiatry*, 68 Suppl 2, 3–9.

Altshuler, L. L., Hendrick, V., & Cohen, L. S. (1998). Course of mood and anxiety disorders during pregnancy and the postpartum period. *The Journal of Clinical Psychiatry*, 59 Suppl 2, 29–33.

American-Psychiatric-Association, Ed. (2013). *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*. Washington, D.C: American Psychiatric Association, 5th ed edition.

Andrade, L., Caraveo-anduaga, J. J., Berglund, P., Bijl, R. V., Graaf, R. D., Vollebergh, W., Dragomirecka, E., Kohn, R., Keller, M., Kessler, R. C., Kawakami, N., Kiliç, C., Offord, D., Ustun, T. B., & Wittchen, H.-U. (2003). The epidemiology of major depressive episodes: Results from the International Consortium of Psychiatric Epidemiology (ICPE) surveys. *International Journal of Methods in Psychiatric Research*, 12(1), 3–21.

Aouad, M., Charlet, A., Rodeau, J.-L., & Poisbeau, P. (2009). Reduction and prevention of vincristine-induced neuropathic pain symptoms by the non-benzodiazepine anxiolytic etifoxine are mediated by 3α-reduced neurosteroids. *PAIN*, 147(1), 54–59.

Armario, A. & Nadal, R. (2013). Individual differences and the characterization of animal models of psychopathology: A strong challenge and a good opportunity. *Frontiers in Pharmacology*, 4, 137.

Ask, H., Torgersen, S., Seglem, K. B., & Waaktaar, T. (2014). Genetic and environmental causes of variation in adolescent anxiety symptoms: A multiple-rater twin study. *Journal of Anxiety Disorders*, 28(4), 363–371.

Bahr, L.-M., Maurer, F., Weigl, J., Weber, K., Melchner, D., Dörfelt, A., Wechsler, T. F., Bauer, O., Reinders, J., Milenkovic, V. M., Wetzel, C. H., Wetter, T. C., Rupprecht, R., Mühlberger, A., & Nothdurfter, C. (2021). Dissociation of endocrine responses to the Trier Social Stress Test in Virtual Reality (VR-TSST) by the benzodiazepine

alprazolam and the translocator protein 18 kDa (TSPO) ligand etifoxine. *Psychoneuroendocrinology*, 124, 105100.

Baldwin, D. S., Ajel, K. I., & Garner, M. (2010). Pharmacological treatment of generalized anxiety disorder. *Current Topics in Behavioral Neurosciences*, 2, 453–467.

Baldwin, D. S., Allgulander, C., Bandelow, B., Ferre, F., & Pallanti, S. (2012). An international survey of reported prescribing practice in the treatment of patients with generalised anxiety disorder. *The World Journal of Biological Psychiatry: The Official Journal of the World Federation of Societies of Biological Psychiatry*, 13(7), 510–516.

Baldwin, D. S. & Polkinghorn, C. (2005). Evidence-based pharmacotherapy of Generalized Anxiety Disorder. *The International Journal of Neuropsychopharmacology*, 8(2), 293–302.

Bale, T. L., Davis, A. M., Auger, A. P., Dorsa, D. M., & McCarthy, M. M. (2001). CNS Region-Specific Oxytocin Receptor Expression: Importance in Regulation of Anxiety and Sex Behavior. *Journal of Neuroscience*, 21(7), 2546–2552.

Balon, R. & Starcevic, V. (2020). Role of Benzodiazepines in Anxiety Disorders. In Y.-K. Kim (Ed.), *Anxiety Disorders: Rethinking and Understanding Recent Discoveries*, Advances in Experimental Medicine and Biology (pp. 367–388). Singapore: Springer.

Banati, R. B., Middleton, R. J., Chan, R., Hatty, C. R., Wai-Ying Kam, W., Quin, C., Graeber, M. B., Parmar, A., Zahra, D., Callaghan, P., Fok, S., Howell, N. R., Gregoire, M., Szabo, A., Pham, T., Davis, E., & Liu, G.-J. (2014). Positron emission tomography and functional characterization of a complete PBR/TSPO knockout. *Nature Communications*, 5(1), 5452.

Bandelow, B. (2014). S3-Leitlinie Angststörungen. https://www.awmf.org/uploads/tx$_s$*zleitlinien*/051 − 028l_S3$_A$*ngststrungen*$_2$014 − 05 − *abgelaufen.pdf*(*Downloadon*16.01.2020).

Bandelow, B. (2020). Current and Novel Psychopharmacological Drugs for Anxiety Disorders. In Y.-K. Kim (Ed.), *Anxiety Disorders: Rethinking and Understanding Recent*

Discoveries, Advances in Experimental Medicine and Biology (pp. 347–365). Singapore: Springer.

Bandelow, B., Baldwin, D., Abelli, M., Bolea-Alamanac, B., Bourin, M., Chamberlain, S. R., Cinosi, E., Davies, S., Domschke, K., Fineberg, N., Grünblatt, E., Jarema, M., Kim, Y.-K., Maron, E., Masdrakis, V., Mikova, O., Nutt, D., Pallanti, S., Pini, S., Ströhle, A., Thibaut, F., Vaghix, M. M., Won, E., Wedekind, D., Wichniak, A., Woolley, J., Zwanzger, P., & Riederer, P. (2017). Biological markers for anxiety disorders, OCD and PTSD: A consensus statement. Part II: Neurochemistry, neurophysiology and neurocognition. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, 18(3), 162–214.

Bandelow, B. & Michaelis, S. (2015). Epidemiology of anxiety disorders in the 21st century. *Dialogues in Clinical Neuroscience*, 17(3), 327–335.

Bandelow, B., Reitt, M., Röver, C., Michaelis, S., Görlich, Y., & Wedekind, D. (2015). Efficacy of treatments for anxiety disorders: A meta-analysis. *International Clinical Psychopharmacology*, 30(4), 183–192.

Bandelow, B., Sher, L., Bunevicius, R., Hollander, E., Kasper, S., Zohar, J., Möller, H.-J., WFSBP Task Force on Mental Disorders in Primary Care, & WFSBP Task Force on Anxiety Disorders, OCD and PTSD (2012). Guidelines for the pharmacological treatment of anxiety disorders, obsessive-compulsive disorder and posttraumatic stress disorder in primary care. *International Journal of Psychiatry in Clinical Practice*, 16(2), 77–84.

Bangasser, D. A. & Cuarenta, A. (2021). Sex differences in anxiety and depression: Circuits and mechanisms. *Nature Reviews Neuroscience*, 22(11), 674–684.

Barbalho, C. A., Nunes-de-Souza, R. L., & Canto-de-Souza, A. (2009). Similar anxiolytic-like effects following intra-amygdala infusions of benzodiazepine receptor agonist and antagonist: Evidence for the release of an endogenous benzodiazepine inverse agonist in mice exposed to elevated plus-maze test. *Brain Research*, 1267, 65–76.

Barron, A. M., Higuchi, M., Hattori, S., Kito, S., Suhara, T., & Ji, B. (2021). Regulation of Anxiety and Depression by Mitochondrial Translocator Protein-Mediated Steroidogenesis: The Role of Neurons. *Molecular Neurobiology*, 58(2), 550–563.

Bartlett, A. A., Singh, R., & Hunter, R. G. (2017). Anxiety and Epigenetics. In R. Delgado-Morales (Ed.), *Neuroepigenomics in Aging and Disease*, Advances in Experimental Medicine and Biology (pp. 145–166). Cham: Springer International Publishing.

Basso, A. M., Gallagher, K. B., Mikusa, J. P., & Rueter, L. E. (2011). Vogel conflict test: Sex differences and pharmacological validation of the model. *Behavioural Brain Research*, 218(1), 174–183.

Bastiaanssen, T. F. S., Cussotto, S., Claesson, M. J., Clarke, G., Dinan, T. G., & Cryan, J. F. (2020). Gutted! Unraveling the Role of the Microbiome in Major Depressive Disorder. *Harvard Review of Psychiatry*, 28(1), 26–39.

Beery, A. K. & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neuroscience & Biobehavioral Reviews*, 35(3), 565–572.

Beiderbeck, D. I., Reber, S. O., Havasi, A., Bredewold, R., Veenema, A. H., & Neumann, I. D. (2012). High and abnormal forms of aggression in rats with extremes in trait anxiety–involvement of the dopamine system in the nucleus accumbens. *Psychoneuroendocrinology*, 37(12), 1969–1980.

Belelli, D., Herd, M. B., Mitchell, E. A., Peden, D. R., Vardy, A. W., Gentet, L., & Lambert, J. J. (2006). Neuroactive steroids and inhibitory neurotransmission: Mechanisms of action and physiological relevance. *Neuroscience*, 138(3), 821–829.

Belelli, D. & Lambert, J. J. (2005). Neurosteroids: Endogenous regulators of the GABA A receptor. *Nature Reviews Neuroscience*, 6(7), 565–575.

Berton, O., Hahn, C.-G., & Thase, M. E. (2012). Are we getting closer to valid translational models for major depression? *Science (New York, N.Y.)*, 338(6103), 75–79.

Bhutani, M. K., Bishnoi, M., & Kulkarni, S. K. (2009). Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. *Pharmacology, Biochemistry, and Behavior*, 92(1), 39–43.

Bicíková, M., Tallová, J., Hill, M., Krausová, Z., & Hampl, R. (2000). Serum concentrations of some neuroactive steroids in women suffering from mixed anxiety-depressive disorder. *Neurochemical Research*, 25(12), 1623–1627.

Birn, R. M., Shackman, A. J., Oler, J. A., Williams, L. E., McFarlin, D. R., Rogers, G. M., Shelton, S. E., Alexander, A. L., Pine, D. S., Slattery, M. J., Davidson, R. J., Fox, A. S., & Kalin, N. H. (2014). Evolutionarily conserved prefrontal-amygdalar dysfunction in early-life anxiety. *Molecular Psychiatry*, 19(8), 915–922.

