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Neurobiology of Anxiety and Anxiety Disorders

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Abstract

Anxiety is one of the most conserved affective states across sentient organisms, yet its biological, historical and pharmacological aspects remain incompletely integrated. This thesis examines anxiety across three domains to establish a unified framework that links anxiety as a physiological affective state and anxiety disorders as psychopathological disorders. An in-depth literature review was conducted to analyse findings across disciplines namely anatomy, pharmacology, psychology, and neuroscience, to dissect anxiety and anxiety disorders, how they are manifested in different species and what underlying changes occur. Each domain revealed conceptual boundaries that reflect both the progress and the persistent gaps in anxiety research. The absence of a clear, cross-disciplinary distinction between physiological anxiety and anxiety disorders has limited translational research, particularly across species. This thesis addresses that gap by integrating historical, neuroanatomical, and psychopharmacological evidence to propose a unified, biologically grounded framework applicable across humans, canines, non-human primates, and rodents.

What emerges is not a disorder of circuits gone wrong, but of circuits that cannot stop being correct, and it is precisely that distinction that future research, across species and disciplines, must be built upon.

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Abbreviations

5-HT 5-Hydroxytryptamine (Serotonin)

5-HT$_{1A}$ Serotonin receptor subtype 1_A

5-HT$_{2A}$ Serotonin receptor subtype 2_A

5-HT$_{2C}$ Serotonin receptor subtype 2_C

5-HT$_3$ Serotonin receptor subtype 3

5-HTR Serotonin Receptor

ACC Anterior Cingulate Cortex

adBNST Anterodorsal Bed Nucleus of the Stria Terminalis

AHA Anterior Hypothalamic Area/Nucleus

AIc Anterior Insular Cortex

alBNST Anterolateral Bed Nucleus of the Stria Terminalis

ALS Amyotrophic Lateral Sclerosis

AN Anterior Thalamic Nucleus

BDNF Brain-Derived Neurotrophic Factor

BDZs Benzodiazepines

BID Twice daily (bis in die)

BL Basolateral amygdala nuclear group

BLA Basolateral Amygdala

BM Basomedial amygdala nuclear group

BMA Basomedial Amygdala

BNST Bed Nucleus of the Stria Terminalis

BOLD Blood-Oxygen-Level-Dependent

CA1, CA2, CA3, CA4 Cornu Ammonis subfields (hippocampus)

CeA Central Nucleus of the Amygdala

CeL Central Lateral Amygdala

CeM Central Medial Amygdala

CGRP Calcitonin Gene-Related Peptide

CM Central Medial intralaminar thalamic nucleus

CNS Central Nervous System

CRH Corticotropin-Releasing Hormone

DAT Dopamine Transporter

dBNST Dorsal Bed Nucleus of the Stria Terminalis

DG Dentate Gyrus

dlPAG Dorsolateral Periaqueductal Grey

dlPFC Dorsolateral Prefrontal Cortex

DMH Dorsomedial Hypothalamus

DRN Dorsal Raphe Nucleus

DSM Diagnostic and Statistical Manual of Mental Disorders

E/I Excitation/Inhibition (balance)

EPM Elevated Plus Maze

FDA Food and Drug Administration

fMRI Functional Magnetic Resonance Imaging

fuBNST Fusiform nucleus of the Bed Nucleus of the Stria Terminalis

GABA Gamma-Aminobutyric Acid

GAD Generalised Anxiety Disorder

GAD65 Glutamic Acid Decarboxylase isoform 65

GAD67 Glutamic Acid Decarboxylase isoform 67

GAT GABA Transporter

GIRK G-protein-coupled Inwardly Rectifying Potassium channel

H Hypothalamus

HAM-A Hamilton Anxiety Rating Scale

HPA Hypothalamic-Pituitary-Adrenal (axis)

HPC Hippocampus

IC Insular Cortex

iGluRs Ionotropic Glutamate Receptors

IL Infralimbic cortex

I.p. Intraperitoneal

I.v. Intravenous

juBNST Juxtacapsular nucleus of the Bed Nucleus of the Stria Terminalis

LA Lateral Nucleus of the Amygdala

LC Locus Coeruleus

LH Lateral Hypothalamus / Lateral Hypothalamic Area

LHb Lateral Habenula

LS Lateral Septum

MDT Mediodorsal Thalamic Nucleus

MeA Medial Nucleus of the Amygdala

mGluR5 Metabotropic Glutamate Receptor 5

mGluRs Metabotropic Glutamate Receptors

mPFC Medial Prefrontal Cortex

mTOR Mammalian Target of Rapamycin

NAA N-Acetylaspartate

NAc Nucleus Accumbens

NAS Neuroactive Steroids

NET Norepinephrine Transporter

NHPs Non-Human Primates

NMDA N-Methyl-D-Aspartate

NMDARs N-Methyl-D-Aspartate Receptors

OCD Obsessive-Compulsive Disorder

OFC / oPFC Orbitofrontal Cortex

ovBNST Oval Nucleus of the Bed Nucleus of the Stria Terminalis

PAG Periaqueductal Grey

PBN Parabrachial Nucleus

PCG Paracingulate Gyrus

PCP Phencyclidine

PD Panic Disorder

PET Positron Emission Tomography

PF Parafascicular nucleus (intralaminar thalamus)

PFC Prefrontal Cortex

PIC Posterior Insular Cortex

PL Prelimbic cortex

PPD Postpartum Depression

PTSD Post-Traumatic Stress Disorder

PUV Pulvinar

PVN Paraventricular Nucleus of the Hypothalamus

PVT Paraventricular Nucleus of the Thalamus

SAD Social Anxiety Disorder

SC Subcutaneous

SERT Serotonin Transporter

SI Primary Somatosensory Cortex

SII Secondary Somatosensory Cortex

SNRIs Serotonin-Norepinephrine Reuptake Inhibitors

SPECT Single-Photon Emission Computed Tomography

SSC Somatosensory Cortex

SSRIs Selective Serotonin Reuptake Inhibitors

SUB Subiculum

TD Tusculan Disputations

TID Three times daily (ter in die)

UCMS Unpredictable Chronic Mild Stress

vBNST Ventral Bed Nucleus of the Stria Terminalis

vCA1 Ventral CA1 (hippocampal subfield)

vHPC Ventral Hippocampus

vlPAG Ventrolateral Periaqueductal Grey

VMH / VMN Ventromedial Hypothalamus / Ventromedial Nucleus

vmPFC Ventromedial Prefrontal Cortex

VTA Ventral Tegmental Area

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Chapter 1.0: The History of Anxiety

1.1 Introduction

From classical antiquity to the early 18th century, anxiety was not formally recognised as it is understood today. Nevertheless, its impact on human life has remained a persistent concern across centuries. The conceptual evolution of anxiety from a vague feeling of anticipation to a well-distinguished affective state was neither linear nor continuous. From Hippocrates and later thinkers such as Burton, Darwin, de Sauvages, and Pavlov, anxiety gradually came to be recognised as a distinct affective state and laid the groundwork to understand pathological anxiety.

On account of physiology, the understanding of anxiety followed a similar trajectory from being interpreted as a component of fear to being dismissed as a symptom of neurasthenia. Evolutionary theory later positioned anxiety as a survival instinct within the nervous system. Importantly, the physiological manifestations of anxiety extend beyond *Homo sapiens*, with comparable responses observed across the animal kingdom, from social to solitary organisms.

1.2 Origin of Anxiety: Inexact origin

Although the exact origins of anxiety cannot be pinpointed, its conceptualisation can be traced back to the early medical texts. In Epidemic V of the Hippocratic Corpus[1], Hippocrates describes the case of a man named Nicanor, who suffered from a "fear of flute girls". While he heard the flute during the day, it had no effect on him, though the anticipation of the music became intolerable and the symptoms recurred2, this condition was later labelled as a phobia.

Between 85 and 370 BCE, Plato, a Roman philosopher, wrote Symposium, a Socratic dialogue in which he also describes the feeling of anticipation3. During the Roman Empire, Cicero categorised anxiety (angor) in Tusculan Disputations (TD) as a disorder amongst other negative affections, drawing an analogy between a troubled mind and a diseased body(4)(2). Another philosopher, Seneca, in his letters to his friend Lucilius, described anxiety as the mental cost of public approval, writing, "at the cost of great mental anxiety" (translation adopted from the Introduction to Anxiety: A Philosophical History, Bettina Bergo). His work, Of Peace of Mind

and On the Shortness of Life, further highlights the cognitive dimension of anxiety in human consciousness. The Roman Stoics associated angustia (narrowness) more closely with melancholy than augustia (hallowed greatness).

Parallel to Stoicism, another school of Philosophy known as Epicureans, Lucretius a philosopher from this school wrote a poetry book called De natura rerum Book III (On the Nature of Things) which promoted the concept of ataraxia - a state of mind free from worry as a therapeutic ideal, focused on eliminating the negative cognition about the past and the fears about the future(5)(6) pointing towards how to limit the excess desire, by using ataraxia. This perspective framed anxiety as a consequence of excessive desire and future-oriented fear, aligning more closely with augustia.

Throughout classical antiquity, fear, phobias, and anxiety were not clearly distinguished, nor was experimental physiological work was being conducted. However, scholars recognised that these states were accompanied by bodily symptoms and attempted to explain them, shaping early understandings of cognition and affect.

1.3 Naming a forgotten Illness

Anxiety has been described using different terms across languages. The term anxiety is derived from the Latin angor and the verb ango (to constrict), referring to a sense of narrowness (angustus/angustia). Over time, related words developed across European languages to describe states of worry, distress or unease[2]. The English term Anxiety derives from the Latin anxĭetas, ‘craving,’ ‘aspiration,’ ‘worry,’ ‘embarrassment,’ which was introduced into modern languages through the French anxiété[7].As time passed, the meanings of angustĭa and anxĭetas have been differentiated, based on somatic manifestations and psychological feelings. From classical antiquity to the early modern era, anxiety was often ignored as a medical problem. Even though people experienced anxiety, medical records rarely mention it, and it mostly appeared in philosophical writings.

1.4 Rediscovering Anxiety - Age of Rational

In the early 17th century, Robert Burton's The Anatomy of Melancholy discussed negative affective states under the concept of melancholy, which is a vast spectrum of human sadness, depression, and touches upon anxiety[8].

The 18th century saw a further development in the description of anxiety and anxiety disorders. George Cheyne described internal anxiety in his works, Treatise of Nervous Diseases of all Kinds, which later became known as The English Malady. His observations included cases that resemble generalised anxiety cases. In the mid-century, Boissier de Sauvages introduced the concept of panophobia, describing persistent worry and panic-like symptoms that resemble *modern generalised anxiety disorders* (GADs) and *panic disorders* (PD). de Sauvages detailed his clinical observations, distinguished different forms of anxiety, chronic worry and panic disorders[2]. William Battie later argued that anxiety could be a component of madness and should be understood as a sensory nervous disturbance[9].

The end of the 18th century saw a turn for anxiety, when William Cullen extensively wrote about "panophobia" and "praeternatural anxiety". In A System of Nosology, he introduced the concept of neurosis as a group of disorders affecting the nervous system[10]. In the following century, G. M. Beard coined the diagnostic label 'neurasthenia' to describe symptoms including fatigue, anxiety, headache, impotence, neuralgia, and depression resulting from nervous exhaustion[11].

1.5 Conceptualisation of Modern Anxiety

The 19th century brought anxiety to the forefront, which is now known as modern anxiety and pathological anxiety. This century saw significant development in psychopathology, with the descriptions of fear-related pain in the chest[12], agoraphobia[13][14], irregularities of the cardiac rhythm in circumstances of war[15], by Kraepelin and Freud. In this era, a careful description of symptoms and syndromes was attempted, followed by attempts to detect underlying mechanisms.

Charles Darwin, widely recognised for his work on evolution and his book "On the Origin of Species," published "The Expression of the Emotions in Man and Animals" in 1874[3]. In this

work, he focused on five emotions, specifically discussing fear. It's important to note that he did not explicitly distinguish between fear and anxiety, treating them as one due to the lack of a clear differentiation at that time. Darwin emphasised that emotions are biologically innate, evolutionarily inherited and shared across humans and animals - pointing towards the affective state.

In 1876, a German psychiatrist, Carl Westphal, first identified Agoraphobia as a dread of public places, which morphed in the 20th century into panic attacks in any place or situation where one feels unsafe or trapped[16].

The work of Emil Kraepelin provides an overview of delineated anxiety - spontaneous anxiety attacks, social phobia, agoraphobia, anxiety related to obsessions and compulsions, and anxieties associated with automatic actions[17]. Parallel to Kraepelin, a French psychopathologist, Pierre Janet, describes anxiety as the ultimate bodily manifestation of a lowering of the level of mental tension, quietly indicating towards the affective status[17].

Cullen's work inspired Freud's work on neurosis. Sigmund Freud, a controversial Austrian neurologist and the founder of psychoanalysis, was among the first to see anxiety as the result of inner threat instead of external. He coined the term 'Anxiety neurosis' or 'névrose d'angoisse'; in short, he postulated that "anxiety neurosis was caused by an accumulation of sexual excitation that could not find discharge in coitus[2]. By 1895, he had established the bifurcation between physiological and psychological disorders, and he abandoned neurosis to focus on the physiology of anxiety. Later, Freud lists the broad symptoms of anxiety neurosis, which also include anxiety attacks, agoraphobia, and diarrhoea[2]. While anxiety neurosis is an outdated concept, this work laid the major foundation of the DSM criteria of GAD and anxiety as an internal affect. Based on Freud's work, a French author, Francis Heckel, adopted anxiety neurosis without the sexual aetiology and redefined it as a paroxysmal manifestation of anxiety.

1.6 Experimental History of Anxiety

Early experimentation research advanced our understanding of anxiety through the use of animal models. Rodents, dogs, and non-human primates have been used to study behavioural and physiological responses associated with anxiety.

Early psychological research used observable emotions to study anxiety. Ivan Pavlov's work on classical conditioning showed that emotions could be learned, triggered, and measured[18][19]. Watson and Rayner extended this principle to humans in the Little Albert study[20]. In this study, little Albert, a child, developed a fear response to neutral stimuli after repeated pairing with aversive stimuli. This study demonstrated acquisition, generalisation, and persistence of fear, which later became central to experimental and clinical models of anxiety.

In the mid-20th century, Adolf Meyer conceptualised a category called anxiety reaction or reactions[21]. Meyer believed that to understand a patient's psychopathology, it becomes important to look into the life history thoroughly and pinpoint the specific stressors. Anxiety reactions were diagnosed when the anxiety was diffuse and neither restricted to situations nor objects, nor controlled by any specific psychological defence mechanism[21]. This became monumental in the history of anxiety diagnosis.

Further work by Howard Liddell extended Pavlovian techniques to diverse animal species and studied pathological emotional states. Liddell focused on conditioned motor reflexes; this early work mirrored Pavlov's work on the dog's neurosis and led to an accidental discovery of agitation in sheep[22]. His findings suggested that anxiety could emerge from a responsive state of worry, which, when prolonged, will become chronic.

Down the years, conflict-based models emerged to test the reliability of anxiolytic drugs. One of the earliest methods was the Geller-Seifter conflict test, in which animals trained to press a lever for food reduce their responding when punishment was introduced. Anxiolytic drugs increase responding in this conflict situation, reducing anxiety. Vogel et al developed the modified Geller-Seifter's test to evaluate benzodiazepines (chlordiazepoxide, diazepam, and oxazepam) as anxiolytics in animal models[23], the result showed a clear anti-anxiety effect.

These experimental approaches helped establish reliable methods for studying anxiety and anxiolytics.

1.7 Standardisation of Anxiety - DSM

From fear of flute girls, to anxiety, multiple terminologies were utilised to describe anxiety, which deemed the importance of a standard guide or psychiatric classification and acted as a reference for the clinicians, which gave birth to "Diagnostics and Statistical Manual of Mental Disorders" better known as the DSM[24]. The first edition, DSM-I, was published in 1952 and included a glossary of diagnostic categories. Later in DSM - II, anxiety reactivity was renamed as 'neuroses', which mentioned that anxiety could be felt directly or controlled unconsciously by various symptoms[25]. DSM-III gave much greater prominence to anxiety disorders by providing a separate section for them, by splitting out separate panic and generalised anxiety disorders, and by dividing phobic neurosis into agoraphobia, social phobias, and simple phobias. DSM - III categorised anxiety neurosis into Generalised Anxiety Disorder (GAD) and Panic Disorder (PD)[26], which was based on the response to medication and pharmacological dissection. Later, it was found that GAD had fewer autonomic symptoms and an earlier, more gradual onset than patients with PD[27].

Psychopharmacological and biological psychiatric research required stringent diagnostic criteria, such as the Feighner criteria, and, together with the research diagnostic criteria, formed the basis of the third revised edition of the DSM, DSM-III-R. It introduced fairly dramatic revisions in the anxiety disorder section. Neurasthenic neurosis and neurosis were discarded completely. There was a reduction in hierarchical exclusions between panic and other disorders, resulting in more frequent diagnosis of anxiety disorders. Priority was given to panic disorder over agoraphobia when both were present, as agoraphobia is often a complication of panic disorder. The threshold for panic disorder was changed from three attacks in 3 weeks to four attacks in 4 weeks or one attack with persistent fear of having additional attacks[28]. Post-traumatic Stress Disorder (PTSD) was first defined in the DSM-III as a syndrome that occurs following a psychologically traumatic event, which would evoke significant distress in almost everyone. DSM III was mainly based on work that was ages-old and on the early psychodynamics of DSM I and II, with few changes. DSM-III-R set to correct the course of the psychiatric world.

On the other hand, the DSM-IV introduced a three-stage empirical process for its revision. This process included clinical trials, systematic literature reviews, and secondary analyses. It was designed to serve as an accurate clinical guide[29], which distinguished GAD in practice, and it continued to include PTSD and OCD under anxiety disorder criteria[30].

In DSM-V, the anxiety criteria were revised to improve diagnostics, reliability and clinical utility through supported symptom refinement and distinct disorder boundaries. The anxiety criteria saw a drastic change; separation anxiety disorder was expanded to include adult presentations, reflecting epidemiological evidence of significant adult onset, which were previously excluded by DSM-IV anxiety criteria[31]. Panic disorder and agoraphobia were separated into distinct diagnoses based on data demonstrating differing phenomenology, course of onset, and treatment response[32]. Panic disorder was set to be used as a transdiagnostic specifier, as it was accompanied by other mental disorders. Selective mutism and separation anxiety disorder were classified within anxiety disorders due to shared fear-based mechanisms and treatment responsiveness[33]. Obsessive-compulsive disorder and post-traumatic stress disorder were removed from the anxiety disorders category in light of evidence for distinct neurobiological and clinical profiles[34]. Criteria for generalised anxiety disorder emphasised uncontrollable worry as the core feature to enhance construct specificity[35].

DSM-V is currently being used in the mental health domain of human medicine; on the contrary, veterinary medicine does not have a standardised manual of psychiatric disorders, which could provide an overall outlook of mental disorders along with diagnostic parameters and treatment protocols.

Anxiety becomes clinically significant when this physiological process significantly interferes with the day-to-day functioning of an organism, leading to anxiety disorders. Understanding anxiety requires integrating the physiological, psychological and evolutionary perspectives.

Throughout this thesis, neuroanatomical comparisons are drawn across species with fundamentally different body plans, including humans, non-human primates, canines and rodents. In quadrupeds, directional terms such as rostral, caudal, and cranial signify a different spatial orientation relative to the neuraxis in bipedal species. For the purpose of this thesis, these

directional terms are treated as functionally equivalent across species, following standard comparative neuroanatomy convention and should be interpreted accordingly.

Regulation of anxiety is an integrative connection between behaviour, the neuroendocrine system, cellular biology and pharmacological interaction. It becomes crucial to take these components into account to understand how anxiety is regulated and affects the brain and behaviour. In a few chapters, we are going to take these interactions into account to establish the neural pathway of physiological anxiety and what physiological anxiety is.