Bitran, D., Hilvers, R. J., & Kellogg, C. K. (1991). Anxiolytic effects of 3α-hydroxy-5α[β]-pregnan-20-one: Endogenous metabolites of progesterone that are active at the GABAA receptor. *Brain Research*, 561(1), 157–161.

Blanchard, D. C. & Blanchard, R. J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative and Physiological Psychology*, 81(2), 281–290.

Blume, A., Bosch, O. J., Miklos, S., Torner, L., Wales, L., Waldherr, M., & Neumann, I. D. (2008). Oxytocin reduces anxiety via ERK1/2 activation: Local effect within the rat hypothalamic paraventricular nucleus. *European Journal of Neuroscience*, 27(8), 1947–1956.

Bormann, J. (2000). The 'ABC' of GABA receptors. *Trends in Pharmacological Sciences*, 21(1), 16–19.

Bosch, O. J. (2011). Maternal nurturing is dependent on her innate anxiety: The behavioral roles of brain oxytocin and vasopressin. *Hormones and Behavior*, 59, 202–212.

Bosch, O. J., Krömer, S. A., & Neumann, I. D. (2006). Prenatal stress: Opposite effects on anxiety and hypothalamic expression of vasopressin and corticotropin-releasing hormone

in rats selectively bred for high and low anxiety. *European Journal of Neuroscience*, 23(2), 541–551.

Bosch, O. J. & Neumann, I. D. (2008). Brain vasopressin is an important regulator of maternal behavior independent of dams' trait anxiety. *Proceedings of the National Academy of Sciences*, 105(44), 17139–17144.

Bosch, O. J. & Neumann, I. D. (2010). Vasopressin released within the central amygdala promotes maternal aggression. *European Journal of Neuroscience*, 31(5), 883–891.

Bosch, O. J. & Neumann, I. D. (2012). Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: From central release to sites of action. *Hormones and Behavior*, 61(3), 293–303.

Boschloo, L., Schoevers, R. A., Beekman, A. T. F., Smit, J. H., van Hemert, A. M., & Penninx, B. W. J. H. (2014). The Four-Year Course of Major Depressive Disorder: The Role of Staging and Risk Factor Determination. *Psychotherapy and Psychosomatics*, 83(5), 279–288.

Bosker, F. J., Hartman, C. A., Nolte, I. M., Prins, B. P., Terpstra, P., Posthuma, D., van Veen, T., Willemsen, G., DeRijk, R. H., de Geus, E. J., Hoogendijk, W. J., Sullivan, P. F., Penninx, B. W., Boomsma, D. I., Snieder, H., & Nolen, W. A. (2011). Poor replication of candidate genes for major depressive disorder using genome-wide association data. *Molecular Psychiatry*, 16(5), 516–532.

Braestrup, C. & Squires, R. F. (1977). Specific benzodiazepine receptors in rat brain characterized by high-affinity (3H)diazepam binding. *Proceedings of the National Academy of Sciences*, 74(9), 3805–3809.

Brambilla, F., Biggio, G., Pisu, M. G., Bellodi, L., Perna, G., Bogdanovich-Djukic, V., Purdy, R. H., & Serra, M. (2003). Neurosteroid secretion in panic disorder. *Psychiatry Research*, 118(2), 107–116.

Brot, M. D., Akwa, Y., Purdy, R. H., Koob, G. F., & Britton, K. T. (1997). The anxiolytic-like effects of the neurosteroid allopregnanolone: Interactions with GABAA receptors. *European Journal of Pharmacology*, 325(1), 1–7.

Brühl, A. B., Delsignore, A., Komossa, K., & Weidt, S. (2014). Neuroimaging in social anxiety disorder—A meta-analytic review resulting in a new neurofunctional model. *Neuroscience & Biobehavioral Reviews*, 47, 260–280.

Brunton, P. J., Donadio, M. V., Yao, S. T., Greenwood, M., Seckl, J. R., Murphy, D., & Russell, J. A. (2015). 5α-Reduced Neurosteroids Sex-Dependently Reverse Central Prenatal Programming of Neuroendocrine Stress Responses in Rats. *The Journal of Neuroscience*, 35(2), 666–677.

Busch, M., Maske, U., Ryl, L., Schlack, R., & Hapke, U. (2013). Prävalenz von depressiver Symptomatik und diagnostizierter Depression bei Erwachsenen in Deutschland: Ergebnisse der Studie zur Gesundheit Erwachsener in Deutschland (DEGS1). *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz*, 56(5-6), 733–739.

Caballero, B., Veenman, L., & Gavish, M. (2013). Role of mitochondrial translocator protein (18 kDa) on mitochondrial- related cell death processes. *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*, 7(2), 86–101.

Carnevali, L., Sgoifo, A., Trombini, M., Landgraf, R., Neumann, I. D., & Nalivaiko, E. (2013). Different Patterns of Respiration in Rat Lines Selectively Bred for High or Low Anxiety. *PLOS ONE*, 8(5), e64519.

Chaouloff, F., Durand, M., & Mormède, P. (1997). Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. *Behavioural Brain Research*, 85(1), 27–35.

Check Hayden, E. (2010). Sex bias blights drug studies. *Nature*, 464(7287), 332–333.

Chelli, B., Pini, S., Abelli, M., Cardini, A., Lari, L., Muti, M., Gesi, C., Cassano, G. B., Lucacchini, A., & Martini, C. (2008). Platelet 18 kDa Translocator Protein density is

reduced in depressed patients with adult separation anxiety. *European Neuropsychopharmacology*, 18(4), 249–254.

Choe, A. Y., Kim, B., Lee, K. S., Lee, J. E., Lee, J.-Y., Choi, T. K., & Lee, S.-H. (2013). Serotonergic Genes (5-HTT and HTR1A) and Separation Life Events: Gene-by-Environment Interaction for Panic Disorder. *Neuropsychobiology*, 67(4), 192–200.

Choi, Y. M. & Kim, K. H. (2015). Etifoxine for Pain Patients with Anxiety. *The Korean Journal of Pain*, 28(1), 4–10.

Choleris, E., Gustafsson, J.-A., Korach, K. S., Muglia, L. J., Pfaff, D. W., & Ogawa, S. (2003). An estrogen-dependent four-gene micronet regulating social recognition: A study with oxytocin and estrogen receptor-alpha and -beta knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, 100(10), 6192–6197.

Clapp, M., Aurora, N., Herrera, L., Bhatia, M., Wilen, E., & Wakefield, S. (2017). Gut microbiota's effect on mental health: The gut-brain axis. *Clinics and Practice*, 7(4), 987.

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, 18(6), 666–673.

Corchs, F., Nutt, D. J., Hince, D. A., Davies, S. J., Bernik, M., & Hood, S. D. (2015). Evidence for serotonin function as a neurochemical difference between fear and anxiety disorders in humans? *Journal of Psychopharmacology*, 29(10), 1061–1069.

Corsi, L., Geminiani, E., & Baraldi, M. (2008). Peripheral benzodiazepine receptor (PBR) new insight in cell proliferation and cell differentiation review. *Current Clinical Pharmacology*, 3(1), 38–45.

Costa, B., Da Pozzo, E., Chelli, B., Simola, N., Morelli, M., Luisi, M., Maccheroni, M., Taliani, S., Simorini, F., Da Settimo, F., & Martini, C. (2011). Anxiolytic properties of a 2-phenylindolglyoxylamide TSPO ligand: Stimulation of in vitro neurosteroid production affecting GABAA receptor activity. *Psychoneuroendocrinology*, 36(4), 463–472.

Costa, B., Pini, S., Gabelloni, P., Da Pozzo, E., Abelli, M., Lari, L., Preve, M., Lucacchini, A., Cassano, G. B., & Martini, C. (2009a). The spontaneous Ala147Thr amino acid substitution within the translocator protein influences pregnenolone production in lymphomonocytes of healthy individuals. *Endocrinology*, 150(12), 5438–5445.

Costa, B., Pini, S., Martini, C., Abelli, M., Gabelloni, P., Landi, S., Muti, M., Gesi, C., Lari, L., Cardini, A., Galderisi, S., Mucci, A., Lucacchini, A., & Cassano, G. B. (2009b). Ala147Thr substitution in translocator protein is associated with adult separation anxiety in patients with depression. *Psychiatric Genetics*, 19(2), 110–111.

Craske, M. G., Stein, M. B., Eley, T. C., Milad, M. R., Holmes, A., Rapee, R. M., & Wittchen, H.-U. (2017). Anxiety disorders. *Nature Reviews. Disease Primers*, 3, 17024.

Crawley, J. & Goodwin, F. K. (1980). Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacology Biochemistry and Behavior*, 13(2), 167–170.

Cremers, H. R., Veer, I. M., Spinhoven, P., Rombouts, S. A. R. B., Yarkoni, T., Wager, T. D., & Roelofs, K. (2015). Altered cortical-amygdala coupling in social anxiety disorder during the anticipation of giving a public speech. *Psychological medicine*, 45(7), 1521–1529.

Cryan, J. F., Markou, A., & Lucki, I. (2002). Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends in Pharmacological Sciences*, 23(5), 238–245.

Cryan, J. F., Valentino, R. J., & Lucki, I. (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neuroscience & Biobehavioral Reviews*, 29(4), 547–569.

DAK-Gesundheit (2019). Gesundheitsreport 2019. https://www.dak.de/dak/bundesthemen/gesundheitsre
2019-2099306.html/(Download on 16.01.2020).

Dalla, C. & Shors, T. J. (2009). Sex differences in learning processes of classical and operant conditioning. *Physiology & behavior*, 97(2), 229–238.

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs Sustained Fear in Rats and Humans: Role of the Extended Amygdala in Fear vs Anxiety. *Neuropsychopharmacology*, 35(1), 105–135.

de Oliveira, D. C., Zuardi, A. W., Graeff, F. G., Queiroz, R. H., & Crippa, J. A. (2012). Anxiolytic-like effect of oxytocin in the simulated public speaking test. *Journal of Psychopharmacology*, 26(4), 497–504.

Deakin, J. (2013). The origins of '5-HT and mechanisms of defence' by Deakin and Graeff: A personal perspective. *Journal of Psychopharmacology (Oxford, England)*, 27(12), 1084–1089.

Deakin, J. F. & Graeff, F. G. (1991). 5-HT and mechanisms of defence. *Journal of Psychopharmacology (Oxford, England)*, 5(4), 305–315.

Dell'Osso, B., Buoli, M., Baldwin, D. S., & Altamura, A. C. (2010). Serotonin norepinephrine reuptake inhibitors (SNRIs) in anxiety disorders: A comprehensive review of their clinical efficacy. *Human Psychopharmacology*, 25(1), 17–29.

Deplanque, D., Machuron, F., Waucquier, N., Jozefowicz, E., Duhem, S., Somers, S., Colin, O., Duhamel, A., & Bordet, R. (2018). Etifoxine impairs neither alertness nor cognitive functions of the elderly: A randomized, double-blind, placebo-controlled crossover study. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, 28(8), 925–932.

Diamant, M., Baars, A. M., Kovács, G. L., & de Wied, D. (1994). Barrel rotation induced by central arginine8-vasopressin treatment: Involvement of neurohypophyseal peptide receptors. *Pharmacology Biochemistry and Behavior*, 47(1), 27–32.

do Rego, J. L., Vaudry, D., & Vaudry, H. (2015). The Non-Benzodiazepine Anxiolytic Drug Etifoxine Causes a Rapid, Receptor-Independent Stimulation of Neurosteroid Biosynthesis. *PLOS ONE*, 10(3), e0120473.

Dolan, R. J. & Vuilleumier, P. (2003). Amygdala Automaticity in Emotional Processing. *Annals of the New York Academy of Sciences*, 985(1), 348–355.

Domonkos, E., Borbélyová, V., Csongová, M., Bosý, M., Kačmárová, M., Ostatníková, D., Hodosy, J., & Celec, P. (2017). Sex differences and sex hormones in anxiety-like behavior of aging rats. *Hormones and Behavior*, 93, 159–165.

D'Souza, D. & Sadananda, M. (2017). Estrous Cycle Phase-Dependent Changes in Anxiety- and Depression-Like Profiles in the Late Adolescent Wistar-Kyoto Rat. *Annals of Neurosciences*, 24(3), 136–145.

Durant, C., Christmas, D., & Nutt, D. (2010). The Pharmacology of Anxiety. In M. B. Stein & T. Steckler (Eds.), *Behavioral Neurobiology of Anxiety and Its Treatment*, Current Topics in Behavioral Neurosciences (pp. 303–330). Berlin, Heidelberg: Springer.

Engelmann, M., Landgraf, R., & Wotjak, C. T. (2004). The hypothalamic–neurohypophysial system regulates the hypothalamic–pituitary–adrenal axis under stress: An old concept revisited. *Frontiers in Neuroendocrinology*, 25(3), 132–149.

Engin, E. & Treit, D. (2007). The anxiolytic-like effects of allopregnanolone vary as a function of intracerebral microinfusion site: The amygdala, medial prefrontal cortex, or hippocampus. *Behavioural Pharmacology*, 18(5-6), 461–470.

Eser, D., Baghai, T. C., Schule, C., & Rupprecht, C. N. a. R. (2008). Neuroactive Steroids as Endogenous Modulators of Anxiety. http://www.eurekaselect.com/68186/article.

Evans, R. M. (1988). The steroid and thyroid hormone receptor superfamily. *Science*, 240(4854), 889–895.

Fan, J., Campioli, E., Sottas, C., Zirkin, B., & Papadopoulos, V. (2020). Amhr2-Cre-Mediated Global Tspo Knockout. *Journal of the Endocrine Society*, 4(2), bvaa001.

Farhane-Medina, N. Z., Luque, B., Tabernero, C., & Castillo-Mayén, R. (2022). Factors associated with gender and sex differences in anxiety prevalence and comorbidity: A systematic review. *Science Progress*, 105(4), 00368504221135469.

Foster, J. A., Baker, G. B., & Dursun, S. M. (2021). The Relationship Between the Gut

Microbiome-Immune System-Brain Axis and Major Depressive Disorder. *Frontiers in Neurology*, 12.

Frank, E., Salchner, P., Aldag, J. M., Salomé, N., Singewald, N., Landgraf, R., & Wigger, A. (2006). Genetic predisposition to anxiety-related behavior determines coping style, neuroendocrine responses, and neuronal activation during social defeat. *Behavioral Neuroscience*, 120(1), 60–71.

Frankiensztajn, L. M., Elliott, E., & Koren, O. (2020). The microbiota and the hypothalamus-pituitary-adrenocortical (HPA) axis, implications for anxiety and stress disorders. *Current Opinion in Neurobiology*, 62, 76–82.

Friedman, E., Berman, M., & Overstreet, D. (2006). Swim test immobility in a genetic rat model of depression is modified by maternal environment: A cross-foster study. *Developmental Psychobiology*, 48(2), 169–177.

Garakani, A., Murrough, J. W., Freire, R. C., Thom, R. P., Larkin, K., Buono, F. D., & Iosifescu, D. V. (2020). Pharmacotherapy of Anxiety Disorders: Current and Emerging Treatment Options. *Frontiers in Psychiatry*, 11.

Gardener, E. K. T., Carr, A. R., MacGregor, A., & Felmingham, K. L. (2013). Sex Differences and Emotion Regulation: An Event-Related Potential Study. *PLOS ONE*, 8(10), e73475.

Geschwind, D. H. & Flint, J. (2015). Genetics and genomics of psychiatric disease. *Science (New York, N.Y.)*, 349(6255), 1489–1494.

Giatti, S., Diviccaro, S., Garcia-Segura, L. M., & Melcangi, R. C. (2019). Sex differences in the brain expression of steroidogenic molecules under basal conditions and after gonadectomy. *Journal of Neuroendocrinology*, 31(6), e12736.

Giatti, S., Diviccaro, S., Serafini, M. M., Caruso, D., Garcia-Segura, L. M., Viviani, B., & Melcangi, R. C. (2020). Sex differences in steroid levels and steroidogenesis in the nervous system: Physiopathological role. *Frontiers in Neuroendocrinology*, 56, 100804.

Girard, C., Liu, S., Adams, D., Lacroix, C., Sinéus, M., Boucher, C., Papadopoulos, V., Rupprecht, R., Schumacher, M., & Groyer, G. (2012). Axonal Regeneration and Neuroinflammation: Roles for the Translocator Protein 18 kDa. *Journal of Neuroendocrinology*, 24(1), 71–81.

Goodyer, I. M., Herbert, J., Tamplin, A., & Altham, P. M. E. (2000). Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. *The British Journal of Psychiatry*, 177(6), 499–504.

Gottschalk, M. G. & Domschke, K. (2017). Genetics of generalized anxiety disorder and related traits. *Dialogues in Clinical Neuroscience*, 19(2), 159–168.

Gryksa, K., Schmidtner, A. K., Masís-Calvo, M., Rodríguez-Villagra, O. A., Havasi, A., Wirobski, G., Maloumby, R., Jägle, H., Bosch, O. J., Slattery, D. A., & Neumann, I. D. (2023). Selective breeding of rats for high (HAB) and low (LAB) anxiety-related behaviour: A unique model for comorbid depression and social dysfunctions. *Neuroscience and Biobehavioral Reviews*, (pp. 105292).

Gupta, R. R., Sen, S., Diepenhorst, L. L., Rudick, C. N., & Maren, S. (2001). Estrogen modulates sexually dimorphic contextual fear conditioning and hippocampal long-term potentiation (LTP) in rats(1). *Brain Research*, 888(2), 356–365.

Gur, T. L., Palkar, A. V., Rajasekera, T., Allen, J., Niraula, A., Godbout, J., & Bailey, M. T. (2019). Prenatal stress disrupts social behavior, cortical neurobiology and commensal microbes in adult male offspring. *Behavioural Brain Research*, 359, 886–894.

Gururajan, A., Reif, A., Cryan, J. F., & Slattery, D. A. (2019). The future of rodent models in depression research. *Nature Reviews Neuroscience*, 20(11), 686–701.

Gustavsson, A., Svensson, M., Jacobi, F., Allgulander, C., Alonso, J., Beghi, E., Dodel, R., Ekman, M., Faravelli, C., Fratiglioni, L., Gannon, B., Jones, D. H., Jennum, P., Jordanova, A., Jönsson, L., Karampampa, K., Knapp, M., Kobelt, G., Kurth, T., Lieb, R., Linde, M., Ljungcrantz, C., Maercker, A., Melin, B., Moscarelli, M., Musayev, A., Norwood, F., Preisig, M., Pugliatti, M., Rehm, J., Salvador-Carulla, L., Schlehofer, B.,

Simon, R., Steinhausen, H.-C., Stovner, L. J., Vallat, J.-M., Van den Bergh, P., den Bergh, P. V., van Os, J., Vos, P., Xu, W., Wittchen, H.-U., Jönsson, B., Olesen, J., & CDBE2010Study Group (2011). Cost of disorders of the brain in Europe 2010. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, 21(10), 718–779.

Hamon, A., Morel, A., Hue, B., Verleye, M., & Gillardin, J. M. (2003). The modulatory effects of the anxiolytic etifoxine on GABA(A) receptors are mediated by the beta subunit. *Neuropharmacology*, 45(3), 293–303.

Haug, M., Spetz, J. F., Ouss-Schlegel, M. L., Baulieu, E. E., & Robel, P. (1988). [The role of dehydroepiandrosterone and pregnenolone in the expression of stress behavior towards lactating females in mice]. *Pathologie-Biologie*, 36(8), 995–1001.

Henniger, M. S. H., Ohl, F., Hölter, S. M., Weißenbacher, P., Toschi, N., Lörscher, P., Wigger, A., Spanagel, R., & Landgraf, R. (2000). Unconditioned anxiety and social behaviour in two rat lines selectively bred for high and low anxiety-related behaviour. *Behavioural Brain Research*, 111(1), 153–163.