References

1. H., Withington, E. T., Potter, P., Smith, W. D. (1923). Hippocrates: Epidemics, Books 2, 4-7. United Kingdom: Harvard University Press.
2. Marc-Antoine Crocq (2015) A history of anxiety: from Hippocrates to DSM, Dialogues in Clinical Neuroscience, 17:3, 319-325, DOI: 10.31887/DCNS.2015.17.3/macrocq
3. Bergo, B. (2020). Introduction. In _Anxiety_ (pp. 1–35). https://doi.org/10.1093/oso/9780197539712.003.0001
4. Cicero, M. T., Anthon, C., ed. (1852) Cicero's Tusculan disputations. New York, Harper & brothers. [Pdf] Retrieved from the Library of Congress, https://www.loc.gov/item/27022571/.
5. Segal, C. (1990). _Lucretius on Death and Anxiety: Poetry and Philosophy in DE RERUM NATURA_. Princeton University Press. http://www.jstor.org/stable/j.ctt7zv12q
6. Lucretius. On the Nature of Things. Transl: Leonard WE. Translation provided by the Internet Classics Archive by Stevenson DC, Web Atomics.
7. López-Ibor, J. J., & López-Ibor, M. (2009). Anxiety and logos: Toward a linguistic analysis of the origins of human thinking. _Journal of Affective Disorders_, _120_(1–3), 1–11. https://doi.org/10.1016/j.jad.2009.02.026
8. Brink, A. (1979). Depression and loss: a theme in Robert Burton's "Anatomy of Melancholy" (1621). _The Canadian Journal of Psychiatry_, _24_(8), 767–772. https://doi.org/10.1177/070674377902400811
9. A treatise on madness. By William Battie . . . 1758 : Battie, William, M.D. : Free Download, Borrow, and Streaming : Internet Archive_. (1758). Internet Archive. https://archive.org/details/bim_eighteenth-century_a-treatise-on-madness-b_battie-william-md_1758/page/n41/mode/2up
10. Flaskerud, J. H. (2007). NEURASTHENIA: HERE AND THERE, NOW AND THEN. Issues in Mental Health Nursing, 28(6), 657–659. https://doi.org/10.1080/01612840701354638
11. Stein, M. B., & Steckler, T. (2010). Behavioral Neurobiology of anxiety and its treatment. In _Current topics in behavioral neurosciences_. https://doi.org/10.1007/978-3-642-02912-7

12. Flemming, C. F. (1848). Über Praekordial-Angst. Allgemeine Zeitschri für Psychiatrie, 5, 341–61.
13. Benedikt, M. (1870). Über “Platzschwindel.” Allgemeine Wiener Medizinische Zeitung, 15, 488–9.
14. Westphal, C. (1872), Die Agoraphobie, eine neuropathische Erscheinung. Archive für Psychiatrie und Nervenkrankheite, 3, 138–61.
15. Da Costa, J. M. (1871). On irritable heart; a clinical study of a form of functional cardiac disorder and its consequences. The American Journal of the Medical Sciences, 71, 17–52.
16. Dowbiggin, I. R. (2009). High anxieties: the social construction of anxiety disorders. _The Canadian Journal of Psychiatry_, _54_(7), 429–436. https://doi.org/10.1177/070674370905400703
17. Glas, G. (2013). Anxiety and phobias. In _Oxford University Press eBooks_. https://doi.org/10.1093/oxfordhb/9780199579563.013.0035
18. VanElzakker, M. B., Dahlgren, M. K., Davis, F. C., Dubois, S., & Shin, L. M. (2013). From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiology of Learning and Memory*, *113*, 3–18. https://doi.org/10.1016/j.nlm.2013.11.014
19. Modecki, K. L., Ryan, K. M., & Waters, A. M. (2022). Fear learning and extinction predicts anxiety in daily life: a study of Pavlovian conditioning and ecological momentary assessment. _Psychological Medicine_, _53_(11), 5301–5311. https://doi.org/10.1017/s0033291722002379
20. Mertens, G., Krypotos, A., & Engelhard, I. M. (2020). A review on mental imagery in fear conditioning research 100 years since the ‘Little Albert’ study. _Behaviour Research and Therapy_, _126_, 103556. https://doi.org/10.1016/j.brat.2020.103556
21. Lanzoni, S. (2015). S. D. Lamb.Pathologist of the Mind: Adolf Meyer and the Origins of American Psychiatry. Journal of the History of Medicine and Allied Sciences, 71(3), 358–360. https://doi.org/10.1093/jhmas/jrv049
22. Kirk, R. G. W., & Ramsden, E. (2018). Working across species down on the farm: Howard S. Liddell and the development of comparative psychopathology, c. 1923–1962. _History & Philosophy of the Life Sciences_, _40_(1), 24. https://doi.org/10.1007/s40656-018-0189-y

23. Vogel, J. R., Beer, B., & Clody, D. E. (1971). A simple and reliable conflict procedure for testing anti-anxiety agents. _Psychopharmacology_, _21_(1), 1–7. https://doi.org/10.1007/bf00403989
24. Craske, M. G., Stein, M. B., Eley, T. C., Milad, M. R., Holmes, A., Rapee, R. M., & Wittchen, H. (2017). Anxiety disorders. Nature Reviews Disease Primers, 3(1), 17024. https://doi.org/10.1038/nrdp.2017.24
25. Stein, M. B., & Sareen, J. (2015). Generalized Anxiety Disorder. New England Journal of Medicine, 373(21), 2059–2068. https://doi.org/10.1056/nejmcp1502514
26. Stein, M. B., & Craske, M. G. (2017). Treating anxiety in 2017. JAMA, 318(3), 235. https://doi.org/10.1001/jama.2017.6996
27. Anderson, D. J., Noyes, R., & Crowe, R. R. (1984). A comparison of panic disorder and generalized anxiety disorder. _American Journal of Psychiatry_, _141_(4), 572–575. https://doi.org/10.1176/ajp.141.4.572
28. Frances, A. (1990). Classification of anxiety disorders: From Freud to DSM-IV. _Journal of Psychiatric Research_, _24_, 9–10. https://doi.org/10.1016/0022-3956(90)90068-2
29. First, M. B. (2016). The DSM revision process: needing to keep an eye on the empirical ball. Psychological Medicine, 47(1), 19–22. https://doi.org/10.1017/s0033291716002129
30. Andrews, G., Hobbs, M. J., Borkovec, T. D., Beesdo, K., Craske, M. G., Heimberg, R. G., Rapee, R. M., Ruscio, A. M., & Stanley, M. A. (2010). Generalized worry disorder: a review of DSM-IV generalized anxiety disorder and options for DSM-V. _Depression and Anxiety_, _27_(2), 134–147. https://doi.org/10.1002/da.20658
31. Silove, D., Alonso, J., Bromet, E., Gruber, M., Sampson, N., Scott, K., Andrade, L., Benjet, C., De Almeida, J. M. C., De Girolamo, G., De Jonge, P., Demyttenaere, K., Fiestas, F., Florescu, S., Gureje, O., He, Y., Karam, E., Lepine, J., Murphy, S., . . . Kessler, R. C. (2015). Pediatric-Onset and Adult-Onset Separation Anxiety Disorder across Countries in the World Mental Health Survey. _American Journal of Psychiatry_, _172_(7), 647–656. https://doi.org/10.1176/appi.ajp.2015.14091185
32. Regier, D. A., Kuhl, E. A., & Kupfer, D. J. (2013). The DSM‐5: Classification and criteria changes. _World Psychiatry_, _12_(2), 92–98. https://doi.org/10.1002/wps.20050
33. Craske, M. G., Kircanski, K., Epstein, A., Wittchen, H., Pine, D. S., Lewis-Fernández, R., Hinton, D., Anxiety, D. V., Spectrum, O., & Group, P. a. D. D. W. (2010). Panic disorder:

a review of DSM-IV panic disorder and proposals for DSM-V. _Depression and Anxiety_, _27_(2), 93–112. https://doi.org/10.1002/da.20654

34. Stein, D. J., Craske, M. G., Friedman, M. J., & Phillips, K. A. (2011). Meta-structure issues for the DSM-5: How do Anxiety Disorders, Obsessive-Compulsive and Related Disorders, Post-Traumatic Disorders, and Dissociative Disorders fit together? _Current Psychiatry Reports_, _13_(4), 248–250. https://doi.org/10.1007/s11920-011-0207-1
35. Ruscio, A. M. (2002). Delimiting the boundaries of generalized anxiety disorder: differentiating high worriers with and without GAD. _Journal of Anxiety Disorders_, _16_(4), 377–400. https://doi.org/10.1016/s0887-6185(02)00130-5

Chapter 2.0: Neuroanatomy of Anxiety

To understand the regulation of anxiety, it is crucial to understand the structural and functional organisation of the underlying brain regions. This chapter focuses on the neuroanatomy of the sensory cortex, thalamus, amygdala, bed nucleus of the stria terminalis, anterior cingulate cortex, prefrontal cortex, insula, and brainstem and also addresses the emerging role of the cerebellum in anxiety modulation.

Anxiety has often been thought to be an outward manifestation of the amygdala alone. However, multiple studies across species have established that surrounding structures are also interlinked in the mechanism underlying the psychosomatic manifestation of anxiety.

2.1 Somatosensory Cortex (SSC)

The sensory cortex is the first point of anatomical contact in the regulation of anxiety, and its role is not limited to emotion processing. Therefore, it is important to understand how it fits in the broader network of anxiety regulation.

Structure and Function: The SSC is located in the parietal lobe of the brain and is divided into two regions. The primary somatosensory cortex (SI) is located in the postcentral sulcus in primates and humans, and in post-cruciate or rostral suprasylvian gyri in dogs. In rodents, the SI is highly differentiated, most notably the barrel cortex, located in the dorsolateral parietal cortex[1]. SI exhibits precise and dense somatotopy for sensation (whiskers, limbs, trunk, face) and receives dense thalamocortical inputs primarily from the ventral posteromedial and posterolateral nuclei.

Functionally, SI serves as the primary recipient of sensory input. In primates, secondary somatosensory cortex (SII) integrates ipsilateral, contralateral and bilateral inputs, which doesn't occur in SI. In dogs, touch elicits a lateralised response in SI and SII, alongside cingulate regions, suggesting integration with affective brain circuits[2].

Projections: Across mammals, SI and SII receive organised thalamic inputs, which become necessary in emotion processing. In rodents, SII receives substantial inputs from the ipsilateral ventrobasal thalamic nucleus and the posterior group of the thalamus[3].

In non-human primates (NHPs), multiple thalamic nuclei project to numerous anterior parietal fields[4] and also to SII and adjacent somatosensory subregions. These projections produce both divergent and convergent thalamic projections into these cortical zones simultaneously. Similarly, somatosensory mapping in humans has revealed a direct thalamocortical projection to both SI and SII[5].

In contrast to thalamocortical projections, SI projections are indirectly relayed to the amygdala via polysynaptic cortical pathways in rodents. SII receives input from the SI and sends projections to the insular cortex and directly into the BLA (lateral and basal nuclei) densely. Similar pathways are seen across other mammals as well. In NHPs, the operculum, together with the insula, acts as a relay structure.

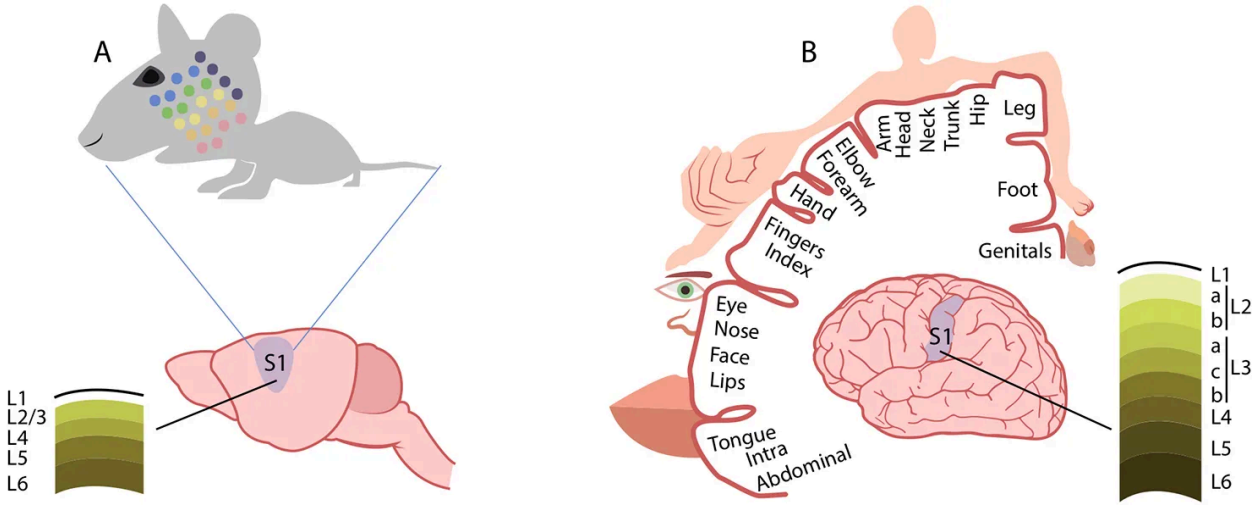

Figure 1. Somatosensory Cortex (SSC) in Rodent and Human.

From Balasco, L., Provenzano, G., & Bozzi, Y. (2020b). Sensory Abnormalities in Autism Spectrum Disorders: a focus on the tactile domain, from genetic mouse models to the clinic. Frontiers in Psychiatry, 10, 1016. https://doi.org/10.3389/fpsyt.2019.01016

2.2 Thalamus (Th):

The thalamus is another structure involved in anxiety regulation and sensory inputs (excluding olfaction). It acts as a relay centre and hub for sensory information, which is relayed to other brain regions, depending on the context and the affect.

Structure and Function: The thalamus is a paired structure, located at the centre of the brain. On each side, it can be divided into three groups of thalamic nuclei: the lateral, medial and anterior nuclear groups. In addition to these nuclei, two more thalamic nuclei are present: the intralaminar nuclei, a Y-shaped structure that separates the three nuclei and the reticular nuclei, which form an envelope around the lateral nuclei. These nuclei can further be subdivided, but the major nuclei involved in anxiety regulation include the pulvinar (PUV) from the lateral mediodorsal nucleus (MDT), paraventricular Nucleus of the thalamus (PVT) from the medial nucleus, anterior thalamic nucleus (AN) from the anterior nucleus, and central medial and Parafascicular from the intralaminar nuclei and Lateral Geniculate Nucleus (LGN) [6].

The human thalamus has a well-defined, subdivided, highly differentiated nuclear structure, and similar subdivisions are seen in non-human primates and in canines, with basic nuclear organisation, with less well-defined structures[7-8]. In comparison, rodent thalamus has a conserved set of core sensory, relay and association nuclei, which are smaller and less subdivided than in primates[9].

Projections: The pulvinar coordinates the cortico-cortical and cortico-limbic information, projecting extensively to visual, parietal, cingulate, and orbitofrontal association cortices indirectly[10]. The mediodorsal nucleus (MDT) projects to the medial prefrontal cortex (mPFC), orbitofrontal cortex, and amygdala, thereby linking thalamic output to executive control and the regulation of fear and anxiety states. The paraventricular nucleus (PVT) sits within the midline thalamus and acts as a limbic integration hub. PVT receives arousal-related hypothalamic[11] and brainstem inputs and sends dense projections to basolateral and central amygdala (CeA), nucleus accumbens (NAc), and bed nucleus of stria terminalis (BNST)[12-13]. The anterior thalamic nuclei (AN), comprising the anteroventral, anteromedial, and anterodorsal subnuclei, receive hippocampal and mammillary body inputs and project to retrosplenial and anterior cingulate cortices. These pathways embed contextual memory and hippocampal limbic information into cingulate and prefrontal networks that shape anxiety-related contextual processing[14].

Finally, intralaminar nuclei such as the central medial (CM) and parafascicular (PF) nuclei form broadly distributed projections to the cingulate, insular, and frontal cortices.

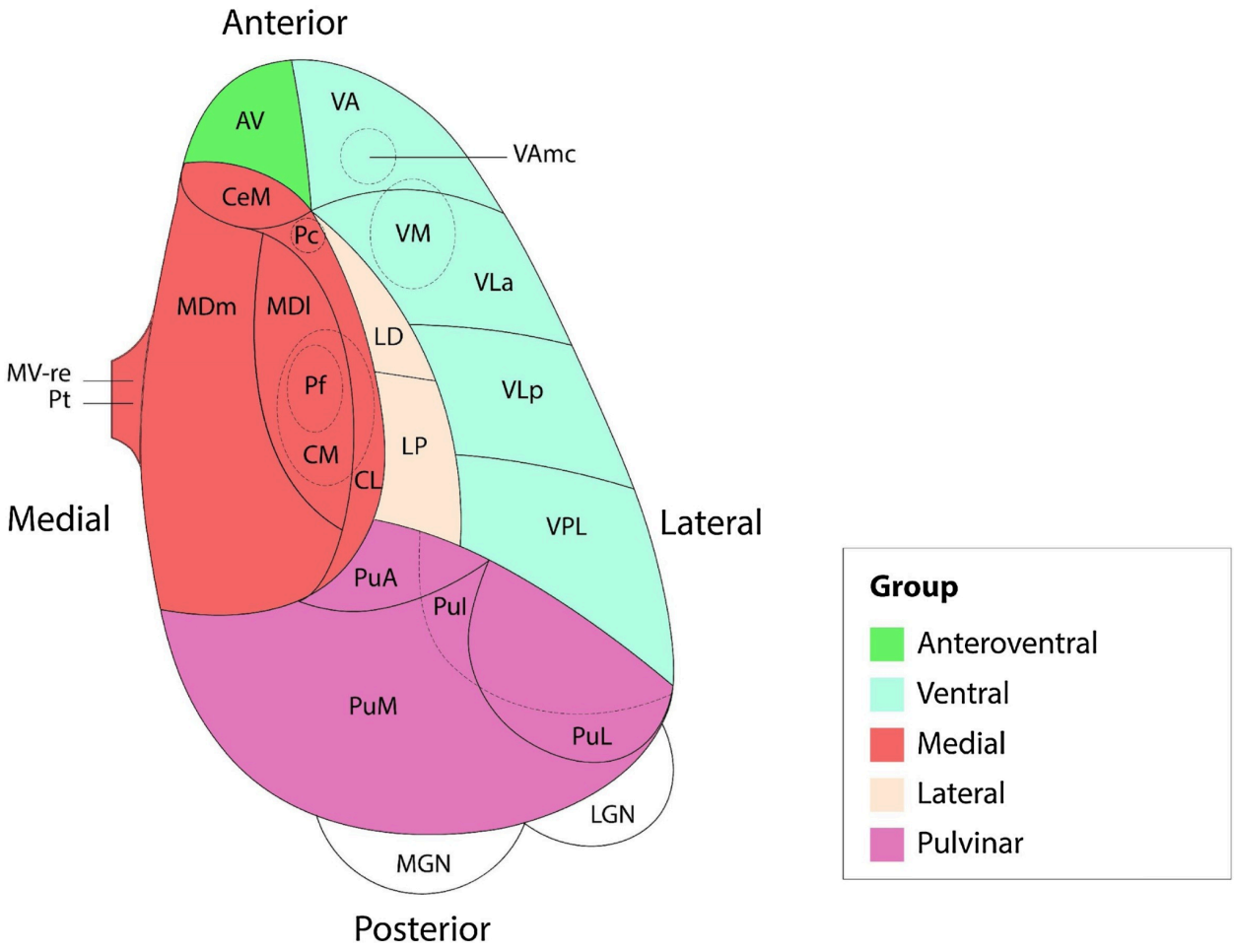

Figure 2. Thalamic Subnuclei.

From Keun, J. T. B., Van Heese, E. M., Laansma, M. A., Weeland, C. J., De Joode, N. T., Van Den Heuvel, O. A., Gool, J. K., Kasprzak, S., Bright, J. K., Vriend, C., & Van Der Werf, Y. D. (2021). Structural assessment of thalamus morphology in brain disorders: A review and recommendation of thalamic nucleus segmentation and shape analysis. Neuroscience & Biobehavioral Reviews, 131, 466–478. https://doi.org/10.1016/j.neubiorev.2021.09.044

2.3 Amygdala (Am):

The amygdala is a complex structure located deep in the temporal lobe of primates. It processes diverse emotions and social behaviour and plays an important role in mood-related disorders, including anxiety.

Structure and function: The amygdala is an almond-shaped complex structure comprising approximately 13 nuclei in primates. These nuclei are divided into five major functional groups: basolateral (BL), basomedial (BM), cortical-like nuclei, central nuclei (CeA), medial nuclei (CeM), and lateral nuclei (CeL)[15]. Among these, the basolateral amygdala (BLA), central nucleus (CeA), and Central medial nucleus (CeM) are the most extensively studied. The basomedial nucleus is sometimes referred to as the accessory nucleus. Across mammals, the amygdala is highly conserved, although regional volumes vary among dog breeds due to selective breeding for different functions[16]. The BLA has a cortical-like profile and primarily contains excitatory neurons, whereas the CeA and MeA have a striatal-like composition consisting largely of inhibitory neurons[17].

Projections: Despite species differences in size and volume, amygdala projections are largely conserved. These projections are broadly categorised into afferent and efferent pathways relevant to anxiety.

Afferent projections arise from the medial prefrontal cortex. In rodents, these projections are direct to the BLA, whereas in primates, the ventromedial, orbitofrontal, and anterior cingulate cortices project directly to the amygdala[18]. The MeA receives fibres from the accessory olfactory bulb[17]. Hippocampal projections originate from the ventral hippocampus (vHPC) to the BLA across species and regulate fear retrieval and renewal. Multimodal sensory inputs are sent to the LA, indicating a role in sensory integration during anxiety processing. The amygdalohippocampal area represents the most caudal region of the amygdala and receives internal connections from the LA, BLA, BMA, and CeA nuclei. The anterior amygdala is relatively underdeveloped in primates[19]. The CeA also receives afferents from the PVT, which regulate the expression of fear memory.

Efferent projections from the CeA target hypothalamic nuclei involved in autonomic and endocrine stress responses. The CeA also projects to the periaqueductal grey (PAG), locus coeruleus (LC), hippocampus (HPC), ventral tegmental area (VTA), and lateral substantia nigra[(20)(21)]. The CeA and MeA connect to the BNST via the stria terminalis. Both nuclei send projections to the lateral division of the BNST. The MeA also projects to the basal forebrain, brainstem, and hypothalamus. The BLA sends a large proportion of its efferent projections to the

nucleus accumbens[22]. The subcortical structures of the amygdala, such as the anterior, medial, and posterior amygdala, are well studied and known to regulate behavioural functions involving emotion, fear processing, and various aspects of social interaction, regulating anxiety, fear, and aggression[(15)(23)]. Amygdala nuclei are interconnected but can function independently. An exception is the lateral CeA, which receives no amygdala inputs[24]. Functionally, the BLA encodes conditioned threat associations whereas the CeA executes phasic fear responses and initiates acute anxiety responses[25].

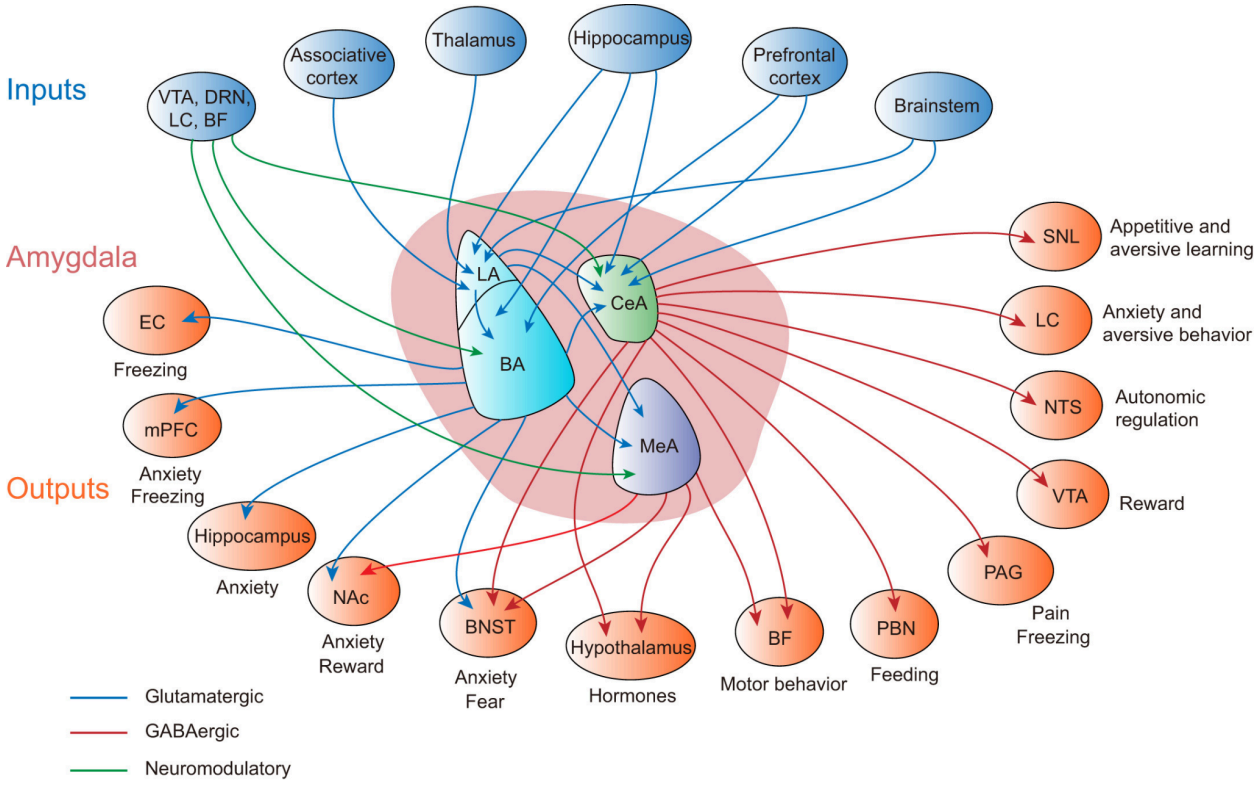

Figure 3. Input and output connections of the major amygdala subnuclei.