Heydari, B. & Le Mellédo, J.-M. (2002). Low pregnenolone sulphate plasma concentrations in patients with generalized social phobia. *Psychological Medicine*, 32(5), 929–933.

Hill, M. N., Hellemans, K. G. C., Verma, P., Gorzalka, B. B., & Weinberg, J. (2012). Neurobiology of chronic mild stress: Parallels to major depression. *Neuroscience & Biobehavioral Reviews*, 36(9), 2085–2117.

Hirsch, J. D., Beyer, C. F., Malkowitz, L., Beer, B., & Blume, A. J. (1989). Mitochondrial benzodiazepine receptors mediate inhibition of mitochondrial respiratory control. *Molecular Pharmacology*, 35(1), 157–163.

Hovens, J. G. F. M., Giltay, E. J., Wiersma, J. E., Spinhoven, P., Penninx, B. W. J. H., & Zitman, F. G. (2012). Impact of childhood life events and trauma on the course of depressive and anxiety disorders. *Acta Psychiatrica Scandinavica*, 126(3), 198–207.

Huang, H., Michetti, C., Busnelli, M., Managò, F., Sannino, S., Scheggia, D., Giancardo, L., Sona, D., Murino, V., Chini, B., Scattoni, M. L., & Papaleo, F. (2014). Chronic and acute intranasal oxytocin produce divergent social effects in mice. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 39(5), 1102–1114.

Huber, D., Veinante, P., & Stoop, R. (2005). Vasopressin and Oxytocin Excite Distinct Neuronal Populations in the Central Amygdala. *Science*, 308(5719), 245–248.

Hunter, R. G. & McEwen, B. S. (2013). Stress and anxiety across the lifespan: Structural plasticity and epigenetic regulation. *Epigenomics*, 5(2), 177–194.

Hyman, S. (2014). Mental health: Depression needs large human-genetics studies. *Nature News*, 515(7526), 189.

Jacobi, F., Höfler, M., Siegert, J., Mack, S., Gerschler, A., Scholl, L., Busch, M. A., Hapke, U., Maske, U., Seiffert, I., Gaebel, W., Maier, W., Wagner, M., Zielasek, J., & Wittchen, H.-U. (2014). Twelve-month prevalence, comorbidity and correlates of mental disorders in Germany: The Mental Health Module of the German Health Interview and Examination Survey for Adults (DEGS1-MH). *International Journal of Methods in Psychiatric Research*, 23(3), 304–319.

Jalnapurkar, I., Allen, M., & Pigott, T. (2018). Sex Differences in Anxiety Disorders: A Review. *Psychiatry, Depression & Anxiety*, 4, 1–9.

Jašarević, E., Howard, C. D., Morrison, K., Misic, A., Weinkopff, T., Scott, P., Hunter, C., Beiting, D., & Bale, T. L. (2018). The maternal vaginal microbiome partially mediates the effects of prenatal stress on offspring gut and hypothalamus. *Nature Neuroscience*, 21(8), 1061–1071.

Jean-Richard-Dit-Bressel, P., Killcross, S., & McNally, G. P. (2018). Behavioral and neurobiological mechanisms of punishment: Implications for psychiatric disorders. *Neuropsychopharmacology*, 43(8), 1639–1650.

Johnson, M. R., Marazziti, D., Brawman-Mintzer, O., Emmanuel, N. P., Ware, M. R., Morton, W. A., Rossi, A., Cassano, G. B., & Lydiard, R. B. (1998). Abnormal peripheral benzodiazepine receptor density associated with generalized social phobia. *Biological Psychiatry*, 43(4), 306–309.

Juruena, M. F., Agustini, B., Cleare, A. J., & Young, A. H. (2017). A translational approach to clinical practice via stress-responsive glucocorticoid receptor signaling. *Stem Cell Investigation*, 4.

Juruena, M. F., Pariante, C. M., Papadopoulos, A. S., Poon, L., Lightman, S., & Cleare, A. J. (2009). Prednisolone suppression test in depression: Prospective study of the role of HPA axis dysfunction in treatment resistance. *The British Journal of Psychiatry*, 194(4), 342–349.

Keck, M. E., Welt, T., Müller, M. B., Uhr, M., Ohl, F., Wigger, A., Toschi, N., Holsboer, F., & Landgraf, R. (2003). Reduction of Hypothalamic Vasopressinergic Hyperdrive Contributes to Clinically Relevant Behavioral and Neuroendocrine Effects of Chronic Paroxetine Treatment in a Psychopathological Rat Model. *Neuropsychopharmacology*, 28(2), 235–243.

Keck, M. E., Wigger, A., Welt, T., Müller, M. B., Gesing, A., Reul, J. M. H. M., Holsboer, F., Landgraf, R., & Neumann, I. D. (2002). Vasopressin mediates the response of the combined dexamethasone/CRH test in hyper-anxious rats: Implications for pathogenesis of affective disorders. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 26(1), 94–105.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, 62(6), 593–602.

Kessler, R. C., Berglund, P. A., Bruce, M. L., Koch, J. R., Laska, E. M., Leaf, P. J., Manderscheid, R. W., Rosenheck, R. A., Walters, E. E., & Wang, P. S. (2001). The

prevalence and correlates of untreated serious mental illness. *Health Services Research*, 36(6 Pt 1), 987–1007.

Khisti, R. T., Chopde, C. T., & Jain, S. P. (2000). Antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice forced swim test. *Pharmacology, Biochemistry, and Behavior*, 67(1), 137–143.

Kinlein, S. A., Phillips, D. J., Keller, C. R., & Karatsoreos, I. N. (2019). Role of corticosterone in altered neurobehavioral responses to acute stress in a model of compromised hypothalamic-pituitary-adrenal axis function. *Psychoneuroendocrinology*, 102, 248–255.

Kita, A. & Furukawa, K. (2008). Involvement of neurosteroids in the anxiolytic-like effects of AC-5216 in mice. *Pharmacology Biochemistry and Behavior*, 89(2), 171–178.

Kogan, C. S., Stein, D. J., Maj, M., First, M. B., Emmelkamp, P. M. G., & Reed, G. M. (2016). The Classification of Anxiety and Fear-Related Disorders in the ICD-11. *Depression and Anxiety*, 33(12), 1141–1154.

Kohn, R., Saxena, S., Levav, I., & Saraceno, B. (2004). The treatment gap in mental health care. *Bulletin of the World Health Organization*, 82(11), 858–866.

Kokras, N. & Dalla, C. (2017). Preclinical sex differences in depression and antidepressant response: Implications for clinical research. *Journal of Neuroscience Research*, 95(1-2), 731–736.

Kosfeld, M., Heinrichs, M., Zak, P. J., Fischbacher, U., & Fehr, E. (2005). Oxytocin increases trust in humans. *Nature*, 435(7042), 673–676.

Kraus, C., Castrén, E., Kasper, S., & Lanzenberger, R. (2017). Serotonin and neuroplasticity - Links between molecular, functional and structural pathophysiology in depression. *Neuroscience and Biobehavioral Reviews*, 77, 317–326.

Labonté, B., Engmann, O., Purushothaman, I., Menard, C., Wang, J., Tan, C., Scarpa, J. R., Moy, G., Loh, Y.-H. E., Cahill, M., Lorsch, Z. S., Hamilton, P. J., Calipari, E. S., Hodes, G. E., Issler, O., Kronman, H., Pfau, M., Obradovic, A. L. J., Dong, Y., Neve,

R. L., Russo, S., Kasarskis, A., Tamminga, C., Mechawar, N., Turecki, G., Zhang, B., Shen, L., & Nestler, E. J. (2017). Sex-specific transcriptional signatures in human depression. *Nature Medicine*, 23(9), 1102–1111.

Lacapère, J. J. & Papadopoulos, V. (2003). Peripheral-type benzodiazepine receptor: Structure and function of a cholesterol-binding protein in steroid and bile acid biosynthesis. *Steroids*, 68(7-8), 569–585.

Lai, C.-H. (2020). Task MRI-Based Functional Brain Network of Anxiety. *Advances in Experimental Medicine and Biology*, 1191, 3–20.

Lambert, J. J., Belelli, D., Hill-Venning, C., & Peters, J. A. (1995). Neurosteroids and GABAA receptor function. *Trends in Pharmacological Sciences*, 16(9), 295–303.

Lambert, J. J., Belelli, D., Peden, D. R., Vardy, A. W., & Peters, J. A. (2003). Neurosteroid modulation of GABAA receptors. *Progress in Neurobiology*, 71(1), 67–80.

Landgraf, R. & Neumann, I. D. (2004). Vasopressin and oxytocin release within the brain: A dynamic concept of multiple and variable modes of neuropeptide communication. *Frontiers in Neuroendocrinology*, 25(3-4), 150–176.

Landgraf, R. & Wigger, A. (2002). High vs low anxiety-related behavior rats: An animal model of extremes in trait anxiety. *Behavior Genetics*, 32(5), 301–314.

Landgraf, R. & Wigger, A. (2003). Born to be anxious: Neuroendocrine and genetic correlates of trait anxiety in HAB rats. *Stress (Amsterdam, Netherlands)*, 6(2), 111–119.

Landgraf, R., Wigger, A., Holsboer, F., & Neumann, I. D. (1999). Hyper-reactive hypothalamo-pituitary-adrenocortical axis in rats bred for high anxiety-related behaviour. *Journal of Neuroendocrinology*, 11(6), 405–407.

Lassé, M., Pilbrow, A. P., Kleffmann, T., Andersson Överström, E., von Zychlinski, A., Frampton, C. M. A., Poppe, K. K., Troughton, R. W., Lewis, L. K., Prickett, T. C. R., Pemberton, C. J., Richards, A. M., & Cameron, V. A. (2021). Fibrinogen and hemoglobin

predict near future cardiovascular events in asymptomatic individuals. *Scientific Reports*, 11(1), 4605.

Lavaque, E., Sierra, A., Azcoitia, I., & Garcia-Segura, L. M. (2006). Steroidogenic acute regulatory protein in the brain. *Neuroscience*, 138(3), 741–747.

Le Merrer, J., Becker, J. A. J., Befort, K., & Kieffer, B. L. (2009). Reward processing by the opioid system in the brain. *Physiological Reviews*, 89(4), 1379–1412.

LeDoux, J. E. & Pine, D. S. (2016). Using Neuroscience to Help Understand Fear and Anxiety: A Two-System Framework. *American Journal of Psychiatry*, 173(11), 1083–1093.

Li, H., Yao, Z., Degenhardt, B., Teper, G., & Papadopoulos, V. (2001). Cholesterol binding at the cholesterol recognition/ interaction amino acid consensus (CRAC) of the peripheral-type benzodiazepine receptor and inhibition of steroidogenesis by an HIV TAT-CRAC peptide. *Proceedings of the National Academy of Sciences of the United States of America*, 98(3), 1267–1272.

Li, S. H. & Graham, B. M. (2017). Why are women so vulnerable to anxiety, trauma-related and stress-related disorders? The potential role of sex hormones. *The Lancet. Psychiatry*, 4(1), 73–82.

Liauzun, E., Delmas, P., Shire, D., & Ferrara, P. (1998). Topological analysis of the peripheral benzodiazepine receptor in yeast mitochondrial membranes supports a five-transmembrane structure. *The Journal of Biological Chemistry*, 273(4), 2146–2152.

Liebsch, G., Linthorst, A. C., Neumann, I. D., Reul, J. M., Holsboer, F., & Landgraf, R. (1998). Behavioral, physiological, and neuroendocrine stress responses and differential sensitivity to diazepam in two Wistar rat lines selectively bred for high- and low-anxiety-related behavior. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 19(5), 381–396.

Liere, P., Pianos, A., Oudinet, J.-P., Schumacher, M., & Akwa, Y. (2017). Differential effects of the 18-kDa translocator protein (TSPO) ligand etifoxine on steroidogenesis in rat brain,

plasma and steroidogenic glands: Pharmacodynamic studies. *Psychoneuroendocrinology*, 83, 122–134.

Lisboa, S. F., Stecchini, M. F., Corrêa, F. M. A., Guimarães, F. S., & Resstel, L. B. M. (2010). Different role of the ventral medial prefrontal cortex on modulation of innate and associative learned fear. *Neuroscience*, 171(3), 760–768.

Lovick, T. A. & Zangrossi, H. (2021). Effect of Estrous Cycle on Behavior of Females in Rodent Tests of Anxiety. *Frontiers in Psychiatry*, 12, 1492.

Löw, K., Crestani, F., Keist, R., Benke, D., Brünig, I., Benson, J. A., Fritschy, J. M., Rülicke, T., Bluethmann, H., Möhler, H., & Rudolph, U. (2000). Molecular and neuronal substrate for the selective attenuation of anxiety. *Science (New York, N.Y.)*, 290(5489), 131–134.

Lungu, O., Potvin, S., Tikàsz, A., & Mendrek, A. (2015). Sex differences in effective fronto-limbic connectivity during negative emotion processing. *Psychoneuroendocrinology*, 62, 180–188.

Luscher, B., Shen, Q., & Sahir, N. (2011). The GABAergic deficit hypothesis of major depressive disorder. *Molecular Psychiatry*, 16(4), 383–406.

Majewska, M. D. (1992). Neurosteroids: Endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. *Progress in Neurobiology*, 38(4), 379–395.

Malhi, G. S. & Mann, J. J. (2018). Depression. *The Lancet*, 392(10161), 2299–2312.

Marcondes, F. K., Bianchi, F. J., & Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: Some helpful considerations. *Brazilian Journal of Biology*, 62(4A), 609–614.

Marrannes, R. & Wauquier, A. (1988). Episodic barrel rotations induced by intrastriatal injection of quinolinic acid in rats. Inhibition by anticonvulsants. *Pharmacology, Biochemistry, and Behavior*, 31(1), 153–162.

McEwen, B. S. (1991). Non-genomic and genomic effects of steroids on neural activity. *Trends in Pharmacological Sciences*, 12(4), 141–147.

Mellon, S. H. & Griffin, L. D. (2002). Neurosteroids: Biochemistry and clinical significance. *Trends in Endocrinology & Metabolism*, 13(1), 35–43.

Ménard, C., Hodes, G. E., & Russo, S. J. (2016). Pathogenesis of depression: Insights from human and rodent studies. *Neuroscience*, 321, 138–162.

Merikangas, A. K. & Almasy, L. (2020). Using the tools of genetic epidemiology to understand sex differences in neuropsychiatric disorders. *Genes, Brain, and Behavior*, 19(6), e12660.

Micallef, J., Soubrouillard, C., Guet, F., Le Guern, M. E., Alquier, C., Bruguerolle, B., & Blin, O. (2001). A double blind parallel group placebo controlled comparison of sedative and mnesic effects of etifoxine and lorazepam in healthy subjects [corrected]. *Fundamental & Clinical Pharmacology*, 15(3), 209–216.

Milad, M. R. & Quirk, G. J. (2012). Fear Extinction as a Model for Translational Neuroscience: Ten Years of Progress. *Annual review of psychology*, 63, 129–151.

Millan, M. J. (2003). The neurobiology and control of anxious states. *Progress in Neurobiology*, 70(2), 83–244.

Mobbs, D., Petrovic, P., Marchant, J. L., Hassabis, D., Weiskopf, N., Seymour, B., Dolan, R. J., & Frith, C. D. (2007). When Fear Is Near. *Science (New York, N.Y.)*, 317(5841), 1079–1083.

Moch, C., Rocher, F., Lainé, P., Lacotte, J., Biour, M., Gouraud, A., Bernard, N., Descotes, J., & Vial, T. (2012). Etifoxine-induced acute hepatitis: A case series. *Clinics and Research in Hepatology and Gastroenterology*, 36(5), e85–e88.

Möhler, H. (2006). GABA(A) receptor diversity and pharmacology. *Cell and Tissue Research*, 326(2), 505–516.

Morohaku, K., Pelton, S. H., Daugherty, D. J., Butler, W. R., Deng, W., & Selvaraj, V. (2014). Translocator protein/peripheral benzodiazepine receptor is not required for steroid hormone biosynthesis. *Endocrinology*, 155(1), 89–97.

Muigg, P., Hoelzl, U., Palfrader, K., Neumann, I., Wigger, A., Landgraf, R., & Singewald, N. (2007). Altered Brain Activation Pattern Associated With Drug-Induced Attenuation of Enhanced Depression-Like Behavior in Rats Bred for High Anxiety. *Biological Psychiatry*, 61(6), 782–796.

Murgatroyd, C., Wigger, A., Frank, E., Singewald, N., Bunck, M., Holsboer, F., Landgraf, R., & Spengler, D. (2004). Impaired repression at a vasopressin promoter polymorphism underlies overexpression of vasopressin in a rat model of trait anxiety. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 24(35), 7762–7770.

Nestler, E. J. & Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. *Nature Neuroscience*, 13(10), 1161–1169.

Neumann, I. D. & Slattery, D. A. (2016). Oxytocin in General Anxiety and Social Fear: A Translational Approach. *Biological Psychiatry*, 79(3), 213–221.

Neumann, I. D., Torner, L., & Wigger, A. (2000). Brain oxytocin: Differential inhibition of neuroendocrine stress responses and anxiety-related behaviour in virgin, pregnant and lactating rats. *Neuroscience*, 95(2), 567–575.

Neumann, I. D., Wegener, G., Homberg, J. R., Cohen, H., Slattery, D. A., Zohar, J., Olivier, J. D. A., & Mathé, A. A. (2011). Animal models of depression and anxiety: What do they tell us about human condition? *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 35(6), 1357–1375.

Nguyen, N., Fakra, E., Pradel, V., Jouve, E., Alquier, C., Guern, M.-E. L., Micallef, J., & Blin, O. (2006). Efficacy of etifoxine compared to lorazepam monotherapy in the treatment of patients with adjustment disorders with anxiety: A double-blind controlled study in general practice. *Human Psychopharmacology: Clinical and Experimental*, 21(3), 139–149.

Nillni, Y. I., Rasmusson, A. M., Paul, E. L., & Pineles, S. L. (2021). The Impact of the Menstrual Cycle and Underlying Hormones in Anxiety and PTSD: What Do We Know and Where Do We Go From Here? *Current Psychiatry Reports*, 23(2), 8.

Nothdurfter, C., Rammes, G., Baghai, T. C., Schüle, C., Schumacher, M., Papadopoulos, V., & Rupprecht, R. (2012). Translocator Protein (18 kDa) as a Target for Novel Anxiolytics with a Favourable Side-Effect Profile. *Journal of Neuroendocrinology*, 24(1), 82–92.

Nudmamud, S., Siripurkpong, P., Chindaduangratana, C., Harnyuttanakorn, P., Lotrakul, P., Laarbboonsarp, W., Srikiatkhachorn, A., Kotchabhakdi, N., & Casalotti, S. O. (2000). Stress, anxiety and peripheral benzodiazepine receptor mRNA levels in human lymphocytes. *Life Sciences*, 67(18), 2221–2231.

Nuss, P., Ferreri, F., & Bourin, M. (2019). An update on the anxiolytic and neuroprotective properties of etifoxine: From brain GABA modulation to a whole-body mode of action. *Neuropsychiatric Disease and Treatment*, 15, 1781–1795.

Nutt, D. J. (1990). The pharmacology of human anxiety. *Pharmacology & Therapeutics*, 47(2), 233–266.

Ohl, F., Holsboer, F., & Landgraf, R. (2001a). The modified hole board as a differential screen for behavior in rodents. *Behavior Research Methods, Instruments, & Computers: A Journal of the Psychonomic Society, Inc*, 33(3), 392–397.

Ohl, F., Toschi, N., Wigger, A., Henniger, M. S. H., & Landgraf, R. (2001b). Dimensions of emotionality in a rat model of innate anxiety. *Behavioral Neuroscience*, 115(2), 429–436.