From Zhang, W., Zhang, J., Holmes, A., & Pan, B. (2021c). Amygdala Circuit substrates for stress adaptation and adversity. Biological Psychiatry, 89(9), 847–856. https://doi.org/10.1016/j.biopsych.2020.12.026

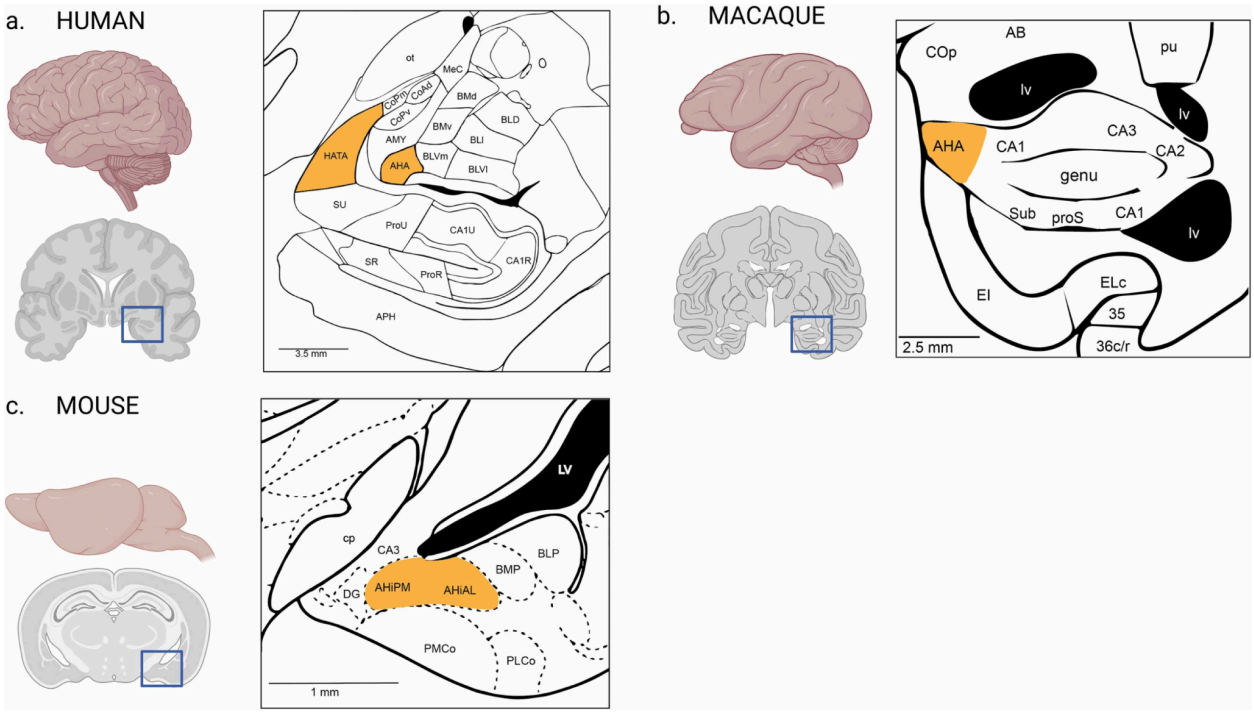

Figure 4. Comparative anatomy of human, macaque and mouse amygdala.

From Sedwick, V. M., & Autry, A. E. (2022b). Anatomical and molecular features of the amygdalohippocampal transition area and its role in social and emotional behavior processes. Neuroscience & Biobehavioral Reviews, 142, 104893. https://doi.org/10.1016/j.neubiorev.2022.104893

2.4 Bed Nucleus of the Stria terminalis (BNST):

The bed nucleus of the stria terminalis (BNST) was previously considered part of the extended amygdala. It is now recognised as a complex structure involved in integrating limbic information and monitoring valence. Although BNST structural connections have been extensively studied in rodents, fewer structural studies exist in non-human primates, dogs, and humans. Cross-species analyses indicate that BNST circuitry is largely conserved, although species-specific cellular differences exist [26].

Structure and Function: The BNST is a small cluster of nuclei located in the basomedial forebrain near the ventral septal region. It borders the anterior hypothalamus and lies adjacent to the anterior commissure[27].

- Anterolateral group (algBNST)

- Ventral BNST (vBNST)

- Oval nucleus (ovBNST)

- Aterodorsal BNST (adBNST).

The anterolateral group includes the oval (ovBNST), anterolateral (alBNST), juxtacapsular (juBNST), and fusiform (fuBNST) nuclei. Each subregion projects to distinct brain regions and exerts distinct regulatory effects[28]. Comparative studies in non-human primates indicate differences in neuron morphology and increased connectivity with the prefrontal cortex. In humans, the BNST is small and difficult to image, although high-resolution imaging has revealed connectivity with brain regions involved in anxiety disorders[26].

Direct studies of the BNST in dogs are limited, although canine neuroanatomy suggests an extended amygdala organisation similar to that in primates.

Projections: Afferent projections to the BNST include:

- CeA to ovBNST

- BLA to juBNST and adBNST

- Hippocampus to BNST,

Efferent projections include:

- ovBNST to CeA and adBNST

- adBNST to Lateral hypothalamus and ventral tegmental area (VTA)

- vBNST to VTA[(29)(30)(31)].

The anterolateral (alBNST) also shows strong reciprocal connectivity with cerebellar cortex and PFC. In rodents, the ACC and mPFC provide some of the largest projections.

Functionally, glutamatergic inputs arise primarily from the BLA, hippocampus, prefrontal cortex, and paraventricular thalamus, while GABAergic inputs arise from the CeA, MeA, and nucleus accumbens. The lateral hypothalamus provides both glutamatergic and GABAergic inputs[26]. BNST functional connectivity was significantly greater for the left and right subnuclei, compared with the left and right laterobasal and the left centromedial subnuclei[32].

The function of BNST is to influence the behavioural states over a longer duration[33], hence sustain anticipation behaviour for a longer duration. BNST has been linked with aBNST primarily regulating specific emotions[34-35]. Anxiety-like behaviours in rats, sustained anxiety anticipation in mice, and behavioural inhibition, threat anticipation and evaluation of threat in Macaques, and anxious temperament (AT), GADs, Social Anxiety, and phasic threat processing in humans, are some of the behavioural functions regulated by the BNST.

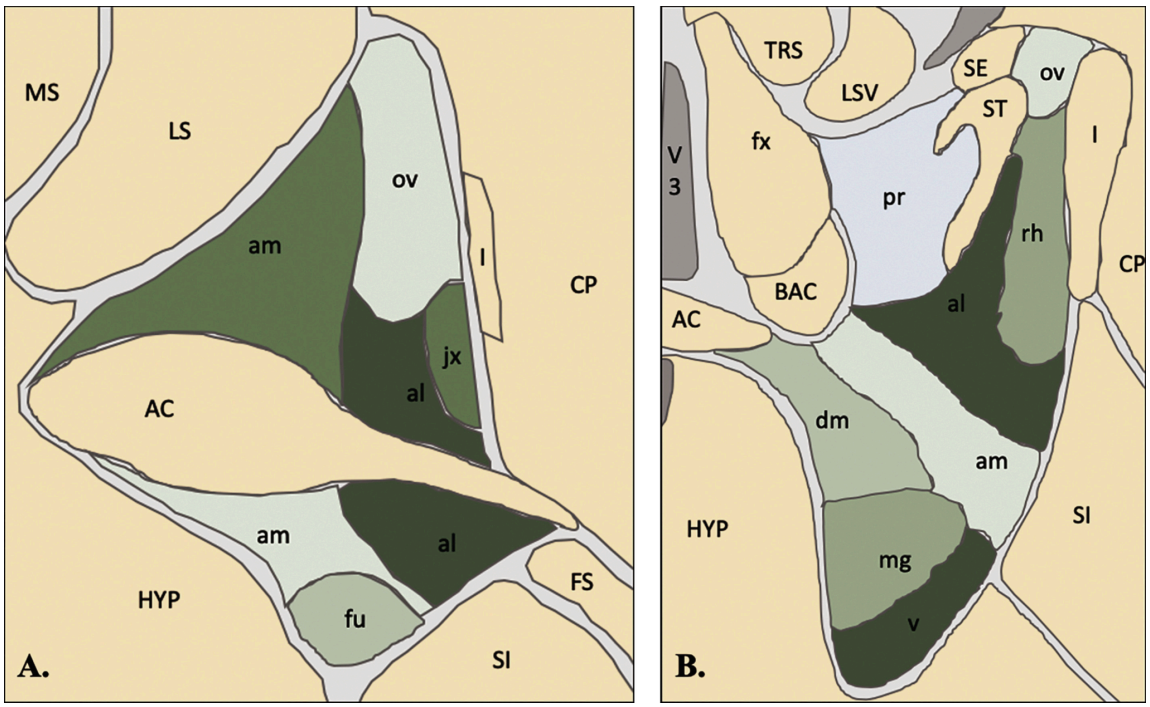

Figure 5. Coronal diagram of the mouse anterior BNST and its subnuclei, shown in green, and surrounding regions, based on the Allen Brain Atlas.

From Maita, I., Bazer, A., Blackford, J. U., & Samuels, B. A. (2021). Functional anatomy of the bed nucleus of the stria terminalis–hypothalamus neural circuitry: Implications for valence surveillance, addiction, feeding, and social behaviors. Handbook of Clinical Neurology, 179, 403–418. https://doi.org/10.1016/b978-0-12-819975-6.00026-1

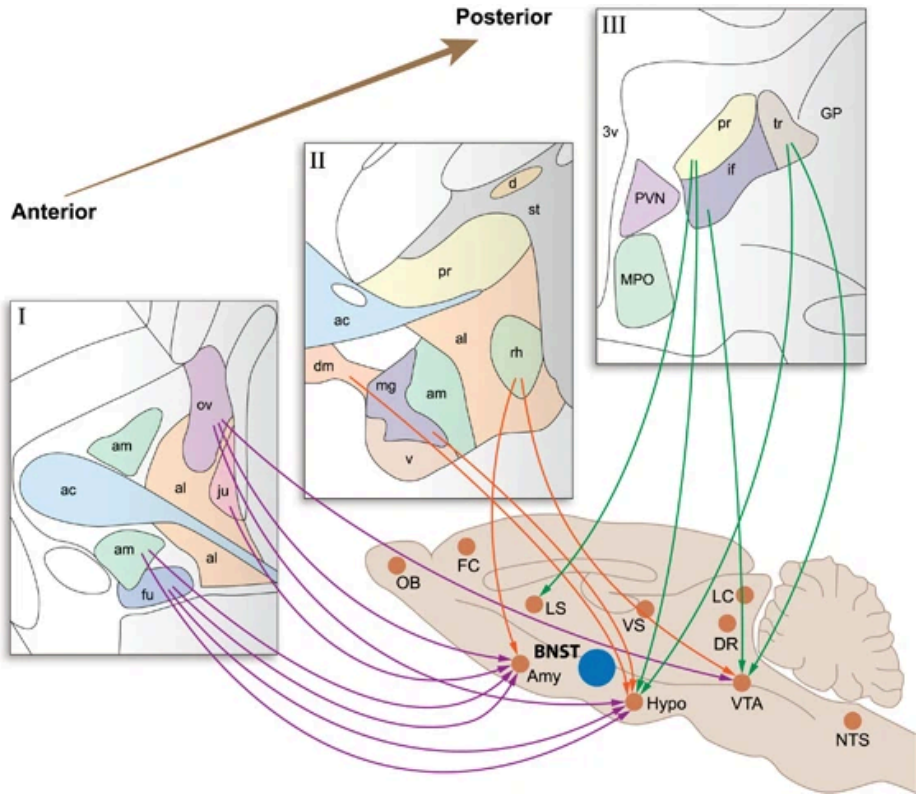

Figure 6. Afferent projections of the rodent BNST

From Lebow, M. A., & Chen, A. (2016). Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. Molecular Psychiatry, 21(4), 450–463. https://doi.org/10.1038/mp.2016.1

2.5 Hippocampus (HPC):

The hippocampus plays an important role in anxiety regulation due to its sensitivity to stress mediators and its strong connections with the amygdala and BNST.

Structure and Function: The hippocampus is a complex structure located in the medial temporal lobe. In rodents, it is composed of:

- dentate gyrus (DG)

- cornu ammonis (CA3, CA2, CA1)

- subiculum (SUB)[36]

In humans and primates, the hippocampus includes an additional region, CA4, which corresponds to cells within the dentate gyrus hilus[33-37]. The tri-synaptic circuit, which includes the entorhinal cortex, dentate gyrus, CA3, CA1 and subiculum, is well-conserved across mammals.

Rodents show strong anterior–posterior differentiation, with the ventral hippocampus (vHPC) more strongly associated with emotion and anxiety. In primates, the hippocampus shows stronger connectivity with prefrontal regions[38].

Projections: As the hippocampus is a major target and region for many processes, some major afferent projections that are essential in anxiety regulation are inputs from the entorhinal cortex, which carries sensory cortex information and environmental threat cues. Primates show greater anterior expansion and stronger limbic integrations as compared to rodents and canids. BLA sends strong projections to the HPC in primates and dogs. The BNST sends inputs to the hippocampus, with a stronger projection in primates. The hypothalamus also sends inputs to the HPC.

Efferent projections from the vCA1 in rodents, and the anterior HPC project into BNST, respectively[(39)(40)]. vCA1 and the subiculum project to BLA, which is involved in emotional context in primates, the HPC regulates cortical release and the autonomic response. Hippocampus sends projections to mPFC, which is dominant in primates, while rodents have limbic control[41]. The unique feature is the amygdalohippocampal transition area, which integrates social and emotional signals, particularly relevant in primates and dogs[42]. The hippocampus, therefore, contributes to contextual regulation of fear and anxiety responses.

2.6 Ventral Tegmental Area (VTA):

VTA comprises multiple subnuclei, which integrate multimodal limbic, cortical and brainstem information which is relevant to the process of anxiety regulation. It contains dopaminergic, GABAergic, and glutamatergic neurons implicated in reward and aversion.

Structure and Function: VTA comprises multiple subnuclei, which integrate multimodal limbic, cortical and brainstem information which is relevant to the process of anxiety regulation[43].

Across rodents, non-human primates (NHPs), humans and dogs, VTA retains a conserved mesencephalic position with relative proportions, and connectivity with cortical expansion is higher in mammals[44].

Projections: Afferent projections to VTA arise from Prefrontal Cortex (PFC), Anterior Cingulate Cortex (ACC), Lateral hypothalamus (LH), Amygdala, and Brainstem, providing glutamatergic and GABAergic regulation. ACC projections can drive aversive processing via activation of VTA GABA neurons, demonstrating top-down modulation of dopaminergic activity in anxiety[45].

Major efferent projections arise from dopaminergic, GABAergic, and glutamatergic neurons to the NAc and the medial prefrontal cortex, modulating anxiety. VTA projects to the shell of the NAc, regulating synaptic transmission and motivated behaviour. Additionally, reciprocal projections exist. The VTA sends inhibitory and excitatory feedback to the PFC and NAc, which act as a *salience hub* linking aversion and anxiety regulation. Stress models have highlighted this bidirectional control, whereby VTA dopaminergic neurons attenuate anxiety-like behaviour, which emphasises its role in anxiety regulation. These circuits regulate motivation, reward processing, and anxiety-related behaviour[45].

2.7 Hypothalamus (H):

The hypothalamus is part of the diencephalon and lies below the hypothalamic sulcus along the medial wall of the third ventricle. It acts as a hub coordinating autonomic, endocrine, and behavioural responses during stress and anxiety.

Structure and Function: The hypothalamus can be divided structurally into three longitudinal zones:

- periventricular

- medial

- lateral zone

These zones extend across anterior-posterior regions, including:

- preoptic

- supraoptic

- tuberal

- mammillary bodies

These regions are highly conserved across species.

The paraventricular nucleus (PVN) is involved in the (Hypothalamic-Pituitary-Adrenal (HPA) axis initiation during stress response, the ventromedial hypothalamus (VMH) in defence behaviour regulation, and the dorsomedial hypothalamus (DMH) in stress integration. The Lateral hypothalamic area (LH) is involved in avoidance behaviours, and the mammillary bodies connect to the limbic structures[46]. Rodents have clearly defined hypothalamic nuclei, whereas primates show more heterogeneous organisation[(47)(48)].

Projections: Primates have strong and expanded cortical connectivity with higher brain regions that support emotional regulation networks. There are subtle differences in humans and NHPs, including expanded integration with the limbic and cortical structures in humans [48].

Afferent projections from different brain regions to different hypothalamic nuclei regulate anxiety. PVN receives hippocampal projections, which regulate inhibitory feedback to the stress response, the amygdala conveys threat information, and the BNST carry forward the sustained anxiety signals to the PVN[49]. VMN receives inputs from PVN, LH and BNST. The PVN projections activate VMH during chronic and sustained stress[50]. Lateral Hypothalamus (LH) and the anterior receives afferent projection from the hippocampus, forming a hippocampus-anterior hypothalamic circuit, which regulates the endocrine and behavioural response during anxiogenic brain activity[49].

On the other hand, PVN sends many efferent projections to key brain regions, the cingulate cortex, PVT, amygdala, and the HPA. The projections from PVN to PVT regulate anxiety[51]. VMN sends projections to PAG and PVT for regulation of defensive /somatic responses and emotion processing in anxiety[52]. The LH sends to the brainstem and limbic structures. PVT acts as the primary output hub by endocrine response, VMH acts as the defensive hub by activating threat circuits, which regulate the threat response behaviours, and the anterior hypothalamus modulates the emotion by integrating hippocampal inputs.

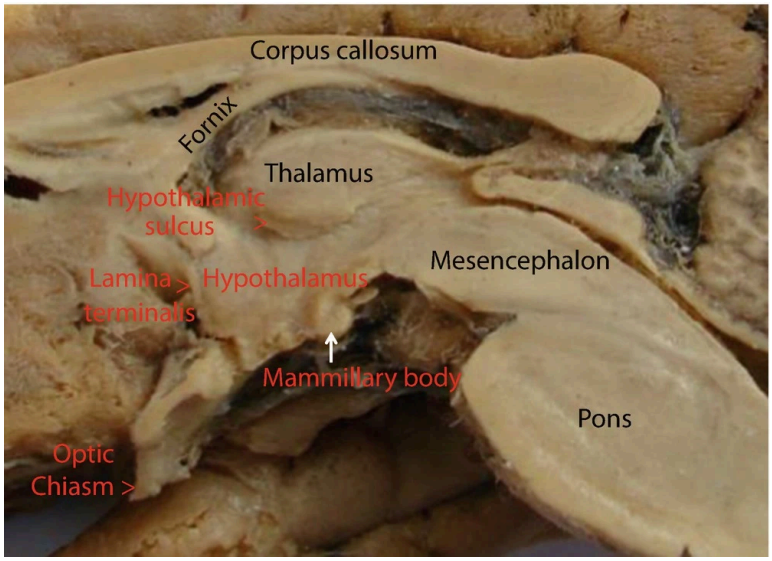

Figure 7. Tuberomammillary region.

From Schröder, H., Moser, N., & Huggenberger, S. (2020). The Mouse Hypothalamus. In Neuroanatomy of the Mouse (pp. 205–230) https://doi.org/10.1007/978-3-030-19898-5_9

2.8 Prefrontal Cortex (PFC):

The prefrontal cortex acts as a central regulator of anxiety by integrating cognitive, emotional, and contextual information and exerting top-down control over limbic structures. Across species, PFC regions receive inputs from limbic structures, which allows the PFC to evaluate the salience and context of potential threats.

Structure and Function: In rodents, the major subregion of the mPFC are prelimbic, which upregulates anxiety-like behaviours, while the infralimbic region is involved in the

down-regulation and the anterior cingulate cortex (ACC)[53]. In Primates, the PFC is more developed and highly specialised cortex regions, which are ventromedial PFC (vmPFC), orbitofrontal PFC (oPFC), Dorsolateral PFC (dlPFC), and anterior cingulate cortex (ACC). dlPFC has lateral convexity, highly granular with extensive connections which are involved in supporting cognitive control[54].

Medial (mPFC) and orbital (oPFC), plays a crucial role in regulating anxiety via different projection. Across species both the regions are divided differently. The vmPFC is located on the medial wall and ventral surface with a thin lamination, with strong structural links to amygdala and the hippocampus[55]. OFC is heterogenous in structure, which acts as an integrative multimodal sensory input. ACC is medially located, which connects the cognitive and affective networks. Paracingulate gyrus (PCG) of the ACC is involved in anxiety modulation in humans as in rodents and primates[56].

Canids have a structurally different PFC, as it lies more anteriorly and is less expanded and less granular than the primate PFC. There is a strong rostral frontal association cortex, a medial PFC region with strong connectivity, and the OFC is for sensory association.

Projections: Afferent Projections in rodents from BLA, BNST, vHP and the PAG projects to the Prelimbic (PL) region of the PFC, while the Infralimbic (IL) receives projections from amygdala, hippocampus and mediodorsal Thalamus (MDT)[57], the ACC also receives projections from hippocampus and MDT. Efferent projections to BLA, striatum, and hypothalamus from PL, in addition, IL projects to BMA and brainstem[58].

The ACC also receives projections from the hippocampus and MDT. Efferent projections to BLA, striatum, and hypothalamus from PL, in addition, IL projects to BMA and brainstem[(59)(60)]. ACC is medially located, which connects the cognitive and affective networks. The paracingulate gyrus (PCG) of the ACC is involved in anxiety modulation in humans, as in rodents and primates[60].

Canids have a structurally different PFC; it lies more anteriorly, is less expanded and less granular than the primate PFC. There is a strong rostral frontal association cortex, a medial PFC

region with strong connectivity, and the OFC is for sensory association[61]. These circuits regulate emotional evaluation and behavioural responses to threat.

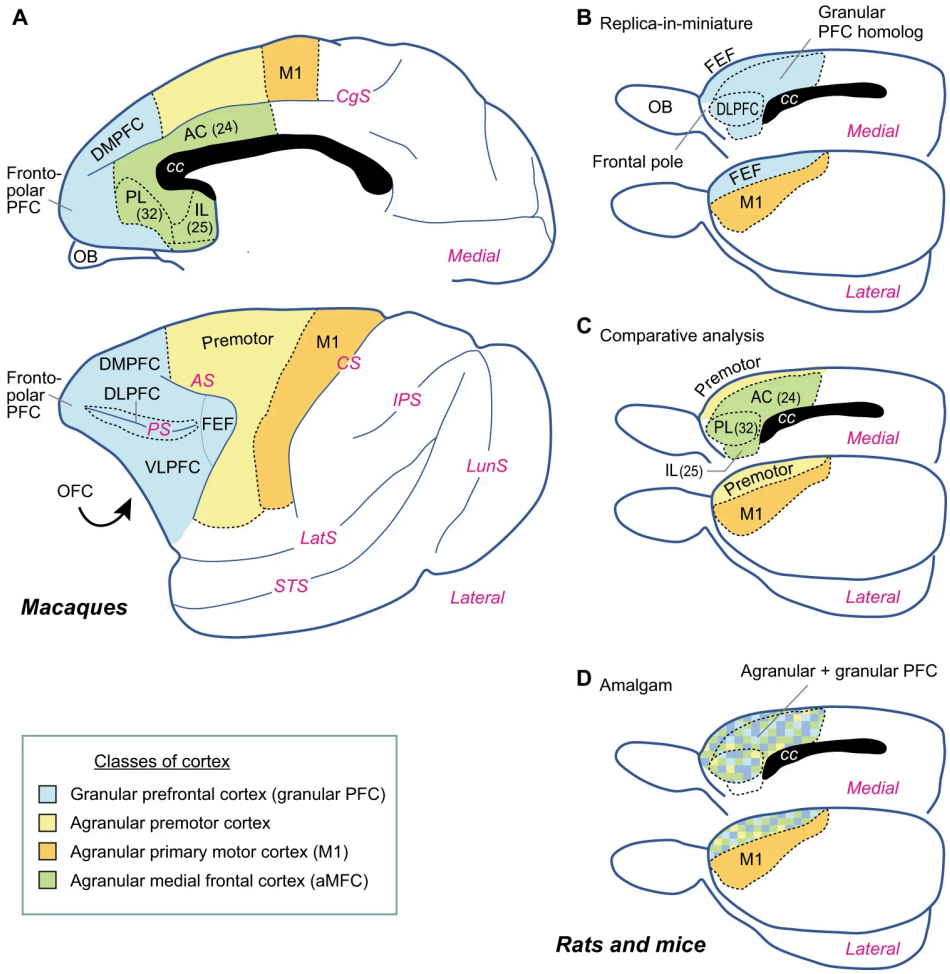

Figure 8. Proposed homologies among frontal areas in primates and rodents.

From Preuss, T. M., & Wise, S. P. (2021). Evolution of prefrontal cortex. Neuropsychopharmacology, 47(1), 3–19. https://doi.org/10.1038/s41386-021-01076-5

2.9 Insula (IC):

The insula is a cortical structure located deep within the lateral sulcus and is sometimes referred to as the "*fifth lobe*" of the brain. Across species, it is conserved, but shows a progressive expansion and laminar complexity from rodents to dogs, and to primates[62].

Structure and Function: In primates, the insula is divided into:

- Anterior (AIC)

- Posterior (PIC) section with a well-defined boundary.

In rodents, the insula is exposed on the cortical surface of the brain and subdivided into agranular, dysgranular, and granular zones [(62)(63)]. In dogs, there is moderate cortical folding and clear section differentiation from rodents but less than primates[63].

The insula integrates visceral and emotional signals from posterior to anterior axis, while the anterior insula integrates this into conscious, affective experience[64]. In a rodent study conducted, it was found that the rostral regions of the insula have an anxiogenic role, while caudal regions have an anxiolytic role, with a transition region that has both anxiolytic and anxiogenic activity based on its activation[65].

Projections: Afferent and efferent projections to and from the insula are similar to those of the above-mentioned brain regions. Afferent projections to the thalamus from the PIC, and sends reciprocal connections to the amygdala and BNST. AIC innervates the PFC and ACC, which send regulatory and contextual signals.

Efferent projections are from the mPFC and oPFC, the BNST, the Amygdala, the NAc, and the brainstem to support cognitive appraisal and emotion regulation, thereby influencing the response[(62)(63)].

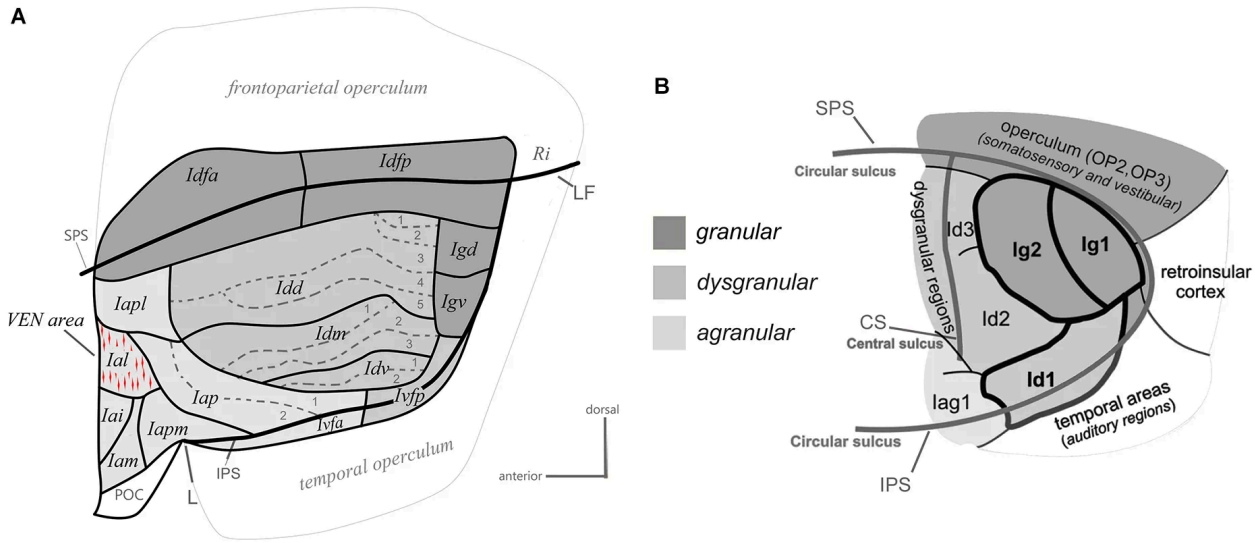

Figure 9. Architectonic parcellation of the insular cortex in the macaque (A) monkey and (B) human.

From Evrard, H. C. (2019). The organization of the Primate Insular Cortex. Frontiers in Neuroanatomy, 13, 43. https://doi.org/10.3389/fnana.2019.00043

2.10 Brainstem:

The mammalian brainstem, which is involved in anxiety regulation, is composed of the Locus coeruleus (LC), Periaqueductal grey (PAG), and Dorsal Raphe Nucleus (DRN). These regions work as independent entities and in sync with other regions to modulate anxiety. Despite species differences, the brainstem is conserved across all mammals, with the noradrenergic nucleus of the locus coeruleus[66]. Parabrachial complex (PBN) is another nuclear group which is involved in the transmission of affective information to the forebrain[67].

Structure and Function: LC is a major nucleus located in the dorsal pontine tegmentum, adjacent to the fourth ventricle. In rodents, the LC organisation is relatively compact and homogenous, while in primates, the LC is larger and more elongated rostrocaudally[68].

Projections: Afferent inputs to LC, from CeA, BNST, PFC, Hypothalamus and internally with the PAG. Efferent projections from LC to amygdala, hippocampus, mPFC, BNST[(69)(70)]. PAG surrounds the cerebral aqueduct in the midbrain. It is organised into longitudinal structures - Dorsolateral (dlPAG), Ventrolateral (vlPAG), Dorsomedial and lateral columns[71]. These columns are conserved in primates and rodents. In primates, a similar columnar layout is observed with

greater cortical inputs from ACCA and mPFC. Afferent inputs to PAG from CeA, BNST, PFC (strong projection in primates), vHP and insula. Efferent projections from PAG to LC[72].

DRN is located in the midline midbrain, ventral to the aqueduct. It is the largest serotonergic nucleus, which is subdivided into dorsal, ventral and ventrolateral[73]. Afferent inputs to DRN from amygdala, BNST, PFC, lateral habenula, PAG and hypothalamus. Efferent projections from amygdala, BNST, hippocampus, mPFC and LC[74].

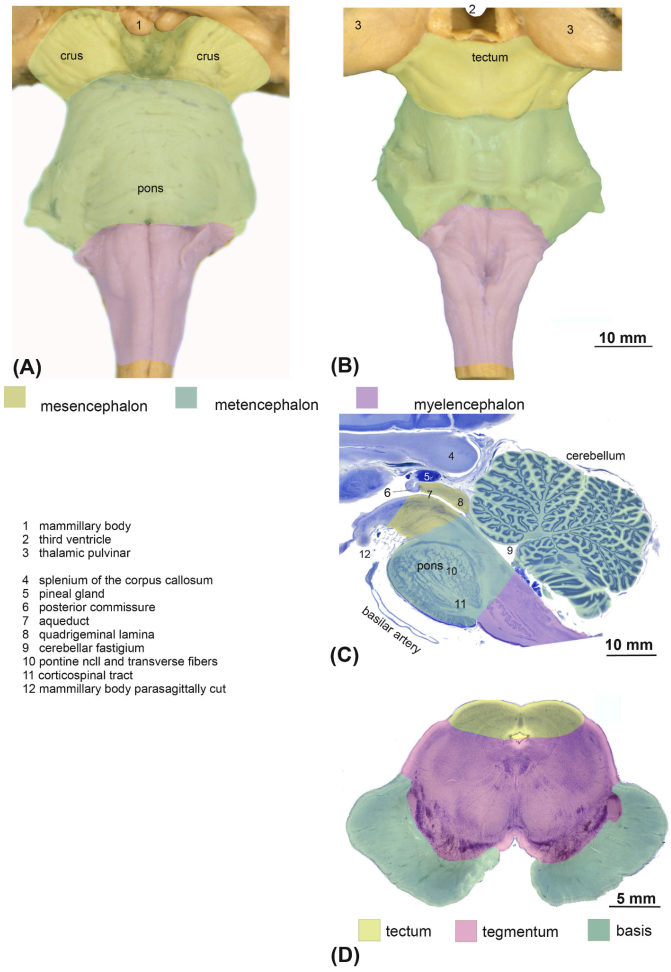

Figure 10. Human Brainstem (A) ventral aspect; (B) dorsal aspect of a human spinal (C) mediosagittal gallocyanin-stained section (D) Mesencephalic tectum tegmentum

From Heinsen, H., Grinberg, L. T., & Bohnert, S. (2026). Structural and functional anatomy of the brainstem. In Handbook of clinical neurology (Vol. 216, pp. 17–57).

https://doi.org/10.1016/b978-0-443-15736-3.00015-9

2.11 Role of Cerebellum:

Cerebellum has been viewed as a structure specialised for motor function, coordination, but recent research indicates that the cerebellum also participates in affective regulation, psychiatric disorders, in this context, anxiety regulation[75]. The cerebellum is a highly conserved brain

structure located dorsal to the brainstem in the posterior cranial fossa. Despite its fundamental anatomy, size, cytology, and circuitry is are conserved across species.

Structure and Function: Anatomically, the cerebellum is divided into the cerebellar hemisphere, vermis, and flocculonodular lobes[76]. Rodents have a small cerebral hemisphere and a large vermal structure. In contrast to primates, mainly humans show extensive hemispheric expansion, and canids have a moderate expansion and vermal structures[77]. In canines, there is a well-developed anterior cerebellar lobes and prominent hemispheres, due to highly coordinated locomotion and complex sensorimotor[78].

Projections: Afferent projection from PFC to cerebellum, reaches via pontine nucleus[79]. Amygdalar projections to cerebellum via brainstem and hypothalamic relays, and the Brainstem and PAG to cerebellum[80]. The nuclear system of the cerebellum is involved in cognitive regulation with dense efferent projections to other regions in anxiety regulation, which are dentate, interposed and fastigial nuclei. The fastigial nucleus to vlPAG, fastigial nucleus innervates the parafasicular nucleus to modulate the amygdala via a polysynaptic pathway. In humans, the dentate nuclei innervates to amygdalar. In NHPs intralaminar and mediodorsal nuclei project to the amygdala. In rodents, the nuclei mediodorsal thalamus to mPFC[81]. The fastigial projects to the hypothalamus and autonomic centres in the brainstem.

For the cerebellum, more species-specific tract studies are needed to elucidate its role in anxiety.

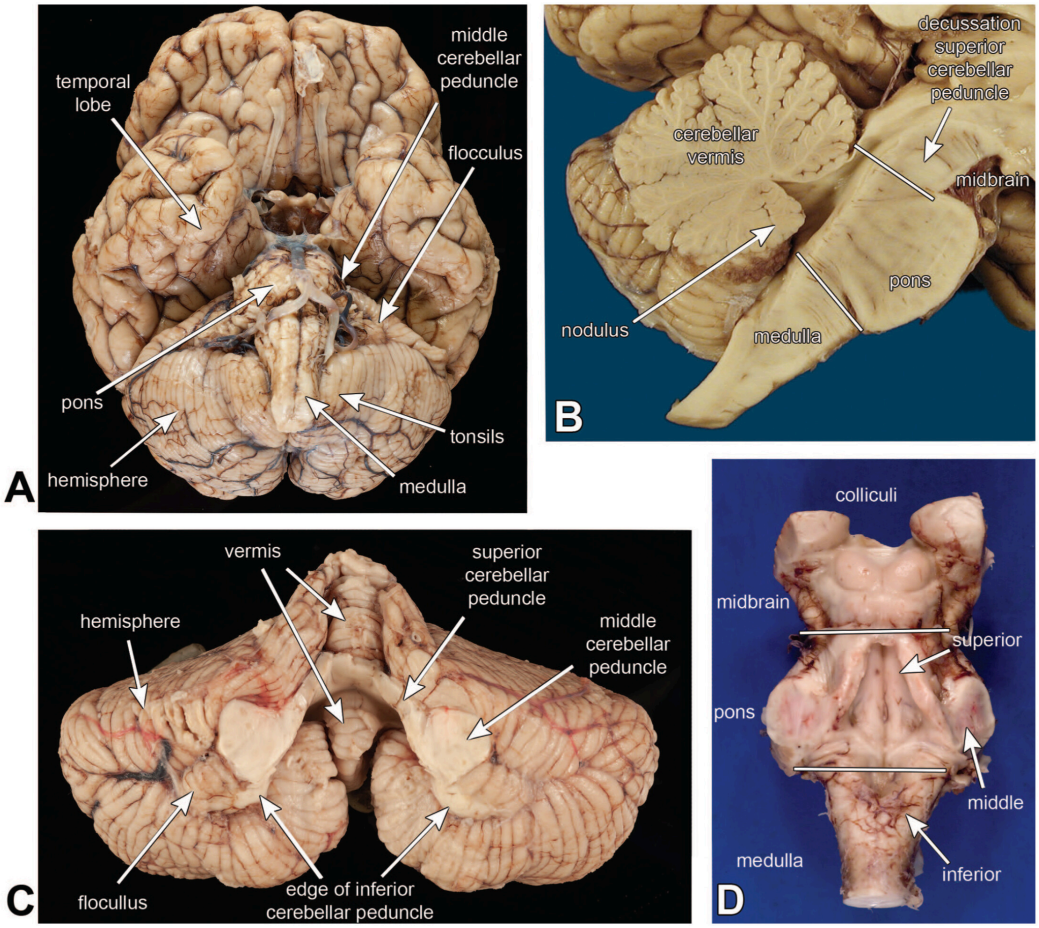

Figure 11. Cerebellar Macroscopic Anatomy**.** From Joseph, J. T. (2025).

The human cerebellum as a semaphore for neuropathology. Toxicologic Pathology, 53(8), 663–681. https://doi.org/10.1177/01926233251386797

2.12 Conclusion

Anatomical understanding of anxiety reveals a complex and highly integrated network of brain regions, demonstrating anxiety is not solely amygdala-centric. Instead, individual neural structures contribute to distinct temporal, cognitive and autonomic functions that collectively shape the generation of anxiety. The broad conservation of anxiety-related circuitry across species further highlights the evolutionary significance of these networks, despite significant differences in morphology; anxiety is conserved across these species. Anatomical studies reveal emerging deep brain structures, including the BNST, thalamic nuclei, and the cerebellum, that

also contribute to anxiety regulation. Continued investigation into these regions will be essential for developing a more comprehensive understanding of the neuroanatomy of anxiety.

References

1. Kropf, E., Syan, S. K., Minuzzi, L., & Frey, B. N. (2018c). From anatomy to function: the role of the somatosensory cortex in emotional regulation. Brazilian Journal of Psychiatry, 41(3), 261–269. https://doi.org/10.1590/1516-4446-2018-0183
2. Guran, C. A., Boch, M., Sladky, R., Lonardo, L., Karl, S., Huber, L., & Lamm, C. (2024). Functional mapping of the somatosensory cortex using noninvasive fMRI and touch in awake dogs. Brain Structure and Function, 229(5), 1193–1207. https://doi.org/10.1007/s00429-024-02798-0
3. O'Connor, D. H., Krubitzer, L., & Bensmaia, S. (2021b). Of mice and monkeys: Somatosensory processing in two prominent animal models. _Progress in Neurobiology_, _201_, 102008. https://doi.org/10.1016/j.pneurobio.2021.102008
4. Padberg, J., Cooke, D. F., Cerkevich, C. M., Kaas, J. H., & Krubitzer, L. (2018b). Cortical connections of area 2 and posterior parietal area 5 in macaque monkeys. *The Journal of Comparative Neurology*, *527*(3), 718–737. https://doi.org/10.1002/cne.24453
5. Klingner, C. M., Brodoehl, S., Huonker, R., & Witte, O. W. (2016). The Processing of Somatosensory Information Shifts from an Early Parallel into a Serial Processing Mode: A Combined fMRI/MEG Study. *Frontiers in Systems Neuroscience*, *10*, 103. https://doi.org/10.3389/fnsys.2016.00103
6. Marcuse, L. V., Langan, M., Hof, P. R., Panov, F., Saez, I., Jimenez-Shahed, J., Figee, M., Mayberg, H., Yoo, J. Y., Ghatan, S., Balchandani, P., & Fields, M. C. (2025). The thalamus: Structure, function, and neurotherapeutics. Neurotherapeutics, 22(2), e00550. https://doi.org/10.1016/j.neurot.2025.e00550
7. Pérez-Santos, I., García-Cabezas, M. Á., & Cavada, C. (2023b). Mapping the primate thalamus: systematic approach to analyze the distribution of subcortical neuromodulatory afferents. _Brain Structure and Function_, _228_(5), 1153–1176. https://doi.org/10.1007/s00429-023-02619-w
8. Pedersen, T. R., Berendt, M., & Rusbridge, C. (2025b). Neuroanatomy of spinal nociception and pain in dogs and cats: a practical review for the veterinary clinician. _Frontiers in Veterinary Science_, _12_, 1534685. https://doi.org/10.3389/fvets.2025.1534685

9. Roy, D. S., Zhang, Y., Halassa, M. M., & Feng, G. (2022b). Thalamic subnetworks as units of function. _Nature Neuroscience_, _25_(2), 140–153. https://doi.org/10.1038/s41593-021-00996-1
10. Cortes, N., Mandel, C., Ikan, L., & Casanova, C. (2025b). The pulvinar nucleus and its role in cognitive functions. _Brain Research Bulletin_, _234_, 111672. https://doi.org/10.1016/j.brainresbull.2025.111672
11. Li, F., Zheng, X., Wang, H., Meng, L., Chen, M., Hui, Y., Liu, D., Li, Y., Xie, K., Zhang, J., & Guo, G. (2024). Mediodorsal thalamus projection to medial prefrontal cortical mediates social defeat stress-induced depression-like behaviors. _Neuropsychopharmacology_, _49_(8), 1318–1329. https://doi.org/10.1038/s41386-024-01829-y
12. Barson JR, Mack NR and Gao W-J (2020) The Paraventricular Nucleus of the Thalamus Is an Important Node in the Emotional Processing Network. Front. Behav. Neurosci. 14:598469. doi: 10.3389/fnbeh.2020.598469
13. Kirouac, G. J. (2021). The paraventricular nucleus of the thalamus as an integrating and relay node in the brain anxiety network. _Frontiers in Behavioral Neuroscience_, _15_, 627633. https://doi.org/10.3389/fnbeh.2021.627633
14. Nelson, A. J. (2021). The anterior thalamic nuclei and cognition: A role beyond space? _Neuroscience & Biobehavioral Reviews_, _126_, 1–11. https://doi.org/10.1016/j.neubiorev.2021.02.047
15. Sedwick, V. M., & Autry, A. E. (2022). Anatomical and molecular features of the amygdalohippocampal transition area and its role in social and emotional behavior processes. _Neuroscience & Biobehavioral Reviews_, _142_, 104893. https://doi.org/10.1016/j.neubiorev.2022.104893
16. Hecht, E. E., Smaers, J. B., Dunn, W. D., Kent, M., Preuss, T. M., & Gutman, D. A. (2019). Significant neuroanatomical variation among domestic dog breeds. _Journal of Neuroscience_, _39_(39), 7748–7758. https://doi.org/10.1523/jneurosci.0303-19.2019
17. Zhang, W., Zhang, J., Holmes, A., & Pan, B. (2021b). Amygdala Circuit substrates for stress adaptation and adversity. Biological Psychiatry, 89(9), 847–856. https://doi.org/10.1016/j.biopsych.2020.12.026