Okuyama, S., Chaki, S., Yoshikawa, R., Ogawa, S., Suzuki, Y., Okubo, T., Nakazato, A., Nagamine, M., & Tomisawa, K. (1999). Neuropharmacological profile of peripheral benzodiazepine receptor agonists, DAA1097 and DAA1106. *Life Sciences*, 64(16), 1455–1464.

Olfson, M., King, M., & Schoenbaum, M. (2015). Benzodiazepine use in the United States. *JAMA psychiatry*, 72(2), 136–142.

Olsen, R. W. & Sieghart, W. (2009). GABA A receptors: Subtypes provide diversity of function and pharmacology. *Neuropharmacology*, 56(1), 141–148.

Ormel, J., Oldehinkel, A. J., Nolen, W. A., & Vollebergh, W. (2004). Psychosocial Disability Before, During, and After a Major DepressiveEpisode: A 3-Wave Population-Based Study of State, Scar, and Trait Effects. *Archives of General Psychiatry*, 61(4), 387.

Otte, C., Gold, S. M., Penninx, B. W., Pariante, C. M., Etkin, A., Fava, M., Mohr, D. C., & Schatzberg, A. F. (2016). Major depressive disorder. *Nature Reviews Disease Primers*, 2(1), 1–20.

Overstreet, D. H. & Griebel, G. (2004). Antidepressant-like effects of CRF1 receptor antagonist SSR125543 in an animal model of depression. *European Journal of Pharmacology*, 497(1), 49–53.

Overstreet, D. H., Rezvani, A. H., & Janowsky, D. S. (1992). Genetic animal models of depression and ethanol preference provide support for cholinergic and serotonergic involvement in depression and alcoholism. *Biological Psychiatry*, 31(9), 919–936.

Owen, D. R., Fan, J., Campioli, E., Venugopal, S., Midzak, A., Daly, E., Harlay, A., Issop, L., Libri, V., Kalogiannopoulou, D., Oliver, E., Gallego-Colon, E., Colasanti, A., Huson, L., Rabiner, E. A., Suppiah, P., Essagian, C., Matthews, P. M., & Papadopoulos, V. (2017). TSPO mutations in rats and a human polymorphism impair the rate of steroid synthesis. *The Biochemical Journal*, 474(23), 3985–3999.

Owen, D. R., Yeo, A. J., Gunn, R. N., Song, K., Wadsworth, G., Lewis, A., Rhodes, C., Pulford, D. J., Bennacef, I., Parker, C. A., StJean, P. L., Cardon, L. R., Mooser, V. E., Matthews, P. M., Rabiner, E. A., & Rubio, J. P. (2012). An 18-kDa Translocator Protein (TSPO) Polymorphism Explains Differences in Binding Affinity of the PET Radioligand PBR28. *Journal of Cerebral Blood Flow & Metabolism*, 32(1), 1–5.

Palumbo, M. A., Salvestroni, C., Gallo, R., Guo, A. L., Genazzani, A. D., Artini, P. G., Petraglia, F., & Genazzani, A. R. (1995). Allopregnanolone concentration in hippocampus

of prepubertal rats and female rats throughout estrous cycle. *Journal of Endocrinological Investigation*, 18(11), 853–856.

Pan, J.-X., Deng, F.-L., Zeng, B.-H., Zheng, P., Liang, W.-W., Yin, B.-M., Wu, J., Dong, M.-X., Luo, Y.-Y., Wang, H.-Y., Wei, H., & Xie, P. (2019). Absence of gut microbiota during early life affects anxiolytic Behaviors and monoamine neurotransmitters system in the hippocampal of mice. *Journal of the Neurological Sciences*, 400, 160–168.

Papadopoulos, V., Amri, H., Boujrad, N., Cascio, C., Culty, M., Garnier, M., Hardwick, M., Li, H., Vidic, B., Brown, A. S., Reversa, J. L., Bernassau, J. M., & Drieu, K. (1997). Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis. *Steroids*, 62(1), 21–28.

Papadopoulos, V., Baraldi, M., Guilarte, T. R., Knudsen, T. B., Lacapère, J.-J., Lindemann, P., Norenberg, M. D., Nutt, D., Weizman, A., Zhang, M.-R., & Gavish, M. (2006). Translocator protein (18kDa): New nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends in Pharmacological Sciences*, 27(8), 402–409.

Papadopoulos, V., Fan, J., & Zirkin, B. (2018). TRANSLOCATOR PROTEIN (18 kDa): AN UPDATE ON ITS FUNCTION IN STEROIDOGENESIS. *Journal of neuroendocrinology*, 30(2).

Papp, M., Nalepa, I., Antkiewicz-Michaluk, L., & Sánchez, C. (2002). Behavioural and biochemical studies of citalopram and WAY 100635 in rat chronic mild stress model. *Pharmacology, Biochemistry, and Behavior*, 72(1-2), 465–474.

Patchev, V. K., Shoaib, M., Holsboer, F., & Almeida, O. F. X. (1994). The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. *Neuroscience*, 62(1), 265–271.

Paul, E. D., Johnson, P. L., Shekhar, A., & Lowry, C. A. (2014). The Deakin/Graeff

hypothesis: Focus on serotonergic inhibition of panic. *Neuroscience & Biobehavioral Reviews*, 46, 379–396.

Paul, S. M. & Purdy, R. H. (1992). Neuroactive steroids. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 6(6), 2311–2322.

Penninx, B. W. J. H., Nolen, W. A., Lamers, F., Zitman, F. G., Smit, J. H., Spinhoven, P., Cuijpers, P., de Jong, P. J., van Marwijk, H. W. J., van der Meer, K., Verhaak, P., Laurant, M. G. H., de Graaf, R., Hoogendijk, W. J., van der Wee, N., Ormel, J., van Dyck, R., & Beekman, A. T. F. (2011). Two-year course of depressive and anxiety disorders: Results from the Netherlands Study of Depression and Anxiety (NESDA). *Journal of Affective Disorders*, 133(1), 76–85.

Perry, D. C. (1987). Barrel rotation in rats induced by intracerebroventricular bradykinin antagonists. *Pharmacology Biochemistry and Behavior*, 28(1), 15–20.

Peters, S., Slattery, D. A., Uschold-Schmidt, N., Reber, S. O., & Neumann, I. D. (2014). Dose-dependent effects of chronic central infusion of oxytocin on anxiety, oxytocin receptor binding and stress-related parameters in mice. *Psychoneuroendocrinology*, 42, 225–236.

Phelps, E. A. (2005). Emotion and Cognition: Insights from Studies of the Human Amygdala. *Annual Review of Psychology*, 57(1), 27–53.

Pillay, S. S., Gruber, S. A., Rogowska, J., Simpson, N., & Yurgelun-Todd, D. A. (2006). fMRI of fearful facial affect recognition in panic disorder: The cingulate gyrus-amygdala connection. *Journal of Affective Disorders*, 94(1-3), 173–181.

Pini, S., Martini, C., Abelli, M., Muti, M., Gesi, C., Montali, M., Chelli, B., Lucacchini, A., & Cassano, G. B. (2005). Peripheral-type benzodiazepine receptor binding sites in platelets of patients with panic disorder associated to separation anxiety symptoms. *Psychopharmacology*, 181(2), 407–411.

Puia, G., Santi, M., Vicini, S., Pritchett, D. B., Purdy, R. H., Paul, S. M., Seeburg, P. H., & Costa, E. (1990). Neurosteroids act on recombinant human GABAA receptors. *Neuron*, 4(5), 759–765.

Purdy, R. H., Morrow, A. L., Moore, P. H., & Paul, S. M. (1991). Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proceedings of the National Academy of Sciences of the United States of America*, 88(10), 4553–4557.

Reddy, D. S., Matthew Carver, C., Clossen, B., & Wu, X. (2019). Extrasynaptic GABA-A receptor-mediated sex differences in the antiseizure activity of neurosteroids in status epilepticus and complex partial seizures. *Epilepsia*, 60(4), 730–743.

Rex, A., Voigt, J. P., & Fink, H. (2005). Anxiety but not arousal increases 5-hydroxytryptamine release in the rat ventral hippocampus in vivo. *European Journal of Neuroscience*, 22(5), 1185–1189.

Rhodes, C. H., Morriell, J. I., & Pfaff, D. W. (1981). Immunohistochemical analysis of magnocellular elements in rat hypothalamus: Distribution and numbers of cells containing neurophysin, oxytocin, and vasopressin. *Journal of Comparative Neurology*, 198(1), 45–64.

Rincel, M. & Darnaudéry, M. (2020). Maternal separation in rodents: A journey from gut to brain and nutritional perspectives. *The Proceedings of the Nutrition Society*, 79(1), 113–132.

Robel, P. & Baulieu, E.-E. (1994). Neurosteroids: Biosynthesis and function. *Trends in Endocrinology & Metabolism*, 5(1), 1–8.

Robinson, O. J., Krimsky, M., Lieberman, L., Allen, P., Vytal, K., & Grillon, C. (2014). Towards a mechanistic understanding of pathological anxiety: The dorsal medial prefrontal-amygdala 'aversive amplification' circuit in unmedicated generalized and social anxiety disorders. *The Lancet. Psychiatry*, 1(4), 294–302.

Rocca, P., Beoni, A. M., Eva, C., Ferrero, P., Zanalda, E., & Ravizza, L. (1998). Peripheral benzodiazepine receptor messenger RNA is decreased in lymphocytes of generalized anxiety disorder patients. *Biological Psychiatry*, 43(10), 767–773.

Romeo, E., Cavallaro, S., Korneyev, A., Kozikowski, A. P., Ma, D., Polo, A., Costa, E., & Guidotti, A. (1993). Stimulation of brain steroidogenesis by 2-aryl-indole-3-acetamide

derivatives acting at the mitochondrial diazepam-binding inhibitor receptor complex. *The Journal of Pharmacology and Experimental Therapeutics*, 267(1), 462–471.