18. Kenwood, M. M., Kalin, N. H., & Barbas, H. (2021). The prefrontal cortex, pathological anxiety, and anxiety disorders. _Neuropsychopharmacology_, _47_(1), 260–275. https://doi.org/10.1038/s41386-021-01109-z
19. Šimić, G., Tkalčić, M., Vukić, V., Mulc, D., Španić, E., Šagud, M., Olucha-Bordonau, F. E., Vukšić, M., & Hof, P. R. (2021). Understanding Emotions: Origins and roles of the amygdala. _Biomolecules_, _11_(6), 823. https://doi.org/10.3390/biom11060823
20. Morel, C., Montgomery, S. E., Li, L., Cuttoli, R. D., Teichman, E. M., Juarez, B., Tzavaras, N., Ku, S. M., Flanigan, M. E., Cai, M., Walsh, J. J., Russo, S. J., Nestler, E. J., Calipari, E. S., Friedman, A. K., & Han, M. (2022). Midbrain projection to the basolateral amygdala encodes anxiety-like but not depression-like behaviors. _Nature Communications_, _13_(1), 1532. https://doi.org/10.1038/s41467-022-29155-1
21. Gilpin, N. W., Herman, M. A., & Roberto, M. (2014). The Central Amygdala as an integrative hub for anxiety and alcohol use disorders. _Biological Psychiatry_, _77_(10), 859–869. https://doi.org/10.1016/j.biopsych.2014.09.008
22. Sangha, S., Diehl, M. M., Bergstrom, H. C., & Drew, M. R. (2019). Know safety, no fear. _Neuroscience & Biobehavioral Reviews_, _108_, 218–230. https://doi.org/10.1016/j.neubiorev.2019.11.006
23. RajMohan, V., & Mohandas, E. (2007). The limbic system. _Indian Journal of Psychiatry_, _49_(2), 132. https://doi.org/10.4103/0019-5545.33264
24. Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2009). Phasic vs Sustained Fear in Rats and Humans: Role of the Extended Amygdala in Fear vs Anxiety. _Neuropsychopharmacology_, _35_(1), 105–135. https://doi.org/10.1038/npp.2009.109
25. Ressler, K. (2010). Amygdala activity, fear, and anxiety: modulation by stress. Biol. Psychiatry 67, 1117–1119. doi: 10.1016/j.biopsych.2010.04.027
26. Van De Poll, Y., Cras, Y., & Ellender, T. J. (2023). The neurophysiological basis of stress and anxiety - comparing neuronal diversity in the bed nucleus of the stria terminalis (BNST) across species. _Frontiers in Cellular Neuroscience_, _17_, 1225758. https://doi.org/10.3389/fncel.2023.1225758
27. Avery, S. N., Clauss, J. A., Winder, D. G., Woodward, N., Heckers, S., & Blackford, J. U. (2014). BNST neurocircuitry in humans. _NeuroImage_, _91_, 311–323. https://doi.org/10.1016/j.neuroimage.2014.01.017

28. Dumais KM, Alonso AG, Immormino MA, Bredewold R, Veenema AH. Involvement of the oxytocin system in the bed nucleus of the stria terminalis in the sex-specific regulation of social recognition. Psychoneuroendocrinology. (2016) 64:7988. doi: 10.1016/j.psyneuen.2015.11.007
29. Johansen, J. P. (2013). Anxiety is the sum of its parts. _Nature_, _496_(7444), 174–175. https://doi.org/10.1038/nature12087
30. Gong, W. (2025). Research progress on the neural circuits mechanisms of anxiety. *Frontiers in Neural Circuits*, *19*, 1609145. https://doi.org/10.3389/fncir.2025.1609145
31. Dedic, N., Kühne, C., Jakovcevski, M., Hartmann, J., Genewsky, A. J., Gomes, K. S., Anderzhanova, E., Pöhlmann, M. L., Chang, S., Kolarz, A., Vogl, A. M., Dine, J., Metzger, M. W., Schmid, B., Almada, R. C., Ressler, K. J., Wotjak, C. T., Grinevich, V., Chen, A., . . . Deussing, J. M. (2018b). Chronic CRH depletion from GABAergic, long-range projection neurons in the extended amygdala reduces dopamine release and increases anxiety. Nature Neuroscience, 21(6), 803–807. https://doi.org/10.1038/s41593-018-0151-z
32. Avery, S. N., Clauss, J. A., Winder, D. G., Woodward, N., Heckers, S., & Blackford, J. U. (2014b). BNST neurocircuitry in humans. _NeuroImage_, _91_, 311–323. https://doi.org/10.1016/j.neuroimage.2014.01.017
33. Shi, H., Wang, S., Wang, X., Zhang, R., & Zhu, L. (2023). Hippocampus: molecular, cellular, and circuit features in anxiety. _Neuroscience Bulletin_, _39_(6), 1009–1026. https://doi.org/10.1007/s12264-023-01020-1
34. Xie, M., Xiong, Y., & Wang, H. (2024). The regulative role and mechanism of BNST in anxiety disorder. _Frontiers in Psychiatry_, _15_, 1437476. https://doi.org/10.3389/fpsyt.2024.1437476
35. Klampfl, S. M., Brunton, P. J., Bayerl, D. S., & Bosch, O. J. (2015). CRF-R1 activation in the anterior-dorsal BNST induces maternal neglect in lactating rats via an HPA axis-independent central mechanism. _Psychoneuroendocrinology_, _64_, 89–98. https://doi.org/10.1016/j.psyneuen.2015.11.015
36. Sedwick, V. M., & Autry, A. E. (2022). Anatomical and molecular features of the amygdalohippocampal transition area and its role in social and emotional behavior

processes. _Neuroscience & Biobehavioral Reviews_, _142_, 104893. https://doi.org/10.1016/j.neubiorev.2022.104893

37. Flattery, C. N. R., Rosen, R. F., Farberg, A. S., Dooyema, J. M., Hof, P. R., Sherwood, C. C., Walker, L. C., & Preuss, T. M. (2020). Quantification of neurons in the hippocampal formation of chimpanzees: comparison to rhesus monkeys and humans. _Brain Structure and Function_, _225_(8), 2521–2531. https://doi.org/10.1007/s00429-020-02139-x
38. Eichert, N., DeKraker, J., Howard, A. F. D., Huszar, I. N., Zhu, S., Sallet, J., Miller, K. L., Mars, R. B., Jbabdi, S., & Bernhardt, B. C. (2024b). Hippocampal connectivity patterns echo macroscale cortical evolution in the primate brain. _Nature Communications_, _15_(1), 5963. https://doi.org/10.1038/s41467-024-49823-8
39. Shi, H., Wang, S., Wang, X., Zhang, R., & Zhu, L. (2023). Hippocampus: molecular, cellular, and circuit features in anxiety. _Neuroscience Bulletin_, _39_(6), 1009–1026. https://doi.org/10.1007/s12264-023-01020-1
40. Avery, S. N., Clauss, J. A., Winder, D. G., Woodward, N., Heckers, S., & Blackford, J. U. (2014b). BNST neurocircuitry in humans. _NeuroImage_, _91_, 311–323. https://doi.org/10.1016/j.neuroimage.2014.01.017
41. Phillips, M. L., Robinson, H. A., & Pozzo-Miller, L. (2019). Ventral hippocampal projections to the medial prefrontal cortex regulate social memory. _eLife_, _8_. https://doi.org/10.7554/elife.44182
42. Sedwick, V. M., & Autry, A. E. (2022). Anatomical and molecular features of the amygdalohippocampal transition area and its role in social and emotional behavior processes. _Neuroscience & Biobehavioral Reviews_, _142_, 104893. https://doi.org/10.1016/j.neubiorev.2022.104893
43. Holly, E. N., & Miczek, K. A. (2015). Ventral tegmental area dopamine revisited: effects of acute and repeated stress. _Psychopharmacology_, _233_(2), 163–186. https://doi.org/10.1007/s00213-015-4151-3
44. Han, X., Jing, M., Zhao, T., Wu, N., Song, R., & Li, J. (2017). Role of dopamine projections from ventral tegmental area to nucleus accumbens and medial prefrontal cortex in reinforcement behaviors assessed using optogenetic manipulation. _Metabolic Brain Disease_, _32_(5), 1491–1502. https://doi.org/10.1007/s11011-017-0023-3

45. Guan, P., Huang, C., Lan, Q., Huang, S., Zhou, P., & Zhang, C. (2022). Activation of ventral tegmental area dopaminergic neurons ameliorates anxiety-like behaviors in single prolonged stress-induced PTSD model rats. _Neurochemistry International_, _161_, 105424. https://doi.org/10.1016/j.neuint.2022.105424
46. Saper, C. B., & Lowell, B. B. (2014). The hypothalamus. _Current Biology_, _24_(23), R1111–R1116. https://doi.org/10.1016/j.cub.2014.10.023
47. Freeman, S. M., & Young, L. J. (2016). Comparative Perspectives on oxytocin and vasopressin receptor research in rodents and primates: Translational implications. Journal of Neuroendocrinology, 28(4). https://doi.org/10.1111/jne.12382
48. Montardy, Q., Kwan, W. C., Mundinano, I. C., Fox, D. M., Wang, L., Gross, C. T., & Bourne, J. A. (2020). Mapping the neural circuitry of predator fear in the nonhuman primate. Brain Structure and Function, 226(1), 195–205. https://doi.org/10.1007/s00429-020-02176-6
49. Bang, J. Y., Zhao, J., Rahman, M., St-Cyr, S., McGowan, P. O., & Kim, J. C. (2022). Hippocampus-Anterior hypothalamic circuit modulates Stress-Induced endocrine and behavioral response. _Frontiers in Neural Circuits_, _16_, 894722. https://doi.org/10.3389/fncir.2022.894722
50. Shao, J., Chen, Y., Gao, D., Liu, Y., Hu, N., Yin, L., Zhang, X., & Yang, F. (2023). Ventromedial hypothalamus relays chronic stress inputs and exerts bidirectional regulation on anxiety state and related sympathetic activity. _Frontiers in Cellular Neuroscience_, _17_, 1281919. https://doi.org/10.3389/fncel.2023.1281919
51. Liu, Y., Rao, B., Li, S., Zheng, N., Wang, J., Bi, L., & Xu, H. (2022b). Distinct hypothalamic paraventricular nucleus inputs to the cingulate cortex and paraventricular thalamic nucleus modulate anxiety and arousal. _Frontiers in Pharmacology_, _13_, 814623. https://doi.org/10.3389/fphar.2022.814623
52. Zhang, J., Chen, D., Sweeney, P., & Yang, Y. (2020). An excitatory ventromedial hypothalamus to paraventricular thalamus circuit that suppresses food intake. _Nature Communications_, _11_(1), 6326. https://doi.org/10.1038/s41467-020-20093-4
53. Suzuki, S., Saitoh, A., Ohashi, M., Yamada, M., Oka, J., & Yamada, M. (2016). The infralimbic and prelimbic medial prefrontal cortices have differential functions in the

expression of anxiety-like behaviors in mice. Behavioural Brain Research, 304, 120–124. https://doi.org/10.1016/j.bbr.2016.01.044

54. Arnsten, A. F., & Rubia, K. (2012). Neurobiological circuits regulating attention, cognitive control, motivation, and emotion: Disruptions in neurodevelopmental psychiatric disorders. Journal of the American Academy of Child & Adolescent Psychiatry_, _51_(4), 356–367. https://doi.org/10.1016/j.jaac.2012.01.008
55. Adhikari, A., Lerner, T. N., Finkelstein, J., Pak, S., Jennings, J. H., Davidson, T. J., Ferenczi, E., Gunaydin, L. A., Mirzabekov, J. J., Ye, L., Kim, S., Lei, A., & Deisseroth, K. (2015). Basomedial amygdala mediates top-down control of anxiety and fear. _Nature_, _527_(7577), 179–185. https://doi.org/10.1038/nature15698
56. Mohanty, A., Engels, A. S., Herrington, J. D., Heller, W., Ho, M. R., Banich, M. T., Webb, A. G., Warren, S. L., & Miller, G. A. (2007). Differential engagement of anterior cingulate cortex subdivisions for cognitive and emotional function. _Psychophysiology_, _44_(3), 343–351. https://doi.org/10.1111/j.1469-8986.2007.00515.x
57. Bloodgood, D. W., Sugam, J. A., Holmes, A., & Kash, T. L. (2018). Fear extinction requires infralimbic cortex projections to the basolateral amygdala. *Translational Psychiatry*, *8*(1), 60. https://doi.org/10.1038/s41398-018-0106-x
58. Vertes, R. P. (2003). Differential projections of the infralimbic and prelimbic cortex in the rat. _Synapse_, _51_(1), 32–58. https://doi.org/10.1002/syn.10279
59. Kenwood, M. M., Oler, J. A., Tromp, D. P. M., Fox, A. S., Riedel, M. K., Roseboom, P. H., Brunner, K. G., Aggarwal, N., Murray, E. A., & Kalin, N. H. (2022). Prefrontal influences on the function of the neural circuitry underlying anxious temperament in primates. *Oxford Open Neuroscience*, *2*. https://doi.org/10.1093/oons/kvac016
60. Kim, M. J., Brown, A. C., Mattek, A. M., Chavez, S. J., Taylor, J. M., Palmer, A. L., Wu, Y., & Whalen, P. J. (2016). The Inverse Relationship between the Microstructural Variability of Amygdala-Prefrontal Pathways and Trait Anxiety Is Moderated by Sex. _Frontiers in Systems Neuroscience_, _10_, 93. https://doi.org/10.3389/fnsys.2016.00093
61. Ramaihgari, B., Pustovyy, O. M., Waggoner, P., Beyers, R. J., Wildey, C., Morrison, E., Salibi, N., Katz, J. S., Denney, T. S., Vodyanoy, V. J., & Deshpande, G. (2018). Zinc nanoparticles enhance brain connectivity in the canine olfactory network: evidence from

an FMRI study in unrestrained awake dogs. _Frontiers in Veterinary Science_, _5_, 127. https://doi.org/10.3389/fvets.2018.00127

62. Laketić, D., Stojanović, N. M., Laketić, I., Pavlović, M., Milosević, B., Starčević, A., & Kapor, S. (2025). Insular Cortex—Biology and its Role in Psychiatric Disorders: A Narrative review. _Brain Sciences_, _15_(8), 793. https://doi.org/10.3390/brainsci15080793
63. Bouras, N. N., Mack, N. R., & Gao, W. (2023). Prefrontal modulation of anxiety through a lens of noradrenergic signaling. _Frontiers in Systems Neuroscience_, _17_, 1173326. https://doi.org/10.3389/fnsys.2023.1173326
64. Zhang, Y., Cai, X., Duan, M., & He, H. (2023). The influence of high worry on static and dynamic insular functional connectivity. _Frontiers in Neuroscience_, _17_, 1062947. https://doi.org/10.3389/fnins.2023.1062947
65. Breton-Provencher, V., Drummond, G. T., & Sur, M. (2021). Locus coeruleus norepinephrine in learned behavior: anatomical modularity and spatiotemporal integration in targets. _Frontiers in Neural Circuits_, _15_, 638007. https://doi.org/10.3389/fncir.2021.638007
66. Palmiter, R. D. (2018). The parabrachial nucleus: CGRP neurons function as a general alarm. _Trends in Neurosciences_, _41_(5), 280–293. https://doi.org/10.1016/j.tins.2018.03.007
67. Poe, G. R., Foote, S., Eschenko, O., Johansen, J. P., Bouret, S., Aston-Jones, G., Harley, C. W., Manahan-Vaughan, D., Weinshenker, D., Valentino, R., Berridge, C., Chandler, D. J., Waterhouse, B., & Sara, S. J. (2020). Locus coeruleus: a new look at the blue spot. _Nature Reviews. Neuroscience_, _21_(11), 644–659. https://doi.org/10.1038/s41583-020-0360-9
68. Breton-Provencher, V., & Sur, M. (2019). Active control of arousal by a locus coeruleus GABAergic circuit. _Nature Neuroscience_, _22_(2), 218–228. https://doi.org/10.1038/s41593-018-0305-z
69. Borodovitsyna, O., Flamini, M. D., & Chandler, D. J. (2018). Acute stress persistently alters locus coeruleus function and anxiety-like behavior in adolescent rats. _Neuroscience_, _373_, 7–19. https://doi.org/10.1016/j.neuroscience.2018.01.020

70. Qian, J., Wu, W., Qiu, L., Liu, X., Luo, Y., Chen, F., Surento, W., Liu, Y., Lu, G., & Qi, R. (2024). Medial prefrontal cortex-periaqueductal gray circuit overcomes anxiety-like behavior in male mice following adversity. _Journal of Affective Disorders_, _372_, 149–159. https://doi.org/10.1016/j.jad.2024.12.017
71. Seo, C., Guru, A., Jin, M., Ito, B., Sleezer, B. J., Ho, Y., Wang, E., Boada, C., Krupa, N. A., Kullakanda, D. S., Shen, C. X., & Warden, M. R. (2019). Intense threat switches dorsal raphe serotonin neurons to a paradoxical operational mode. _Science_, _363_(6426), 538–542. https://doi.org/10.1126/science.aau8722
72. Ren, J., Isakova, A., Friedmann, D., Zeng, J., Grutzner, S. M., Pun, A., Zhao, G. Q., Kolluru, S. S., Wang, R., Lin, R., Li, P., Li, A., Raymond, J. L., Luo, Q., Luo, M., Quake, S. R., & Luo, L. (2019). Single-cell transcriptomes and whole-brain projections of serotonin neurons in the mouse dorsal and median raphe nuclei. _eLife_, _8_. https://doi.org/10.7554/elife.49424
73. Rudolph, S., Badura, A., Lutzu, S., Pathak, S. S., Thieme, A., Verpeut, J. L., Wagner, M. J., Yang, Y., & Fioravante, D. (2023). Cognitive-Affective functions of the cerebellum. _Journal of Neuroscience_, _43_(45), 7554–7564. https://doi.org/10.1523/jneurosci.1451-23.2023
74. Shipman, M. L., & Green, J. T. (2019). Cerebellum and cognition: Does the rodent cerebellum participate in cognitive functions? Neurobiology of Learning and Memory, 170, 106996. https://doi.org/10.1016/j.nlm.2019.02.006
75. Rudolph, S., Badura, A., Lutzu, S., Pathak, S. S., Thieme, A., Verpeut, J. L., Wagner, M. J., Yang, Y., & Fioravante, D. (2023). Cognitive-Affective functions of the cerebellum. _Journal of Neuroscience_, _43_(45), 7554–7564. https://doi.org/10.1523/jneurosci.1451-23.2023
76. Jardim-Messeder, D., Lambert, K., Noctor, S., Pestana, F. M., De Castro Leal, M. E., Bertelsen, M. F., Alagaili, A. N., Mohammad, O. B., Manger, P. R., & Herculano-Houzel, S. (2017). Dogs Have the Most Neurons, Though Not the Largest Brain: Trade-Off between Body Mass and Number of Neurons in the Cerebral Cortex of Large Carnivoran Species. _Frontiers in Neuroanatomy_, _11_, 118. https://doi.org/10.3389/fnana.2017.00118

77. Bostan, A. C., & Strick, P. L. (2010). The Cerebellum and Basal Ganglia are Interconnected. _Neuropsychology Review_, _20_(3), 261–270. https://doi.org/10.1007/s11065-010-9143-9
78. Kelly, E., Meng, F., Fujita, H., Morgado, F., Kazemi, Y., Rice, L. C., Ren, C., Escamilla, C. O., Gibson, J. M., Sajadi, S., Pendry, R. J., Tan, T., Ellegood, J., Basson, M. A., Blakely, R. D., Dindot, S. V., Golzio, C., Hahn, M. K., Katsanis, N., . . . Tsai, P. T. (2020). Regulation of autism-relevant behaviors by cerebellar–prefrontal cortical circuits. _Nature Neuroscience_, _23_(9), 1102–1110. https://doi.org/10.1038/s41593-020-0665-z
79. Frontera, J. L., Aissa, H. B., Sala, R. W., Mailhes-Hamon, C., Georgescu, I. A., Léna, C., & Popa, D. (2020). Bidirectional control of fear memories by cerebellar neurons projecting to the ventrolateral periaqueductal grey. _Nature Communications_, _11_(1), 5207. https://doi.org/10.1038/s41467-020-18953-0
80. Frontera, J. L., Sala, R. W., Georgescu, I. A., Aissa, H. B., D'Almeida, M. N., Popa, D., & Léna, C. (2023). The cerebellum regulates fear extinction through thalamo-prefrontal cortex interactions in male mice. _Nature Communications_, _14_(1), 1508. https://doi.org/10.1038/s41467-023-36943-w

Chapter 3.0: Neural Connectivity of Anxiety: An Affective State Pathway

Anxiety and anxiety-like behaviours are an outcome of an intricate, distributed neural process involving multiple interconnected brain regions and large-scale neural networks. Here, *anxiety-like behaviours* refer to experimentally observable defensive and avoidance behaviours in animal models, which serve as proxies for state anxiety as reported by humans. Rather than operating through strictly segregated pathways, anxiety processing emerges from dynamic and recurrent interactions across thalamo–cortico–limbic circuits with distinct temporal dynamics. While earlier frameworks described a "high-road" (cortical) and "low-road" (subcortical) pathway, contemporary evidence suggests that these processes are highly integrated and continuously interacting[1].

This chapter focuses on the *high-road mechanism* due to its role in cognitive appraisal, contextual integration, and the interconnected feedback mechanism. The underlying neural mechanisms are organised into five functional domains: detection, interpretation, evaluation, response initiation, and response[2], although these stages are non-linear and involve dynamic feedback loops, which will be discussed in depth in this chapter.

3.1 Interpretation and Evaluation:

At the systems level, interpretation is closely linked to the salience network, primarily involving the anterior insular cortex (AIc) and anterior cingulate cortex (ACC), which integrates interoceptive, emotional, and cognitive signals to prioritise behaviourally relevant stimuli[3]. Signals are selectively integrated and modulated within the thalamic nuclei, while higher-order contextualisation is primarily mediated by the cortical and hippocampal circuits. Reticular nuclei of the thalamus regulate and filter the transmission of sensory signals, controlling attentional selection rather than simply enhancing transmission[4]. Thalamic contextualisation can be divided into 3 steps: (1) Hierarchical Context Representation via Mediodorsal thalamus-prefrontal interactions (MDT-PFC), (2) Cortico-Reticular Gating via arousal modulation in Paraventricular Nucleus of the Thalamus (PVT), and (3) Active Signal Modulation via salience processing in the Pulvinar Thalamus (PUV)[5]. The PUV receives signals from the sensory cortex and colliculus and contributes to attentional selection and salience processing, rather than acting as a threat-specific detector[4-5]. Signals from the brainstem are processed by the Paraventricular Nucleus of the

Thalamus (PVT), which is implicated in arousal and stress-related modulation of behaviour[6-7]. The MDT supports prefrontal-thalamic interactions involved in contextual signals rather than directly encoding context alone. The central MDT and parafascicular nucleus integrate arousal and alertness signals to the stimuli[7]. The processed stimuli is further transmitted to the Basolateral Amygdala (BLA).

Concurrently, interpretation within the BLA assigns *affective meaning* and *salience* to stimuli through integration with distributed circuits rather than in isolation. The stimuli are encoded as anxiogenic when sensory cues from the thalamus, environmental context from the ventral hippocampus (vHPC) and prior emotional learning from the BLA are integrated across hippocampal-amygdala-prefrontal networks[8]. The reciprocal projections from the vHPC to the BLA convey contextual and threat-related information via glutamatergic projections rather than encoding anxiety as a discrete state[9]. The Thalamus-vHPC-BLA forms a key circuit for context-emotion integration, in which sensory inputs are combined with contextual information to bias affective processing[10]. Within the BLA, stimuli are encoded as salient or potentially threatening rather than definitively interpreted as anxiety at this stage[7].

While the ventral Bed Nucleus of the Stria Terminalis (vBNST) is indirectly involved in interpretation, it is more accurately described as contributing to *sustained anxiety states* and threat anticipation rather than primary stimulus interpretation[(10)(11)]. The anterodorsal nucleus of the BNST (adBNST) contributes to anxiety and has been implicated in modulating responses to uncertainty and sustained threat, although its precise functional role remains circuit- and cell-type-specific[12] rather than exerting a uniform anxiogenic effect. Distinct neuronal populations within the BNST can either promote or suppress anxiety-like behaviours depending on their projection targets and neurochemical identity. Microcircuits involving the oval nucleus of the BNST (ovBNST) and the adBNST contribute to the regulation of sustained threat responses[13], highlighting the BNST's role in modulating anticipatory and prolonged anxiety states rather than primary stimulus interpretation[14]. Inputs from the BLA to the ventral tegmental area (VTA), which are primarily glutamatergic, modulate dopaminergic signaling and influence affective states[15]. This interpretation is further broken down into *phasic fear*, which is BLA-driven, and *sustained anxiety*, which is BNST-driven. The thalamus also projects directly to the Medial Prefrontal Cortex (mPFC). Additionally, the Anterior Insular cortex (AIc) integrates

interoceptive signals, uncertainty, and subjective awareness, contributing to the anticipation of internal threat states. The insular cortex (Ic) projects directly to the amygdala and BNST, and AIc mediates anticipation by receiving ascending projections from the Parabrachial Nucleus (PBN) and projecting to the mPFC[16]. The Lateral Septum (LS) acts as a node between the vHPC and the Anterior Hypothalamic Nucleus (AHA), which relays anxiogenic stimuli via GABAergic neuron activation[17].

Information labelled salient or threat-related from the BLA engages with limbic circuits[12], which is further processed. BLA projects to the Lateral Nuclei (CeL) and Medial Nuclei (MeA) of the amygdala. In the CeL, the labelled stimuli affect the inhibitory population of the GABAergic neurons, which activate the CeL-ON neurons, which then inhibit the neighbouring CeL-OFF populations of cells, resulting in disinhibition of the Central Medial amygdala (CeM), contributing to downstream autonomic and behavioural responses rather than directly encoding anxiety itself[18]. CeM further sends the interpreted stimuli to the brainstem to initiate responses. The central nucleus of the amygdala (CeA) projects to the dorsal BNST (dBNST) via an excitatory pathway, which increases anxiety. These microcircuits have been primarily characterised in fear-conditioning paradigms, and their generalisation to sustained anxiety states may depend on broader network interactions[19].

In parallel with contextualisation, evaluation transforms affectively tagged stimuli into action-relevant representations. Unlike interpretation, which assigns salience, evaluation involves computing the behavioural relevance, controllability, and predicted outcomes, integrating both internal state and external context to response selection. This process is mediated by a distributed cortico-limbic-striatial-hypothalamic evaluation network, wherein processed stimuli are represented as anxiety. The prelimbic (PL) cortex, along with other medial prefrontal regions including infralimbic (IL) and orbitofrontal cortices, contributes to integrating contextual, emotional, and value-based information for action selection[20]. PL neurons projecting to MDT amplify the signals and sustain these representations, while PL-BLA interactions support the retrieval of fear-related representations[6].

A strong inhibitory pathway from the BLA innervates the Nucleus Accumbens (NAc), which suppresses the NAc, a ventral striatal structure composed primarily of GABAergic medium spiny neurons and receiving dopaminergic input from the VTA. The NAc-VTA circuit mediates

stress-induced anxiety and directly triggers somatic anxiety[21]. Reciprocal projections from HPC, Thalamus, Amygdala, and Insula converge onto the ACC (Anterior Cingulate Cortex), and together these projections and their associated signals, as a part of the salience network[43], integrate cognitive and affective signals and contribute to threat evaluation, behavioural adaptation, and generalisation[(22)(23)]. Stimuli from the LS and adBNST are projected to the anterior area (AHA) and the lateral hypothalamus (LH), respectively[17]. These coordinated interactions support ongoing evaluation of threat, context and action selection. The vBNST and adBNST further contribute by integrating limbic inputs and linking sustained threat processing to this evaluation$_{12}$. These stimuli further proceed to response initiation. Emerging frameworks conceptualise anxiety as a disorder of predictive processing, where heightened uncertainty and altered precision-weighting of threat-related predictions bias perception and interpretation toward potential danger[24].

Evaluation processes function across multiple timescales. Amygdala circuits support rapid, phasic threat responses. BNST and neuromodulatory systems contribute to sustained anxiety states[25].

3.2 Detection of Stimuli:

Detection involves assessment of physical, visual, olfactory, auditory, and interoceptive signals by specialised peripheral receptors, while genetic and epigenetic factors modulate system sensitivity rather than acting as direct stimuli[(26)(27)]. Sensory information is transmitted through an afferent pathway to modality-specific relay nuclei in the thalamus, which serve as a dynamic hub for sensory integration and gating for most sensory modalities (with the exception of olfaction), rather than a purely passive relay[28]. Importantly, thalamic processing is shaped by extensive corticothalamic feedback, permitting dynamic filtering, gain control, and attentional modulation of incoming sensory information[29]. Parallel subcortical pathways involving structures such as the superior colliculus (visual processing) and inferior colliculus (auditory processing), which contribute to rapid detection and orienting but do not serve as general relay stations for all sensory inputs. Further, thalamic relay neurons project predominantly to layer 4 of the corresponding primary sensory cortices, where initial cortical processing of sensory input occurs[30]. From these regions, after detection, the information is further integrated within cortical areas and limbic structures that participate in interpretation and evaluation[31].

3.3 Response Initiation and Response:

Response initiation represents the transformation of the evaluated threat into coordinated behavioural, autonomic, and endocrine outputs, mediated by the hypothalamic and brainstem effector systems. A central component of this process is the activation of the HPA via the paraventricular nucleus of the hypothalamus (PVN), leading to corticotropin-releasing hormone (CRH) release, which triggers the endocrine system (adrenocorticotropic hormone and cortisol release)[32][33]. CRH-expressing and GABAergic long-range projecting neurons in the amygdala, which innervate the VTA, alter anxiety following chronic CRH depletion, leading to reduced dopamine release and increased anxiety[42]. This endocrine response plays a central role in sustaining anxiety. Anxiety-related signals emerging from cortico-limbic-striatal-hypothalamic networks, including the NAc-VTA-PFC-LH axis, are relayed via the AHA to the PVN and downstream brainstem nuclei. In parallel, coordinated inputs from the amygdala and BNST converge onto hypothalamic and brainstem effectors to elicit defensive behavioural responses, including projections to the medulla and autonomic control centres[34]. These pathways collectively regulate sympathetic activation, cardiovascular responses and respiratory adjustments associated with anxiety states.

In parallel, hypothalamic projections to the lateral hypothalamus and the brainstem regulate autonomic and arousal-related responses, including sympathetic activation and cardiovascular and respiratory changes. The locus coeruleus (LC), a primary source of noradrenergic output, projects to the cortex, amygdala, hippocampus, and hypothalamus[35]. LC activation interacts with limbic and cortical circuits to modulate arousal and uncertainty[36], while serotonergic projections from the DRN regulate affective and behavioural responses in a context-dependent manner by innervating the amygdala, BNST, PFC, and PAG[37].

At the behavioural level, the periaqueductal grey (PAG) functions as a key integrative hub. The ventrolateral PAG (vlPAG), under inhibitory control of the GABAergic projections from the vBNST, mediates passive coping responses such as freezing, whereas the dorsal PAG (dlPAG) supports panic behaviour[38]. Dopaminergic projections from the VTA to the BLA modulate anxiety-like behaviours and exploration activity, linking motivational state with threat processing[12]. Additionally, the Lateral Habenula (LHb) receives afferent inputs from key areas of the hypothalamus, BNST, LC, and amygdala, which promote aversive states, including social

avoidance behaviour and reduced exploration[39][40]. The LHb exerts modulatory control over the dopaminergic system via projections to the VTA, thereby modulating mood, motivation, and behavioural responses[41].

Importantly, response generation is not strictly feedforward but involves dynamic connections. Glucocorticoid release via the HPA axis exerts feedback effects on the PFC, hippocampus, and amygdala, shaping anxiety as a behavioural output. Additionally, reciprocal interactions among the LC, DRN, and cortico-limbic systems sustain anxiety by modulating arousal, emotional salience, and cognitive control.

Together, these integrated system circuits coordinate the expression of anxiety as a physiological process across species, enabling preparatory and defensive responses while supporting sustained vigilance and anticipatory behaviour under conditions of uncertainty. Dysregulation within these connected loops, whether via altered gain in neuromodulatory systems, impaired top-down control, or persistent activation of stress pathways, may lead to anxiety disorders characterised by exaggerated threat perception, sustained arousal, and impaired behavioural flexibility.

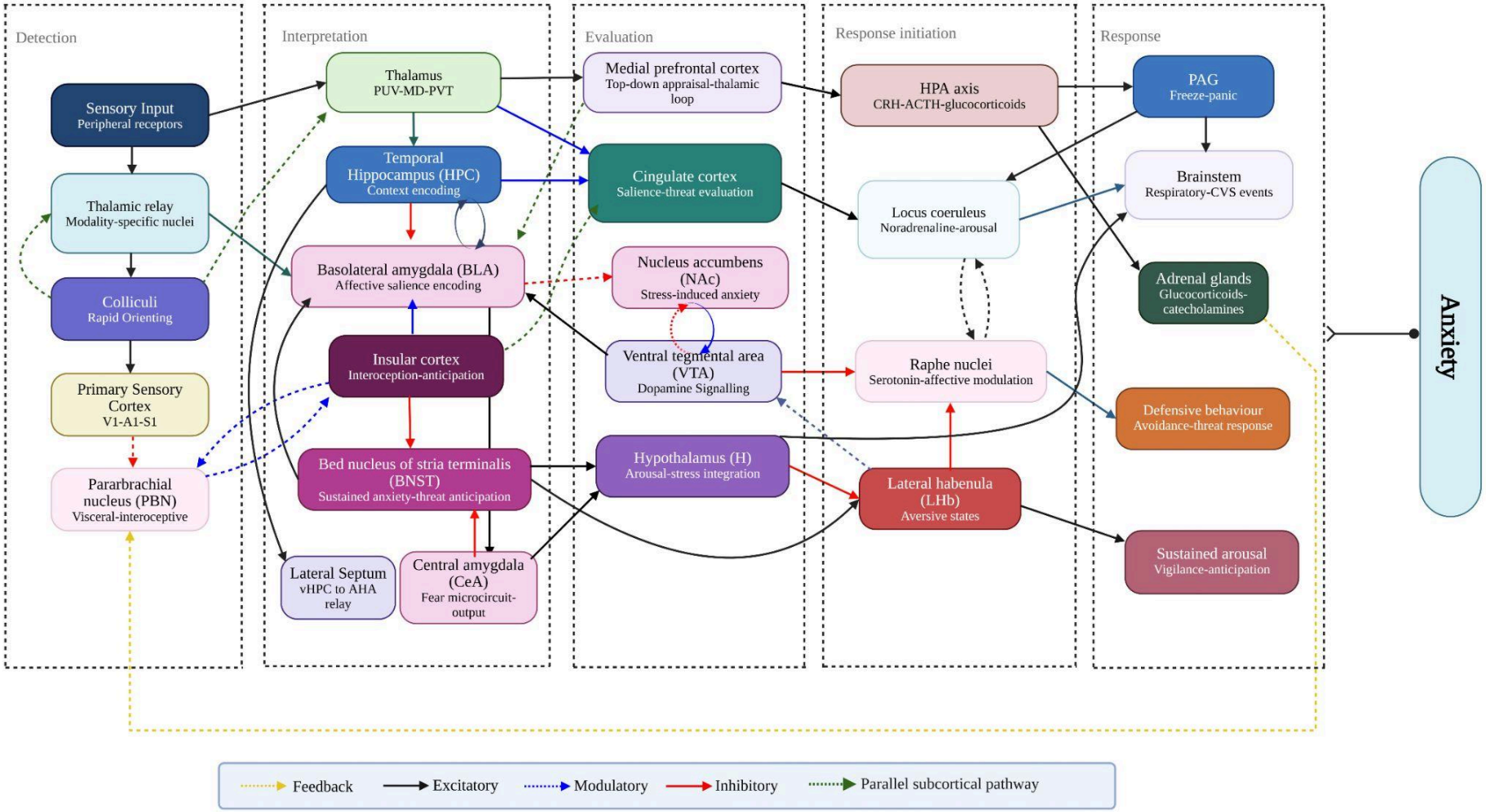

Figure 12. Neural Pathway of Anxiety. Created with BioRender.com.

References

1. 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. https://doi.org/10.1176/appi.ajp.2016.16030353
2. Pessoa, L. (2017). A network model of the emotional brain. Trends in Cognitive Sciences, 21(5), 357–371. https://doi.org/10.1016/j.tics.2017.03.002
3. Seeley, W. W. (2019). The salience network: a neural system for perceiving and responding to homeostatic demands. _Journal of Neuroscience_, _39_(50), 9878–9882. https://doi.org/10.1523/jneurosci.1138-17.2019
4. Briggs, F., & Usrey, W. M. (2008). Emerging views of corticothalamic function. _Current Opinion in Neurobiology_, _18_(4), 403–407. https://doi.org/10.1016/j.conb.2008.09.002
5. Han, X., & Bonin, V. (2024). Higher-order cortical and thalamic pathways shape visual processing streams in the mouse cortex. _Current Biology_, _34_(24), 5671-5684.e6. https://doi.org/10.1016/j.cub.2024.10.048
6. Zhang, S., Wu, D., Luo, R., Wu, J., Chen, H., Li, Z., Zhuang, J., Hu, N., Li, X., Yang, J., Gao, T., & Chen, Y. (2024). A prelimbic Cortex-Thalamus circuit bidirectionally regulates innate and Stress-Induced Anxiety-Like behavior. _Journal of Neuroscience_, _44_(29), e2103232024. https://doi.org/10.1523/jneurosci.2103-23.2024
7. Janak, P. H., & Tye, K. M. (2015b). From circuits to behaviour in the amygdala. _Nature_, _517_(7534), 284–292. https://doi.org/10.1038/nature14188
8. Wang, Z., Zheng, X., Fong, T. H., Liu, X., Gong, Z., Zhou, Q., Liao, J., & Zhang, Y. (2024b). Contribution of prefrontal cortex and ventral hippocampus to anxiety in young epileptic mice. _Biochemical and Biophysical Research Communications_, _734_, 150789. https://doi.org/10.1016/j.bbrc.2024.150789
9. Liu, J., Totty, M. S., Bayer, H., & Maren, S. (2025). Integrating aversive memories in the basolateral amygdala. _Biological Psychiatry_, _98_(10), 746–755. https://doi.org/10.1016/j.biopsych.2025.03.019
10. Calhoon, G. G., & Tye, K. M. (2015). Resolving the neural circuits of anxiety. _Nature Neuroscience_, _18_(10), 1394–1404. https://doi.org/10.1038/nn.4101

11. Goode, T. D., Ressler, R. L., Acca, G. M., Miles, O. W., & Maren, S. (2019). Bed nucleus of the stria terminalis regulates fear to unpredictable threat signals. _eLife_, _8_. https://doi.org/10.7554/elife.46525
12. Gong, W. (2025b). Research progress on the neural circuits mechanisms of anxiety. _Frontiers in Neural Circuits_, _19_, 1609145. https://doi.org/10.3389/fncir.2025.1609145
13. Kim, S., Adhikari, A., Lee, S. Y., Marshel, J. H., Kim, C. K., Mallory, C. S., Lo, M., Pak, S., Mattis, J., Lim, B. K., Malenka, R. C., Warden, M. R., Neve, R., Tye, K. M., & Deisseroth, K. (2013b). Diverging neural pathways assemble a behavioural state from separable features in anxiety. Nature, 496(7444), 219–223. https://doi.org/10.1038/nature12018
14. Goode, T. D., Ressler, R. L., Acca, G. M., Miles, O. W., & Maren, S. (2019). Bed nucleus of the stria terminalis regulates fear to unpredictable threat signals. _eLife_, _8_. https://doi.org/10.7554/elife.46525
15. Kim, S.-R., and Kim, S.-Y. (2021). Functional dissection of glutamatergic and GABAergic neurons in the bed nucleus of the Stria terminalis. Mol. Cells 44, 63–67. doi: 10.14348/molcells.2021.0006
16. Alvarez, R. P., Kirlic, N., Misaki, M., Bodurka, J., Rhudy, J. L., Paulus, M. P., & Drevets, W. C. (2015). Increased anterior insula activity in anxious individuals is linked to diminished perceived control. _Translational Psychiatry_, _5_(6), e591. https://doi.org/10.1038/tp.2015.84
17. Wang, D., Pan, X., Zhou, Y., Wu, Z., Ren, K., Liu, H., Huang, C., Yu, Y., He, T., Zhang, X., Yang, L., Zhang, H., Han, M., Liu, C., Cao, J., & Yang, C. (2023b). Lateral septum-lateral hypothalamus circuit dysfunction in comorbid pain and anxiety. Molecular Psychiatry, 28(3), 1090–1100. https://doi.org/10.1038/s41380-022-01922-y
18. Calhoon, G. G., & Tye, K. M. (2015). Resolving the neural circuits of anxiety. _Nature Neuroscience_, _18_(10), 1394–1404. https://doi.org/10.1038/nn.4101
19. Tovote, P., Fadok, J. P., & Lüthi, A. (2015). Neuronal circuits for fear and anxiety. Nature Reviews. Neuroscience, 16(6), 317–331. https://doi.org/10.1038/nrn3945
20. Wang, Z., Zheng, X., Fong, T. H., Liu, X., Gong, Z., Zhou, Q., Liao, J., & Zhang, Y. (2024). Contribution of prefrontal cortex and ventral hippocampus to anxiety in young

epileptic mice. _Biochemical and Biophysical Research Communications_, _734_, 150789. https://doi.org/10.1016/j.bbrc.2024.150789

21. Qi, G., Zhang, P., Li, T., Li, M., Zhang, Q., He, F., Zhang, L., Cai, H., Lv, X., Qiao, H., Chen, X., Ming, J., & Tian, B. (2022). NAc-VTA circuit underlies emotional stress-induced anxiety-like behavior in the three-chamber vicarious social defeat stress mouse model. _Nature Communications_, _13_(1), 577. https://doi.org/10.1038/s41467-022-28190-2
22. Nagayoshi, T., Ishikawa, R., & Kida, S. (2022). Anterior cingulate cortex projections to the dorsal hippocampus positively control the expression of contextual fear generalization. _Learning & Memory_, _29_(3), 77–82. https://doi.org/10.1101/lm.053440.121
23. Journée, S. H., Mathis, V. P., Fillinger, C., Veinante, P., & Yalcin, I. (2023). Janus effect of the anterior cingulate cortex: Pain and emotion. _Neuroscience & Biobehavioral Reviews_, _153_, 105362. https://doi.org/10.1016/j.neubiorev.2023.105362
24. Holley, D., Varga, E. A., Boorman, E. D., & Fox, A. S. (2024). Temporal dynamics of uncertainty cause anxiety and avoidance. Computational Psychiatry, 8(1), 85–91. https://doi.org/10.5334/cpsy.105
25. Shackman, A. J., & Fox, A. S. (2016). Contributions of the central extended amygdala to fear and anxiety. *Journal of Neuroscience*, *36*(31), 8050–8063. https://doi.org/10.1523/jneurosci.0982-16.2016
26. Rutter, L. A., Norton, D. J., & Brown, T. A. (2021). Visual attention toward emotional stimuli: Anxiety symptoms correspond to distinct gaze patterns. _PLoS ONE_, _16_(5), e0250176. https://doi.org/10.1371/journal.pone.0250176
27. Abraira, V. E., & Ginty, D. D. (2013). The sensory neurons of touch. _Neuron_, _79_(4), 618–639. https://doi.org/10.1016/j.neuron.2013.07.051
28. Hornix, B. E., Havekes, R., & Kas, M. J. (2018). Multisensory cortical processing and dysfunction across the neuropsychiatric spectrum. _Neuroscience & Biobehavioral Reviews_, _97_, 138–151. https://doi.org/10.1016/j.neubiorev.2018.02.010
29. Halassa, M. M., & Kastner, S. (2017). Thalamic functions in distributed cognitive control. _Nature Neuroscience_, _20_(12), 1669–1679. https://doi.org/10.1038/s41593-017-0020-1