Romeo, E., Ströhle, A., Spalletta, G., di Michele, F., Hermann, B., Holsboer, F., Pasini, A., & Rupprecht, R. (1998). Effects of antidepressant treatment on neuroactive steroids in major depression. *The American Journal of Psychiatry*, 155(7), 910–913.

Rupprecht, R. (2003). Neuroactive steroids: Mechanisms of action and neuropsychopharmacological properties. *Psychoneuroendocrinology*, 28(2), 139–168.

Rupprecht, R. & Holsboer, F. (1999). Neuroactive steroids: Mechanisms of action and neuropsychopharmacological perspectives. *Trends in Neurosciences*, 22(9), 410–416.

Rupprecht, R., Papadopoulos, V., Rammes, G., Baghai, T. C., Fan, J., Akula, N., Groyer, G., Adams, D., & Schumacher, M. (2010). Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nature Reviews. Drug Discovery*, 9(12), 971–988.

Rupprecht, R., Pradhan, A. K., Kufner, M., Brunner, L. M., Nothdurfter, C., Wein, S., Schwarzbach, J., Puig, X., Rupprecht, C., & Rammes, G. (2023). Neurosteroids and translocator protein 18 kDa (TSPO) in depression: Implications for synaptic plasticity, cognition, and treatment options. *European Archives of Psychiatry and Clinical Neuroscience*, 273(7), 1477–1487.

Rupprecht, R., Wetzel, C. H., Dorostkar, M., Herms, J., Albert, N. L., Schwarzbach, J., Schumacher, M., & Neumann, I. D. (2022). Translocator protein (18kDa) TSPO: A new diagnostic or therapeutic target for stress-related disorders? *Molecular Psychiatry*, 27(7), 2918–2926.

Salomé, N., Viltart, O., Darnaudéry, M., Salchner, P., Singewald, N., Landgraf, R., Sequeira, H., & Wigger, A. (2002). Reliability of high and low anxiety-related behaviour:: Influence of laboratory environment and multifactorial analysis. *Behavioural Brain Research*, 136(1), 227–237.

Sanders, S. K. & Shekhar, A. (1995). Regulation of anxiety by GABAA receptors in the rat amygdala. *Pharmacology Biochemistry and Behavior*, 52(4), 701–706.

Saridjan, N. S., Huizink, A. C., Koetsier, J. A., Jaddoe, V. W., Mackenbach, J. P., Hofman, A., Kirschbaum, C., Verhulst, F. C., & Tiemeier, H. (2010). Do social disadvantage and early family adversity affect the diurnal cortisol rhythm in infants? The Generation R Study. *Hormones and Behavior*, 57(2), 247–254.

Scherma, M., Giunti, E., Fratta, W., & Fadda, P. (2019). Gene knockout animal models of depression, anxiety and obsessive compulsive disorders. *Psychiatric Genetics*, 29(5), 191.

Schlichter, R., Rybalchenko, V., Poisbeau, P., Verleye, M., & Gillardin, J.-M. (2000). Modulation of GABAergic synaptic transmission by the non-benzodiazepine anxiolytic etifoxine. *Neuropharmacology*, 39(9), 1523–1535.

Schmidt, P. J., Purdy, R. H., Moore, Jr, P. H., Paul, S. M., & Rubinow, D. R. (1994). Circulating levels of anxiolytic steroids in the luteal phase in women with premenstrual syndrome and in control subjects. *The Journal of Clinical Endocrinology & Metabolism*, 79(5), 1256–1260.

Schmidtner, A. K., Slattery, D. A., Gläsner, J., Hiergeist, A., Gryksa, K., Malik, V. A., Hellmann-Regen, J., Heuser, I., Baghai, T. C., Gessner, A., Rupprecht, R., Di Benedetto, B., & Neumann, I. D. (2019). Minocycline alters behavior, microglia and the gut microbiome in a trait-anxiety-dependent manner. *Translational Psychiatry*, 9(1), 1–12.

Schnatz, P. F., Whitehurst, S. K., & O'Sullivan, D. M. (2010). Sexual dysfunction, depression, and anxiety among patients of an inner-city menopause clinic. *Journal of Women's Health (2002)*, 19(10), 1843–1849.

Schofield, C. A., Abdul-Chani, M., & Gaudiano, B. A. (2020). Impact of causal explanations for social anxiety disorder on stigma and treatment perceptions. *Journal of Mental Health*, 29(1), 84–91.

Schüle, C., Nothdurfter, C., & Rupprecht, R. (2014). The role of allopregnanolone in depression and anxiety. *Progress in Neurobiology*, 113, 79–87.

Schumacher, M., Weill-Engerer, S., Liere, P., Robert, F., Franklin, R. J. M., Garcia-Segura, L. M., Lambert, J. J., Mayo, W., Melcangi, R. C., Parducz, A., Suter, U., Carelli, C., Baulieu, E. E., & Akwa, Y. (2003). Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. *Progress in Neurobiology*, 71(1), 3–29.

Schweizer, E. & Rickels, K. (1998). Benzodiazepine dependence and withdrawal: A review of the syndrome and its clinical management. *Acta Psychiatrica Scandinavica. Supplementum*, 393, 95–101.

Seedat, S., Scott, K. M., Angermeyer, M. C., Berglund, P., Bromet, E. J., Brugha, T. S., Demyttenaere, K., de Girolamo, G., Haro, J. M., Jin, R., Karam, E. G., Kovess-Masfety, V., Levinson, D., Medina Mora, M. E., Ono, Y., Ormel, J., Pennell, B.-E., Posada-Villa, J., Sampson, N. A., Williams, D., & Kessler, R. C. (2009). Cross-National Associations Between Gender and Mental Disorders in the World Health Organization World Mental Health Surveys. *Archives of General Psychiatry*, 66(7), 785.

Semeniuk, T., Jhangri, G. S., & Le Mellédo, J. M. (2001). Neuroactive steroid levels in patients with generalized anxiety disorder. *The Journal of Neuropsychiatry and Clinical Neurosciences*, 13(3), 396–398.

Seney, M. L. & Sibille, E. (2014). Sex differences in mood disorders: Perspectives from humans and rodent models. *Biology of Sex Differences*, 5, 17.

Serra, M., Madau, P., Chessa, M. F., Caddeo, M., Sanna, E., Trapani, G., Franco, M., Liso, G., Purdy, R. H., Barbaccia, M. L., & Biggio, G. (1999). 2-Phenyl-imidazo[1,2-a]pyridine derivatives as ligands for peripheral benzodiazepine receptors: Stimulation of neurosteroid synthesis and anticonflict action in rats. *British Journal of Pharmacology*, 127(1), 177–187.

Servant, D., Graziani, P. L., Moyse, D., & Parquet, P. J. (1998). [Treatment of adjustment disorder with anxiety: efficacy and tolerance of etifoxine in a double-blind controlled study]. *L'Encephale*, 24(6), 569–574.

Setiawan, E., Wilson, A. A., Mizrahi, R., Rusjan, P. M., Miler, L., Rajkowska, G., Suridjan, I., Kennedy, J. L., Rekkas, P. V., Houle, S., & Meyer, J. H. (2015). Role of Translocator Protein Density, a Marker of Neuroinflammation, in the Brain During Major Depressive Episodes. *JAMA Psychiatry*, 72(3), 268–275.

Shader, R. I. & Greenblatt, D. J. (2010). Use of Benzodiazepines in Anxiety Disorders. https://www.nejm.org/doi/10.1056/NEJM199305133281907.

Shehadeh, M., Palzur, E., Apel, L., & Soustiel, J. F. (2019). Reduction of Traumatic Brain Damage by Tspo Ligand Etifoxine. *International Journal of Molecular Sciences*, 20(11).

Shimada-Sugimoto, M., Otowa, T., & Hettema, J. M. (2015). Genetics of anxiety disorders: Genetic epidemiological and molecular studies in humans. *Psychiatry and Clinical Neurosciences*, 69(7), 388–401.

Shoshan-Barmatz, V., Israelson, A., & Sheu, D. B. a. S. S. (2006). The Voltage-Dependent Anion Channel (VDAC): Function in Intracellular Signalling, Cell Life and Cell Death. https://www.eurekaselect.com/56234/article.

Sigma-Aldrich (2021). Etifoxine hydrochloride SML0272. https://www.sigmaaldrich.com/catalog/product/sigma/sml0272?lang=deregion=DE.

Slattery, D. A. & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols*, 7(6), 1009–1014.

Slattery, D. A. & Neumann, I. D. (2010). Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats. *Neuropharmacology*, 58(1), 56–61.

Soreni, N., Apter, A., Weizman, A., Don-Tufeled, O., Leschiner, S., Karp, L., & Gavish, M. (1999). Decreased platelet peripheral-type benzodiazepine receptors in adolescent inpatients with repeated suicide attempts. *Biological Psychiatry*, 46(4), 484–488.

Stein, D. J. (2015). Etifoxine Versus Alprazolam for the Treatment of Adjustment Disorder with Anxiety: A Randomized Controlled Trial. *Advances in Therapy*, 32(1), 57–68.

Stetler, C. & Miller, G. E. (2011). Depression and Hypothalamic-Pituitary-Adrenal Activation: A Quantitative Summary of Four Decades of Research. *Psychosomatic Medicine*, 73(2), 114.

Ströhle, A., Gensichen, J., & Domschke, K. (2018). The Diagnosis and Treatment of Anxiety Disorders. *Deutsches Arzteblatt International*, 155(37), 611–620.

Ströhle, A., Romeo, E., di Michele, F., Pasini, A., Hermann, B., Gajewsky, G., Holsboer, F., & Rupprecht, R. (2003). Induced Panic Attacks Shift γ-Aminobutyric Acid Type A Receptor Modulatory Neuroactive Steroid Composition in Patients With Panic Disorder: Preliminary Results. *Archives of General Psychiatry*, 60(2), 161–168.