30. Sun, W., Tan, Z., Mensh, B. D., & Ji, N. (2015). Thalamus provides layer 4 of primary visual cortex with orientation- and direction-tuned inputs. _Nature Neuroscience_, _19_(2), 308–315. https://doi.org/10.1038/nn.4196
31. Moser, E. I., Moser, M., & McNaughton, B. L. (2017). Spatial representation in the hippocampal formation: a history. Nature Neuroscience, 20(11), 1448–1464. https://doi.org/10.1038/nn.4653
32. Ariño-Braña, P., et al. (2025). Influence of the HPA axis on anxiety-related processes: An RDoC overview. Current Psychiatry Reports, 27(10), 593–611. https://doi.org/10.1007/s11920-025-01633-5
33. Tafet, G. E., & Nemeroff, C. B. (2020). Pharmacological treatment of anxiety disorders: The role of the HPA axis. _Frontiers in Psychiatry_, _11_, 443. https://doi.org/10.3389/fpsyt.2020.00443
34. Gu, Y., Piper, W. T., Branigan, L. A., Vazey, E. M., Aston-Jones, G., Lin, L., LeDoux, J. E., & Sears, R. M. (2019). A brainstem-central amygdala circuit underlies defensive responses to learned threats. _Molecular Psychiatry_, _25_(3), 640–654. https://doi.org/10.1038/s41380-019-0599-6
35. Borodovitsyna, O., Flamini, M. D., & Chandler, D. J. (2018b). Acute stress persistently alters locus coeruleus function and anxiety-like behavior in adolescent rats. _Neuroscience_, _373_, 7–19. https://doi.org/10.1016/j.neuroscience.2018.01.020
36. Morris, L. S., McCall, J. G., Charney, D. S., & Murrough, J. W. (2020). The role of the locus coeruleus in the generation of pathological anxiety. _Brain and Neuroscience Advances_, _4_, 239821282093032. https://doi.org/10.1177/2398212820930321
37. Zangrossi, H., Del Ben, C. M., Graeff, F. G., & Guimarães, F. S. (2020). Serotonin in panic and anxiety disorders. In _Handbook of behavioral neuroscience_ (pp. 611–633). https://doi.org/10.1016/b978-0-444-64125-0.00036-0
38. Zhang, H., Zhu, Z., Ma, W., Kong, L., Yuan, P., Bu, L., Han, J., Huang, Z., & Wang, Y. (2024). The contribution of periaqueductal gray in the regulation of physiological and pathological behaviors. _Frontiers in Neuroscience_, _18_. https://doi.org/10.3389/fnins.2024.1380171

39. Tan, W., Ikoma, Y., Takahashi, Y., Konno, A., Hirai, H., Hirase, H., & Matsui, K. (2024). Anxiety control by astrocytes in the lateral habenula. _Neuroscience Research_, _205_, 1–15. https://doi.org/10.1016/j.neures.2024.01.006
40. Baker, P. M., Mathis, V., Lecourtier, L., Simmons, S. C., Nugent, F. S., Hill, S., & Mizumori, S. J. Y. (2022). Lateral Habenula Beyond Avoidance: Roles in Stress, Memory, and Decision-Making with implications for psychiatric disorders. _Frontiers in Systems Neuroscience_, _16_, 826475. https://doi.org/10.3389/fnsys.2022.826475
41. Dolzani, S. D., Baratta, M. V., Amat, J., Agster, K. L., Saddoris, M. P., Watkins, L. R., et al. (2016). Activation of a habenulo–raphe circuit is critical for the behavioral and neurochemical consequences of uncontrollable stress in the male rat. eNeuro 3:229. doi: 10.1523/ENEURO.0229-16.2016
42. Dedic, N., Kühne, C., Jakovcevski, M., Hartmann, J., Genewsky, A. J., Gomes, K. S., Anderzhanova, E., Pöhlmann, M. L., Chang, S., Kolarz, A., Vogl, A. M., Dine, J., Metzger, M. W., Schmid, B., Almada, R. C., Ressler, K. J., Wotjak, C. T., Grinevich, V., Chen, A., . . . Deussing, J. M. (2018c). Chronic CRH depletion from GABAergic, long-range projection neurons in the extended amygdala reduces dopamine release and increases anxiety. Nature Neuroscience, 21(6), 803–807. https://doi.org/10.1038/s41593-018-0151-z
43. Schimmelpfennig, J., Topczewski, J., Zajkowski, W., & Jankowiak-Siuda, K. (2023). The role of the salience network in cognitive and affective deficits. *Frontiers in Human Neuroscience*, *17*, 1133367. https://doi.org/10.3389/fnhum.2023.1133367

Chapter 4.0: Neurotransmitter Systems and Synaptic Regulation in Anxiety

Anxiety disorders arise from dysregulation across distributed neural circuits, particularly the amygdala–hippocampus–medial prefrontal cortex (mPFC) axis, mediated by interacting neurotransmitter systems. At a molecular level, synaptic transmission is governed by neurotransmitter release, receptor activation, and transporter-mediated reuptake, which together determine extracellular signalling dynamics. Transporter proteins such as serotonin transporter (SERT), noradrenaline transporter (NET), dopamine transporter (DAT), and GABA transporter (GAT) are critical regulators of synaptic tone. Molecular imaging studies showed that SERT has the strongest and most consistent association with anxiety disorders, demonstrated by altered transporter availability in clinical populations[1]. The fact that transporter alterations do not translate linearly onto symptom severity suggests that circuit-level adaptations drive pathological anxiety, rather than transporter density alone. This chapter puts the interaction of the neurotransmitter system and the specific pharmacological agents under the lens, relevant to anxiety.

4.1 Serotonergic Neurotransmitter System in Anxiety:

Serotonin, better known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter which regulates brain network. 5-HT exerts bidirectional and context-dependent effects on anxiety, reflecting receptor heterogeneity and region-specific expression patterns. A high-distinction interpretation prioritises functional receptor roles within circuits over taxonomy.

5-HT receptors (5-HTR) have been categorized into seven families and 14 subtypes so far[(2)(3)]. Serotonergic neurons modulate aggression, anxiety and reward processing.

4.1.1 5-HT_{1A} Receptors: Inhibitory Control of Serotonergic Tone

5-HT_{1A} receptors exist as presynaptic autoreceptors in the dorsal raphe nucleus and as postsynaptic heteroreceptors in the mPFC, hippocampus, and amygdala4. Presynaptic 5-HT_{1A} auto-receptors exert inhibitory control over serotonergic firing[5]. In anxiety, enhanced auto-receptor function or reduced postsynaptic receptor availability leads to diminished serotonergic output, which contributes to limbic hyperreactivity. The traditional view of 5-HT_{1A}

receptors as uniformly anxiolytic is overly simplistic. Their net effect depends on spatial localisation and network context, highlighting a broader limitation of receptor-centric models. Based on the localisation, anxiolytic effects are achieved by two complementary pharmacological actions. Partial agonism at postsynaptic 5-HT_{1A} heteroreceptors by moderately activating receptors in the mPFC, hippocampus and amygdala. Partial agonists produce a delayed onset. While full agonism at presynaptic 5-HT_{1A} receptors produces stronger activation by simultaneously engaging the dorsal raphe nucleus receptors and suppressing serotonergic firing.

4.1.2 5-HT_{2A} Receptors: Excitatory Drive and Threat Amplification

5-HT_{2A} receptors are Gq-coupled, which enable glutamatergic transmission within cortico-limbic circuits. Activation enhances excitatory signalling in the amygdala and associated regions, promoting threat salience and anxiety-like behaviour[6][7]. 5-HT_{2A} receptors are distributed across the forebrain, particularly within the cortices, caudate nucleus, and hippocampus, where they are predominantly postsynaptic and excitatory, with the highest density in the neocortex[2].

Pharmacological antagonism produces anxiolytic effects, yet clinical translation remains inconsistent, suggesting that 5-HT_{2A}-mediated anxiogenesis is necessary but not sufficient for pathological anxiety. In preclinical studies, elevated 5-HT_{2A} receptor activity has been linked to generalised anxiety disorder (GAD); pharmacological antagonism consistently produces anxiolytic outcomes. Acute stimulation of 5-HT_{2A} projecting from the raphe nucleus to amygdala, and limbic cortex causes anxiety and panic attacks[8].

4.1.3 5-HT_{2C} Receptors: Gating of Monoaminergic Systems

5-HT_{2C} receptors are mainly located throughout the amygdala, choroid plexus, cortex, basal ganglia, thalamus, and hippocampus, where they modulate dopamine and noradrenaline release via inhibitory control of catecholaminergic neurons[8]. Their activation is generally associated with anxiogenic outcomes, particularly within the amygdala[9]. Evidence indicates that 5-HT_{2C} receptor dysregulation is involved in anxiety and increased 5-HT_{2C} receptor signalling has been associated with anxiogenic effect[10]. Pharmacological effects are by 5-HT_{2C} blockade by antagonists, disinhibiting mesocortical dopamine and noradrenaline transmission.

4.1.4 5-HT_3 Receptors: Ionotropic Modulation and Rapid Effects

Unlike other serotonergic receptors, 5-HT_3 receptors are ligand-gated ion channels, and they modulate fast synaptic transmission and influence dopamine release in reward-related regions. Antagonism of 5-HT_3 receptors produces anxiolytic effects, likely via attenuation of excitatory neurotransmission, although their role in chronic anxiety states is less well defined[11]. Post-synaptic receptors blocking within the hippocampus and NAc causes anxiolytic effect[(12)(13)].

4.2 Pharmacological Modulation of Anxiety Through Serotonin Neurotransmission:

Selective serotonin reuptake inhibitors (SSRIs) are first-line treatments for anxiety disorders. While their primary action is SERT inhibition, increasing extracellular serotonin[14], this mechanism alone does not explain their clinical efficacy.

A critical insight in the drug action is the temporal lag between immediate pharmacological action which begins from minutes to hours, and exhibit a delayed clinical response (weeks). This delay reflects neuroadaptive processes at receptor, circuit, and structural levels, rather than simple monoamine increase.

Individual variability suggests genetic and circuit-level moderators of treatment responses, resistance rates vary considerably by anxiety disorder subtype and are not uniformly established across anxiety[15].

4.2.1 Sertraline

Sertraline selectively inhibits SERT, which decreases the presynaptic reuptake and increases extracellular 5-HT across raphe projections to cortico-limbic regions. In the acute phase, increased serotonin activates 5-HT_{1A} autoreceptors, transiently suppressing serotonergic firing[16]. With chronic treatment, these autoreceptors undergo desensitisation, restoring serotonergic tone[17].

This leads to:

- Reduced amygdala hyperreactivity

- Enhanced mPFC-mediated top-down control

- Restoration of excitation–inhibition balance

Functional imaging studies support reduced amygdala responsiveness following SSRI treatment[16][18][19].

4.2.2 Citalopram

Citalopram selectively inhibits SERT and demonstrates a clear dose–occupancy relationship, supporting its pharmacological specificity. It primarily acts by potentiating the serotonergic activity within the CNS, as it has weak affinity towards noradrenaline and dopamine neuronal reuptake[20]. Due to its massive positive effect with little or no side effects as compared to sertraline as an antidepressant, it was on the WHO list of essential drugs.

It has been indicated that citalopram normalises cortico-limbic network dynamics implicated in anxiety, reducing perceived threat-related amygdala responses and strengthening prefrontal regulatory control over limbic regions[21]. Acute and short-term citalopram challenges modulate amygdala responses to aversive stimuli, consistent with early affective-processing shifts that precede full clinical change. It has been found that citalopram exhibits a dose–occupancy relationship, with high SERT occupancy achieved at clinically used doses and a hyperbolic dose–occupancy curve (rapid occupancy at low doses, then a plateau)[22]. These data mechanistically support increased serotonin tone in anxiety-relevant networks.

Chronic treatment results in:

- 5-HT_{1A} auto-receptor desensitisation

- Increased serotonergic transmission

- Reduced limbic hyperreactivity

A comparative study found that escitalopram, the S-enantiomer of citalopram, demonstrated significantly greater efficacy[23][24].

4.2.3 Fluoxetine

Fluoxetine inhibits SERT, but its therapeutic effects depend on delayed neuroadaptation (3–6 weeks), rather than immediate monoamine elevation[25][26]. PET studies have demonstrated that 20-40 mg/day fluoxetine achieves an 80% threshold that is sufficient to alter serotonergic tone in limbic circuits[22]. Increased extracellular 5-HT in the amygdala, ventromedial prefrontal cortex (vmPFC), and hippocampus is associated with attenuated fear responses and improved emotional regulation.

Recent live-cell imaging studies show that fluoxetine localises within neuronal membranes and vesicular organelles, creating slow-release intracellular reservoirs that extend its pharmacodynamic window. This, in turn, leads to stable brain concentrations that minimise withdrawal effects but also delay dose-response adjustments[27].

Key mechanisms of Fluoxetine involve:

- 5-HT_{1A} auto receptor desensitisation: Under baseline conditions, Gi/o-coupled 5-HT_{1A} auto receptors suppress serotonergic neuron firing via GIRK channel-mediated hyperpolarisation. Chronic fluoxetine reduces autoreceptor density on DRN neurons, uncouples G-protein signalling from receptors, and diminishes downstream GIRK conductance[28].

- 5-HT_{2C} modulation: 5-HT_{2C} heteroreceptors on GABAergic interneurons tonically suppress dopaminergic and noradrenergic neuron firing[29]. Fluoxetine attenuates this inhibition through partial inverse agonism at 5-HT_{2C} and adaptive downregulation following chronic serotonergic stimulation[30][31].

- Noradrenergic activity recalibration: Net locus coeruleus (LC) firing is typically reduced under chronic SSRI exposure via increased serotonergic input activating inhibitory α_2 auto receptors and 5-HT_{1A} receptors on LC neurons. This corrects hyperactive noradrenergic drive rather than augmenting it[30] an important distinction for understanding anxiolytic rather than anxiogenic outcomes.

4.3 Glutamate Neurotransmitter System in Anxiety

Glutamate is the chief excitatory neurotransmitter in the central nervous system (CNS) and is often considered the "master switch" of the brain, as it can excite and activate most of the CNS neurons. Under baseline conditions, during synaptic transmission, glutamate is released from presynaptic neurons via the glutamate–glutamine cycle and activates postsynaptic glutamate receptors, mediating excitatory neurotransmission. This process is modulated by metabotropic glutamate receptors (mGluRs)[32][33].

Glutamate plays a prominent role in learning, memory, neural development, cell proliferation, and migration[34][35]. Evidence indicates that dysregulation of the glutamate system plays a significant role in the pathophysiology of anxiety, with excessive glutamate being intrinsically anxiogenic in nature[36][37].

4.3.1 Glutamate Receptors:

Glutamatergic systems in corticolimbic circuits interact extensively with GABAergic, serotonergic, dopaminergic, and other systems involved in the stress response, implying a central role in the circuitry underlying anxiety disorders[38]. These receptors are classified into two distinct groups, namely metabotropic receptors (mGluRs) and ionotropic receptors (iGluRs). Research demonstrates abnormal glutamate activity in key areas of the limbic system in mood disorders related to anxiety, particularly involving metabotropic glutamate receptors[39][40].

NMDARs (N-methyl-D-aspartate) receptor system, which is an iGluRs receptor, in rodents, the cerebral cortex, hippocampus, amygdala, striatum, nucleus accumbens, cerebellum, brainstem, spinal cord, and peripheral nervous system contain pre-NMDARs[41]. NMDARs mediates learning and memory, including fear consolidation[42][43]. This mechanism is particularly relevant to anxiety disorders, where maladaptive fear responses and hyperexcitability in neural circuits contribute to symptom manifestation. In a study conducted in mice, it was demonstrated that the activation of NMDA receptors leads to anxiety-like behaviours[44].

Current evidence supports that, by mediating brain excitability, neuroplasticity, and social cognition, abnormal metabotropic glutamate activity may predispose individuals to a broad range of psychiatric disorders[39][45]. Metabotropic glutamate receptors 5 (mGluR5) are G-protein-coupled receptors that have emerged as a particularly important target in anxiety pathology. Positron Emission Tomography (PET) studies indicate abnormal glutamate activity related to positive and negative valence systems, suggesting that antagonistic mGluR5 intervention may produce prominent anxiolytic effects[45][46]. Findings across preclinical and imaging studies consistently support the hypothesis that glutamate function is abnormal in key limbic regions in anxiety-related disorders, with this dysfunction likely related to the mGluR5 receptor[47][48]. The increasing utilisation of glutamate-modulating drugs in psychiatric conditions reflects this growing body of evidence[32].

4.4 Pharmacological Modulation of Anxiety Through Glutamate Neurotransmission:

An in-depth examination of four pharmacological agents: Gabapentinoids, ketamine, lamotrigine, and riluzole, highlights their mechanisms of action within the glutamate neurotransmission system and their therapeutic implications for anxiety modulation.

4.4.1 Gabapentinoids (Pregabalin, Gabapentin):

Gabapentinoids are prototypical examples of indirect glutamate modulation via voltage-gated calcium channel inhibition. Although structurally analogous to GABA, a historical naming convention rather than a reflection of functional GABAergic activity. Their pharmacological action does not involve direct interaction with GABAergic receptors. It should be noted that pregabalin and gabapentin differ meaningfully in oral bioavailability and pharmacokinetic profile, with gabapentin exhibiting substantially more variable absorption and a weaker anxiety-specific evidence base. Pregabalin exerts its anxiolytic effects by binding with high affinity to the α_2-δ auxiliary subunit of voltage-gated calcium channels throughout the CNS[32]. By reducing calcium influx at presynaptic nerve terminals, pregabalin diminishes the calcium-dependent release of excitatory neurotransmitters, particularly glutamate. It is worth noting that noradrenaline, substance P, and CGRP release are similarly attenuated, and these

reductions may contribute independently to anxiolysis. This reduction in presynaptic glutamate release represents the primary mechanism underlying its anxiolytic effects[49].

Clinical trials have demonstrated consistent improvement in anxiety symptoms in generalised anxiety disorder (GAD), with reductions in Hamilton Anxiety Rating Scale (HAM-A) scores comparable to lorazepam and superior to placebo[50]. Furthermore, pregabalin shows higher compliance rates and better tolerability compared to benzodiazepines[51]. Neuroimaging studies indicate that pregabalin attenuates activation in limbic structures, particularly the amygdala and insula, during emotional processing tasks, suggesting that modulation of glutamate release translates into measurable changes in anxiety-relevant neural circuits[52].

4.4.2 Ketamine:

Ketamine occupies a unique mechanistic position among anxiolytic agents due to its direct, non-competitive antagonism of NMDA receptors. It binds to the phencyclidine (PCP) site within the open channel pore, effectively blocking ion flux in a use-dependent manner[53].

Critically, current evidence supports a disinhibition model as the primary driver of ketamine's rapid therapeutic effects, particularly involving parvalbumin-positive interneurons that disinhibit pyramidal neurons, producing a glutamate surge in the PFC[54]. This mechanism enables rapid, robust antidepressant and anxiolytic effects, often within hours of administration. Beyond reducing excitatory transmission, NMDA receptor blockade initiates downstream neuroplastic processes, including increased brain-derived neurotrophic factor (BDNF) release, activation of the mammalian target of rapamycin (mTOR) pathway, and enhanced synaptogenesis in prefrontal cortical regions[55]. These neuroplastic changes likely underlie ketamine’s sustained therapeutic effects beyond its pharmacokinetic half-life. Its rapid anxiolytic action is particularly valuable in treatment-resistant cases. However, dissociative and psychotomimetic effects, along with concerns regarding abuse potential and long-term safety, necessitate careful patient selection and monitoring.

4.4.3 Lamotrigine:

Lamotrigine is a multifaceted pharmacological agent that modulates both glutamatergic and GABAergic neurotransmission. It functions primarily through voltage-gated sodium channel blockade, preventing sustained repetitive neuronal firing and reducing presynaptic glutamate release[56].

In addition, lamotrigine facilitates GABA release while simultaneously reducing glutamate release, though this finding is not consistently reported across studies, and should be considered preliminary, shifting the excitatory–inhibitory balance toward inhibition[57]. Electrophysiological studies demonstrate that lamotrigine decreases NMDA receptor-mediated excitatory postsynaptic potentials in amygdala neurons, providing direct evidence of its ability to dampen glutamatergic transmission in anxiety-relevant circuits[(58)(59)].

Preclinical studies consistently demonstrate anxiolytic effects across animal models. In rodents, lamotrigine produces anxiolysis and enhances conditioned emotional responses during presentations of previously aversive stimuli, suggesting facilitation of fear extinction processes[(60)(61)].

Clinical evidence suggests preliminary efficacy in panic disorder, though findings remain limited[58]. Lamotrigine may exhibit relatively modest anxiolytic effects compared to agents acting directly on GABA_A receptors. Its therapeutic effects are likely mediated by neuroprotective mechanisms that reduce excitotoxic glutamate transmission.

4.4.4 Riluzole:

Riluzole represents one of the most comprehensive modulators of glutamatergic neurotransmission, employing multiple mechanisms to reduce synaptic glutamate concentrations and mitigate excitotoxicity[62]. Originally approved for Amyotrophic lateral sclerosis (ALS), its psychiatric repurposing is conceptually grounded in a shared glutamatergic target; however, ALS involves frank motor neuron excitotoxicity, whereas anxiety reflects circuit-level dysregulation of glutamate signalling without neurodegeneration. These are distinct pathological processes[63].

Its mechanisms include blockade of voltage-gated sodium and calcium channels, reduction of presynaptic glutamate release, and enhancement of glutamate transporter activity, particularly in glial cells, thereby increasing synaptic glutamate clearance[63]. This dual mechanism lowers extracellular glutamate concentrations and reduces postsynaptic receptor activation. Additionally, riluzole modulates excitatory amino acid receptors and calcium channel subtypes, contributing to overall suppression of glutamatergic neurotransmission.

Preclinical studies demonstrate consistent anxiolytic-like effects in animal models, including the elevated plus maze and fear-potentiated startle paradigms[(60)(64)]. Clinical evidence remains limited, with small open-label studies in GAD suggesting potential benefit[58].

Neuroimaging findings indicate changes in hippocampal volume and N-acetylaspartate (NAA) levels correlating with symptom improvement. As a marker of neuronal integrity and metabolic function, NAA suggests that riluzole's effects may involve neuroprotection and restoration of glutamate homeostasis[65].

The development of riluzole analogues reflects ongoing efforts to optimise glutamate-targeted therapeutics for psychiatric disorders.

4.5 GABA Neurotransmitter System in Anxiety

Gamma-aminobutyric acid (GABA) is the chief inhibitory neurotransmitter in the central nervous system (CNS) and serves as a critical regulator of neuronal excitability66. Within anxiety-related neurocircuitry, GABA functions as a counterbalance to excitatory glutamatergic transmission, maintaining stability across corticolimbic networks. Under physiological conditions, GABA is released into the synaptic cleft where it activates ionotropic and metabotropic receptors to produce inhibitory postsynaptic effects[(67)(68)]. The GABAergic hypothesis of anxiety indicates that impaired inhibitory tone within limbic structures permits excessive excitatory drive and maladaptive threat processing[69]. However, emerging evidence suggests a more complex framework in which specific GABAergic interneurons and projections may also promote anxiety depending on their microcircuit identity and downstream targets[70]. This has shifted the field away from global GABA enhancement toward receptor- and circuit-specific modulation.