Ströhle, A., Romeo, E., di Michele, F., Pasini, A., Yassouridis, A., Holsboer, F., & Rupprecht, R. (2002). GABAA Receptor-Modulating Neuroactive Steroid Composition in Patients With Panic Disorder Before and During Paroxetine Treatment. *American Journal of Psychiatry*, 159(1), 145–147.

Sudakov, S. K., Nazarova, G. A., Alekseeva, E. V., & Bashkatova, V. G. (2013). Estimation of the Level of Anxiety in Rats: Differences in Results of Open-Field Test, Elevated Plus-Maze Test, and Vogel's Conflict Test. *Bulletin of Experimental Biology and Medicine*, 155(3), 295–297.

ten Have, M., Penninx, B. W. J. H., Tuithof, M., van Dorsselaer, S., Kleinjan, M., Spijker, J., & de Graaf, R. (2017). Duration of major and minor depressive episodes and associated risk indicators in a psychiatric epidemiological cohort study of the general population. *Acta Psychiatrica Scandinavica*, 136(3), 300–312.

Tuisku, J., Plavén-Sigray, P., Gaiser, E. C., Airas, L., Al-Abdulrasul, H., Brück, A., Carson, R. E., Chen, M.-K., Cosgrove, K. P., Ekblad, L., Esterlis, I., Farde, L., Forsberg, A., Halldin, C., Helin, S., Kosek, E., Lekander, M., Lindgren, N., Marjamäki, P., Rissanen, E., Sucksdorff, M., Varrone, A., Collste, K., Gallezot, J.-D., Hillmer, A., Huang, Y., Höglund, C. O., Johansson, J., Jucaite, A., Lampa, J., Nabulsi, N., Pittman, B., Sandiego, C. M., Stenkrona, P., Rinne, J., Matuskey, D., & Cervenka, S. (2019). Effects of age, BMI

and sex on the glial cell marker TSPO — a multicentre [11C]PBR28 HRRT PET study. *European Journal of Nuclear Medicine and Molecular Imaging*, 46(11), 2329–2338.

Ugale, R. R., Sharma, A. N., Kokare, D. M., Hirani, K., Subhedar, N. K., & Chopde, C. T. (2007). Neurosteroid allopregnanolone mediates anxiolytic effect of etifoxine in rats. *Brain Research*, 1184, 193–201.

Van de Velde, S., Bracke, P., & Levecque, K. (2010). Gender differences in depression in 23 European countries. Cross-national variation in the gender gap in depression. *Social Science & Medicine*, 71(2), 305–313.

Veenman, L., Papadopoulos, V., & Gavish, M. (2007). Channel-like functions of the 18-kDa translocator protein (TSPO): Regulation of apoptosis and steroidogenesis as part of the host-defense response. *Current Pharmaceutical Design*, 13(23), 2385–2405.

Verbe, J., Dubertret, C., El-Hage, W., Bonnet-Brilhault, F., & Duriez, P. (2019). [GABAergic approach of postpartum depression: A translational review of literature]. *L'Encephale*.

Verleye, M., Akwa, Y., Liere, P., Ladurelle, N., Pianos, A., Eychenne, B., Schumacher, M., & Gillardin, J.-M. (2005). The anxiolytic etifoxine activates the peripheral benzodiazepine receptor and increases the neurosteroid levels in rat brain. *Pharmacology Biochemistry and Behavior*, 82(4), 712–720.

Verleye, M. & Gillardin, J.-M. (2004). Effects of etifoxine on stress-induced hyperthermia, freezing behavior and colonic motor activation in rats. *Physiology & Behavior*, 82(5), 891–897.

Verleye, M., Schlichter, R., & Gillardin, J. (1999). Interactions of etifoxine with the chloride channel coupled to the GABA(A) receptor complex. *Neuroreport*, 10(15), 3207–3210.

Verleye, M., Schlichter, R., Neliat, G., Pansart, Y., & Gillardin, J. M. (2001). Functional modulation of gamma-aminobutyric acid(A) receptors by etifoxine and allopregnanolone in rodents. *Neuroscience Letters*, 301(3), 191–194.

Verma, A., Nye, J. S., & Snyder, S. H. (1987). Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptor. *Proceedings of the National Academy of Sciences of the United States of America*, 84(8), 2256–2260.

Viviani, D., Charlet, A., van den Burg, E., Robinet, C., Hurni, N., Abatis, M., Magara, F., & Stoop, R. (2011). Oxytocin Selectively Gates Fear Responses Through Distinct Outputs from the Central Amygdala. *Science*, 333(6038), 104–107.

Vogel, J. R., Beer, B., & Clody, D. E. (1971). A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia*, 21(1), 1–7.

Vos, T., Haby, M. M., Barendregt, J. J., Kruijshaar, M., Corry, J., & Andrews, G. (2004). The burden of major depression avoidable by longer-term treatment strategies. *Archives of General Psychiatry*, 61(11), 1097–1103.

Wang, M., Seippel, L., Purdy, R. H., & Bãckström, T. (1996). Relationship between symptom severity and steroid variation in women with premenstrual syndrome: Study on serum pregnenolone, pregnenolone sulfate, 5 alpha-pregnane-3,20-dione and 3 alpha-hydroxy-5 alpha-pregnan-20-one. *The Journal of Clinical Endocrinology & Metabolism*, 81(3), 1076–1082.

Weissman, M. M., Bland, R. C., Canino, G. J., Faravelli, C., Greenwald, S., Hwu, H. G., Joyce, P. R., Karam, E. G., Lee, C. K., Lellouch, J., Lépine, J. P., Newman, S. C., Rubio-Stipec, M., Wells, J. E., Wickramaratne, P. J., Wittchen, H., & Yeh, E. K. (1996). Cross-national epidemiology of major depression and bipolar disorder. *JAMA*, 276(4), 293–299.

Wigger, A., Loerscher, P., Weissenbacher, P., Holsboer, F., & Landgraf, R. (2001). Cross-Fostering and Cross-Breeding of HAB and LAB Rats: A Genetic Rat Model of Anxiety. *Behavior Genetics*, 31(4), 371–382.

Will, T. R., Proaño, S. B., Thomas, A. M., Kunz, L. M., Thompson, K. C., Ginnari, L. A., Jones, C. H., Lucas, S.-C., Reavis, E. M., Dorris, D. M., & Meitzen, J. (2017). Problems

and Progress regarding Sex Bias and Omission in Neuroscience Research. *eNeuro*, 4(6), ENEURO.0278–17.2017.

Willner, P. (2016). The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiology of Stress*, 6, 78–93.

Willner, P., Scheel-Krüger, J., & Belzung, C. (2013). The neurobiology of depression and antidepressant action. *Neuroscience & Biobehavioral Reviews*, 37(10, Part 1), 2331–2371.

Wittchen, H.-U. & Jacobi, F. (2005). Size and burden of mental disorders in Europe–a critical review and appraisal of 27 studies. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, 15(4), 357–376.

World-Health-Organization (2017). Depression and other common mental disorders. https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf(Download on 16.01.2020).

World-Health-Organization, Ed. (2018). *Mental Health Atlas 2017*. Geneva, Switzerland: World Health Organization.

World-Health-Organization (2019a). Global health estimates 2020: Disease burden by cause, age, sex, by country and by region.

World-Health-Organization (2019b). Suicide in the world.

Yoon, S. & Kim, Y.-K. (2020). The Role of the Oxytocin System in Anxiety Disorders. In Y.-K. Kim (Ed.), *Anxiety Disorders: Rethinking and Understanding Recent Discoveries*, Advances in Experimental Medicine and Biology (pp. 103–120). Singapore: Springer.

Young, L. J., Wang, Z., Donaldson, R., & Rissman, E. F. (1998). Estrogen receptor alpha is essential for induction of oxytocin receptor by estrogen. *Neuroreport*, 9(5), 933–936.

Zak, P. J., Kurzban, R., & Matzner, W. T. (2005). Oxytocin is associated with human trustworthiness. *Hormones and Behavior*, 48(5), 522–527.

Zheng, P. (2009). Neuroactive steroid regulation of neurotransmitter release in the CNS: Action, mechanism and possible significance. *Progress in Neurobiology*, 89(2), 134–152.

Zimmerberg, B., Rackow, S. H., & George-Friedman, K. P. (1999). Sex-dependent behavioral effects of the neurosteroid allopregnanolone (3alpha,5alpha-THP) in neonatal and adult rats after postnatal stress. *Pharmacology, Biochemistry, and Behavior*, 64(4), 717–724.

Acknowledgements

My gratitude goes to my mentor, Prof. Dr. Oliver Bosch, who has significantly supported and promoted this work and valuable academic input throughout my thesis work.

I especially want to thank my supervisor, Prof. Dr. Rainer Rupprecht, for his guidance and encouragement.

Special thanks to Prof. Dr. Christian Wetzel and Prof. Dr. Inga Neumann for their supervision and support. I sincerely thank Andrea Havasi, Martina Fuchs, and Rodrigue Maloumby for their magnificent guidance and support in the laboratory.

Furthermore, I thank Dr. Gryksa, Dr. Boi, and Dr. Pohl for their excellent experimental and statistical advice and support.

To my colleagues from the Institute of Neurobiology and Behavioral Biology at the Universität Regensburg, thank you for all your efforts and great support in my practical work. Further, I would like to thank PB for his endless emotional and academic support and the never ending loving support of my parents and my family.

Oath

1. The dissertation on the subject of *"The role of the Translocatorprotein (TSPO) 18 kDa in a psychopathological animal model for anxiety and depression"* is my independent work.

2. I have only used the sources and aids indicated and have not made use of any unauthorized help from third parties. In particular, I have marked as such contents taken literally or analogously from other works.

3. I have not yet submitted the work or parts of it to a university in Germany or abroad as part of an examination or qualification.

4. I confirm the correctness of the above statements.

5. I am aware of the meaning of the affidavit and the criminal consequences of an incorrect or incomplete affidavit. I affirm in lieu of an oath that I have declared the absolute truth to the best of my knowledge and have not concealed anything.

Date and Location

Signature