GABA synthesis, release, and clearance are tightly regulated processes. Glutamic acid decarboxylase (GAD) exists in two isoforms GAD65, which supports activity-dependent synaptic GABA release and is critically implicated in anxiety circuits, and GAD67[71][72], which maintains tonic intracellular GABA pools. Metabolism occurs via GABA transaminase, linking GABA turnover to cellular energy metabolism. These processes are dynamically influenced by stress, neurosteroids, and neuronal activity, with chronic stress reducing GABA synthesis and release in limbic regions[73].

GABAergic inhibition is divided into phasic (synaptic) and tonic (extrasynaptic) forms. Phasic inhibition is mediated by synaptic GABA_A receptors, whereas tonic inhibition arises from extrasynaptic receptors that respond to ambient GABA levels[74]. Neurosteroids preferentially enhance tonic inhibition, modulating network excitability and stress responsiveness[75]. Dysregulation of tonic inhibition has been implicated in anxiety-like behaviours[76].

GABAergic systems interact extensively with glutamatergic, serotonergic, and dopaminergic systems within corticolimbic circuits. These interactions are essential for emotional regulation, with GABAergic interneurons in the prefrontal cortex coordinating oscillatory activity required for top-down control of the amygdala[77].

4.6 Pharmacological Modulation of Anxiety Through GABAergic Neurotransmission

4.6.1. Benzodiazepines (BDZs):

Benzodiazepines (diazepam, lorazepam) represent the prototypical GABAergic anxiolytics. They act as positive allosteric modulators at GABA_A receptors, increasing the frequency of chloride channel opening in response to GABA binding[78]. This enhances inhibitory tone across neural circuits and produces rapid anxiolytic effects. Clinically, benzodiazepines are effective in generalised anxiety disorder (GAD), panic disorder, and social anxiety[79][81]. In a comparative study of the efficacy of BDZs versus other anxiolytics in adult anxious individuals, BDZs were among the most effective pharmacotherapeutic agents for GAD treatment[82]. However, chronic use is associated with tolerance, dependence, and withdrawal phenomena. These limitations are partly attributable to subunit-specific adaptations, including reduced sensitivity of α2-containing receptors following prolonged exposure[83].

4.6.2 Neurosteroids (NAS) and Emerging GABAergic Modulators (Allopregnanolone, Brexanolone):

Neurosteroids represent an emerging class of GABAergic modulators that enhance tonic inhibition by acting on extrasynaptic GABA_A receptors. Neurosteroids influence stress responsiveness, emotional regulation and have demonstrated therapeutic potential in mood and anxiety disorders[84]. In preclinical and clinical studies, Exogenous NAS have shown to exert anxiolytic effects[85]. NAS such as allopregnanolone modulate δ-subunit–containing GABA_A receptors, which are highly expressed extrasynaptically and are critical for maintaining inhibitory tone under conditions of stress[86]. Dysregulation of neurosteroid signalling has been implicated in the pathophysiology of anxiety and stress-related disorders, further supporting their therapeutic relevance[87]. Brexanolone treatment has been demonstrated to achieve rapid antidepressant effects and reduce anxiety and insomnia in patients[88]. It should be noted that brexanolone is currently FDA-approved specifically for postpartum depression, not for anxiety disorders broadly; its inclusion here is mechanistically relevant but does not represent an established anxiolytic indication

No peer-reviewed anxiety-specific data for canine and NHPs on the pharmacological action of allopregnanolone. Overall, the GABAergic system plays a central and multifaceted role in the neurobiology of anxiety. Rather than reflecting a simple deficiency of inhibition, anxiety disorders involve complex dysregulation of inhibitory–excitatory balance across distributed neural circuits. Advances in receptor pharmacology, circuit neuroscience, and translational imaging continue to refine the understanding of GABAergic mechanisms, guiding the development of more selective and effective therapeutic strategies.

4.7 Conclusion

Anxiety emerges not from isolated neurotransmitter deficits, but from a pharmacologically tractable imbalance in synaptic regulation across cortico-limbic circuits, where receptor-specific signalling, neurotransmitter flux, and adaptive plasticity converge. The collective evidence demonstrates that transporters, receptors, and intracellular signalling cascades operate as an

integrated system in which perturbations at any level propagate through the amygdala–hippocampus–mPFC axis, biasing neural processing toward threat.

Pharmacological dissection reveals several unifying principles that pharmacological agents do not simply “increase” or “decrease” neurotransmitters; rather, they reconfigure circuit dynamics over time through receptor desensitisation, synaptic plasticity, and network-level recalibration. This is exemplified by SSRIs, in which initial SERT blockade triggers autoreceptor-mediated suppression, followed by delayed desensitisation that restores serotonergic tone and enhances prefrontal inhibitory control over the amygdala. Similarly, glutamate-targeting agents (e.g., NMDA antagonists, calcium channel modulators) and GABAergic drugs (e.g., benzodiazepines, neurosteroids) converge mechanistically to reduce limbic hyperreactivity and restore E/I equilibrium, albeit through distinct molecular entry points.

It is important to note that the noradrenergic system, centred on the locus coeruleus and its projections to the amygdala, mPFC, and hippocampus, plays a central role in acute anxiety, hyperarousal, and fear consolidation, and is the direct pharmacological target of SNRIs, propranolol, prazosin, and α_2 agonists. A comprehensive account of anxiety pharmacotherapy must treat noradrenergic mechanisms substantively rather than in passing.

Finally, the progression from molecular interaction to clinical anxiety reflects a hierarchical cascade often reinforced by maladaptive plasticity. Effective pharmacotherapies, therefore, are those that normalise, not simply suppress network function, reinstating adaptive flexibility within emotional processing circuits.

In sum, anxiety should be understood as a disorder of dysregulated synaptic integration and circuit-level homeostasis, in which pharmacological interventions exert their effects by modulating receptor dynamics and neurotransmitter systems to restore functional balance across distributed neural networks.

References

1. Frick, A., Åhs, F., Engman, J., Jonasson, M., Alaie, I., Björkstrand, J., Frans, Ö., Faria, V., Linnman, C., Appel, L., Wahlstedt, K., Lubberink, M., Fredrikson, M., & Furmark, T. (2015). Serotonin synthesis and reuptake in social anxiety disorder. JAMA Psychiatry, 72(8), 794. https://doi.org/10.1001/jamapsychiatry.2015.0125
2. Lin, J., Liu, W., Guan, J., Cui, J., Shi, R., Wang, L., Chen, D., & Liu, Y. (2023). Latest updates on the serotonergic system in depression and anxiety. _Frontiers in Synaptic Neuroscience_, _15_. https://doi.org/10.3389/fnsyn.2023.1124112
3. Pourhamzeh, M., Moravej, F. G., Arabi, M., Shahriari, E., Mehrabi, S., Ward, R., Ahadi, R., & Joghataei, M. T. (2021). The roles of serotonin in neuropsychiatric disorders. _Cellular and Molecular Neurobiology_, _42_(6), 1671–1692. https://doi.org/10.1007/s10571-021-01064-9
4. Yevtushenko, O. O., & Reynolds, G. P. (2010). Functional pharmacogenetics of serotonin receptors in psychiatric drug action. In Handbook of behavioral neuroscience (pp. 791–806). https://doi.org/10.1016/s1569-7339(10)70110-7
5. Turcotte-Cardin, V., Vahid-Ansari, F., Luckhart, C., Daigle, M., Geddes, S. D., Tanaka, K. F., Hen, R., James, J., Merali, Z., Béïque, J., & Albert, P. R. (2018). Loss of adult 5-HT1A autoreceptors results in a paradoxical anxiogenic response to antidepressant treatment. Journal of Neuroscience, 39(8), 1334–1346. https://doi.org/10.1523/jneurosci.0352-18.2018
6. Jastrzębski, M. K., Wójcik, P., Grudzińska, A., Andreozzi, G., Vetrò, T., Asim, A., Mudgal, A., Czapiński, J., Wróbel, T. M., Bartuzi, D., Targowska-Duda, K. M., & Kaczor, A. A. (2025). Biased signaling via serotonin 5-HT2A receptor: From structural aspects to in vitro and in vivo pharmacology. _Acta Pharmaceutica Sinica B_, _15_(9), 4438–4455. https://doi.org/10.1016/j.apsb.2025.07.002
7. De Almeida, J., & Mengod, G. (2007). Quantitative analysis of glutamatergic and GABAergic neurons expressing 5‐HT2A receptors in human and monkey prefrontal cortex. Journal of Neurochemistry, 103(2), 475–486. https://doi.org/10.1111/j.1471-4159.2007.04768.x

8. Liu, Y., Zhao, J., & Guo, W. (2018). Emotional roles of Mono-Aminergic neurotransmitters in major depressive disorder and anxiety disorders. _Frontiers in Psychology_, _9_, 2201. https://doi.org/10.3389/fpsyg.2018.02201
9. Chagraoui, A., Thibaut, F., Skiba, M., Thuillez, C., & Bourin, M. (2015). 5-HT2C receptors in psychiatric disorders: A review. _Progress in Neuro-Psychopharmacology and Biological Psychiatry_, _66_, 120–135. https://doi.org/10.1016/j.pnpbp.2015.12.006
10. Martin, C. B., Martin, V. S., Trigo, J. M., Chevarin, C., Maldonado, R., Fink, L. H., Cunningham, K. A., Hamon, M., Lanfumey, L., & Mongeau, R. (2015). 5-HT2C receptor desensitization moderates anxiety in 5-HTT deficient mice: From Behavioral to Cellular evidence. _The International Journal of Neuropsychopharmacology_, _18_(3). https://doi.org/10.1093/ijnp/pyu056
11. Bhatt, S., Devadoss, T., Manjula, S. N., & Rajangam, J. (2020). 5-HT3 Receptor Antagonism: A Potential Therapeutic Approach for the Treatment of Depression and other Disorders. _Current Neuropharmacology_, _19_(9), 1545–1559. https://doi.org/10.2174/1570159x18666201015155816
12. Fakhfouri, G., Rahimian, R., Dyhrfjeld-Johnsen, J., Zirak, M. R., & Beaulieu, J. (2019). 5-HT3 Receptor Antagonists in Neurologic and Neuropsychiatric Disorders: The Iceberg Still Lies beneath the Surface. _Pharmacological Reviews_, _71_(3), 383–412. https://doi.org/10.1124/pr.118.015487
13. Stefański, R., Pałejko, W., Bidzinski, A., Kostowski, W., & Płaźnik, A. (1993). Serotonergic innervation of the hippocampus and nucleus accumbens septi and the anxiolytic-like action of the 5-ht3 receptor antagonists. _Neuropharmacology_, _32_(10), 987–993. https://doi.org/10.1016/0028-3908(93)90063-9
14. Tseilikman, V. E., Tseilikman, O. B., Karpenko, M. N., Traktirov, D. S., Obukhova, D. A., Shatilov, V. A., Zhukov, M. S., Manuilov, G. V., Yegorov, O. N., Aristov, M. R., Lipatov, I. A., Buksha, I. A., Epitashvili, A. E., Pashkov, A. A., & Novak, J. (2024). Unraveling the Serotonergic Mechanism of Stress-Related Anxiety: Focus on Co-Treatment with Resveratrol and Selective Serotonin Reuptake Inhibitors. _Biomedicines_, _12_(11), 2455. https://doi.org/10.3390/biomedicines12112455
15. Vahid-Ansari, F., Zhang, M., Zahrai, A., & Albert, P. R. (2019). Overcoming resistance to selective serotonin reuptake inhibitors: targeting serotonin, Serotonin-1A receptors and

adult neuroplasticity. Frontiers in Neuroscience, 13, 404. https://doi.org/10.3389/fnins.2019.00404

16. MacQueen, G., Born, L., & Steiner, M. (2001). The selective serotonin reuptake inhibitor Sertraline: Its profile and use in psychiatric disorders. CNS Drug Reviews, 7(1), 1–24. https://doi.org/10.1111/j.1527-3458.2001.tb00188.x
17. Piazza, G. G., Allegrini, A. G., Duffy, L., Lewis, G., Lewis, G., Roiser, J. P., & Pingault, J. (2025). The effect of sertraline on networks of mood and anxiety symptoms: secondary analysis of the PANDA randomized controlled trial. _Nature Mental Health_, _3_(11), 1417–1424. https://doi.org/10.1038/s44220-025-00528-x
18. Tseilikman, V. E., Tseilikman, O. B., Karpenko, M. N., Traktirov, D. S., Obukhova, D. A., Shatilov, V. A., Zhukov, M. S., Manuilov, G. V., Yegorov, O. N., Aristov, M. R., Lipatov, I. A., Buksha, I. A., Epitashvili, A. E., Pashkov, A. A., & Novak, J. (2024b). Unraveling the Serotonergic Mechanism of Stress-Related Anxiety: Focus on Co-Treatment with Resveratrol and Selective Serotonin Reuptake Inhibitors. Biomedicines, 12(11), 2455. https://doi.org/10.3390/biomedicines12112455
19. Willard, S. L., Uberseder, B., Clark, A., Daunais, J. B., Johnston, W. D., Neely, D., Massey, A., Williamson, J. D., Kraft, R. A., Bourland, J. D., Jones, S. R., & Shively, C. A. (2015). Long term sertraline effects on neural structures in depressed and nondepressed adult female nonhuman primates. _Neuropharmacology_, _99_, 369–378. https://doi.org/10.1016/j.neuropharm.2015.06.011
20. Citalopram and escitalopram. (2015). In Elsevier eBooks (pp. 383–387). https://doi.org/10.1016/b978-0-444-53717-1.00509-6
21. Selvaraj, S., Walker, C., Arnone, D., Cao, B., Faulkner, P., Cowen, P. J., Roiser, J. P., & Howes, O. (2017). Effect of citalopram on emotion processing in humans: A combined 5-HT1A [11C]CUMI-101 PET and functional MRI study. Neuropsychopharmacology, 43(3), 655–664. https://doi.org/10.1038/npp.2017.166
22. Sørensen, A., Ruhé, H. G., & Munkholm, K. (2021). The relationship between dose and serotonin transporter occupancy of antidepressants—a systematic review. _Molecular Psychiatry_, _27_(1), 192–201. https://doi.org/10.1038/s41380-021-01285-w
23. Du, Y., Du, B., Diao, Y., Yin, Z., Li, J., Shu, Y., Zhang, Z., & Chen, L. (2021). Comparative efficacy and acceptability of antidepressants and benzodiazepines for the

treatment of panic disorder: A systematic review and network meta-analysis. Asian Journal of Psychiatry, 60, 102664. https://doi.org/10.1016/j.ajp.2021.102664

24. Lukow, P. B., Lowther, M., Pike, A. C., Yamamori, Y., Chavanne, A. V., Gormley, S., Aylward, J., Vera, C. E., McCloud, T., Goble, T., Rodriguez-Sanchez, J., Tuominen, E. W., Buehler, S. K., Kirk, P., & Robinson, O. J. (2025). Brain activation and connectivity after 2–3 weeks of escitalopram administration in anxiety disorders: A randomised trial. _Journal of Affective Disorders_, _394_(Pt B), 120682. https://doi.org/10.1016/j.jad.2025.120682
25. Verkhratsky, A., & Butt, A. M. (2023). Neuropsychiatric disorders. In _Neuroglia_ (pp. 599–627). https://doi.org/10.1016/b978-0-12-821565-4.00001-8
26. Malhi, G. S., Bell, E., Morris, G., & Hamilton, A. (2020). The delay in response to antidepressant therapy: A window of opportunity? _Australian & New Zealand Journal of Psychiatry_, _54_(2), 127–129. https://doi.org/10.1177/0004867419900313
27. Blumenfeld, Z., Bera, K., Castrén, E., & Lester, H. A. (2023b). Antidepressants enter cells, organelles, and membranes. Neuropsychopharmacology, 49(1), 246–261. https://doi.org/10.1038/s41386-023-01725-x
28. Turcotte-Cardin, V., Vahid-Ansari, F., Luckhart, C., Daigle, M., Geddes, S. D., Tanaka, K. F., Hen, R., James, J., Merali, Z., Béïque, J., & Albert, P. R. (2018b). Loss of adult 5-HT1A autoreceptors results in a paradoxical anxiogenic response to antidepressant treatment. _Journal of Neuroscience_, _39_(8), 1334–1346. https://doi.org/10.1523/jneurosci.0352-18.2018
29. Sant'Ana, A. B., Vilela-Costa, H. H., Vicente, M. A., Hernandes, P. M., De Andrade, T. G. C. S., & Zangrossi, H. (2019). Role of 5-HT2C receptors of the dorsal hippocampus in the modulation of anxiety- and panic-related defensive responses in rats. _Neuropharmacology_, _148_, 311–319. https://doi.org/10.1016/j.neuropharm.2019.01.026
30. Gunduz-Cinar, O., Flynn, S., Brockway, E., Kaugars, K., Baldi, R., Ramikie, T. S., Cinar, R., Kunos, G., Patel, S., & Holmes, A. (2015). Fluoxetine facilitates fear extinction through amygdala endocannabinoids. _Neuropsychopharmacology_, _41_(6), 1598–1609. https://doi.org/10.1038/npp.2015.318

31. Pejchal, T., Foley, M. A., Kosofsky, B. E., & Waeber, C. (2002). Chronic fluoxetine treatment selectively uncouples raphe 5‐HT1A receptors as measured by [35S]‐GTPγS autoradiography. _British Journal of Pharmacology_, _135_(5), 1115–1122. https://doi.org/10.1038/sj.bjp.0704555
32. Singewald, N., Schmuckermair, C., Whittle, N., Holmes, A., & Ressler, K. (2014). Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. _Pharmacology & Therapeutics_, _149_, 150–190. https://doi.org/10.1016/j.pharmthera.2014.12.004
33. Nasir, M., Trujillo, D., Levine, J., Dwyer, J. B., Rupp, Z. W., & Bloch, M. H. (2020). Glutamate Systems in DSM-5 Anxiety Disorders: Their role and a review of Glutamate and GABA Psychopharmacology. _Frontiers in Psychiatry_, _11_, 548505. https://doi.org/10.3389/fpsyt.2020.548505
34. Borodovitsyna, O., Flamini, M. D., & Chandler, D. J. (2018c). Acute stress persistently alters locus coeruleus function and anxiety-like behavior in adolescent rats. _Neuroscience_, _373_, 7–19. https://doi.org/10.1016/j.neuroscience.2018.01.020
35. Moore, C., Helms, M. L., Nipper, M. A., Winfrey, L. C., Finn, D. A., & Meshul, C. K. (2024). Dopamine loss alters glutamate synapses and transporters in the medial prefrontal cortex and anxiety‐related behaviour in a male MPTP rodent model of Parkinson's disease. _European Journal of Neuroscience_, _60_(9), 6195–6215. https://doi.org/10.1111/ejn.16577
36. Venkataraman, A., & Dias, B. G. (2022). Expanding the canon: An inclusive neurobiology of thalamic and subthalamic fear circuits. _Neuropharmacology_, _226_, 109380. https://doi.org/10.1016/j.neuropharm.2022.109380
37. Wengrovitz, A., Ivantsova, E., Crespo, N., Patel, M., Souders, C. L., & Martyniuk, C. J. (2023). Differential effects of dopamine receptor agonists ropinirole and quinpirole on locomotor and anxiolytic behaviors in larval zebrafish (Danio rerio): A role for the GABAergic and glutamate system? _Neurotoxicology and Teratology_, _98_, 107183. https://doi.org/10.1016/j.ntt.2023.107183
38. Gong, W. (2025c). Research progress on the neural circuits mechanisms of anxiety. _Frontiers in Neural Circuits_, _19_, 1609145. https://doi.org/10.3389/fncir.2025.1609145

39. He, Y., Jiang, L., Liu, H., Bu, Q., Kuang, W., Zhao, Y., Chen, Y., Zhang, N., Xiao, Y., Li, S., Chen, R., Han, S., Zhou, Y., Zhang, J., Wan, X., Xu, R., Wang, S., Zhang, H., Gu, H., . . . Cen, X. (2023). Hippocampal CIRCANK3 deficiency causes anxiety-like behaviors and social deficits by regulating the MIR-7080-3P/IQGAP1 pathway in mice. _Biological Psychiatry_, _95_(9), 896–908. https://doi.org/10.1016/j.biopsych.2023.10.017
40. Terbeck, S., Akkus, F., Chesterman, L. P., & Hasler, G. (2015). The role of metabotropic glutamate receptor 5 in the pathogenesis of mood disorders and addiction: combining preclinical evidence with human Positron Emission Tomography (PET) studies. _Frontiers in Neuroscience_, _9_, 86. https://doi.org/10.3389/fnins.2015.00086
41. Bouvier, G., Bidoret, C., Casado, M., & Paoletti, P. (2015). Presynaptic NMDA receptors: Roles and rules. _Neuroscience_, _311_, 322–340. https://doi.org/10.1016/j.neuroscience.2015.10.033
42. Gafford, G. M., & Ressler, K. J. (2015). GABA and NMDA receptors in CRF neurons have opposing effects in fear acquisition and anxiety in central amygdala vs. bed nucleus of the stria terminalis. _Hormones and Behavior_, _76_, 136–142. https://doi.org/10.1016/j.yhbeh.2015.04.001
43. Mousavi, S. L., Rezayof, A., Alijanpour, S., Delphi, L., & Sahafi, O. H. (2023). Activation of mediodorsal thalamic dopamine receptors inhibited nicotine-induced anxiety in rats: A possible role of corticolimbic NMDA neurotransmission and BDNF expression. _Pharmacology Biochemistry and Behavior_, _232_, 173650. https://doi.org/10.1016/j.pbb.2023.173650
44. Lorigooini, Z., Boroujeni, S. N., Balali-Dehkordi, S., Ebrahimi, L., Bijad, E., Rahimi-Madiseh, M., & Amini-Khoei, H. (2020). Possible involvement of NMDA receptor in the anxiolytic-like effect of caffeic acid in mice model of maternal separation stress. _Heliyon_, _6_(9), e04833. https://doi.org/10.1016/j.heliyon.2020.e04833
45. Nasir, M., Trujillo, D., Levine, J., Dwyer, J. B., Rupp, Z. W., & Bloch, M. H. (2020b). Glutamate Systems in DSM-5 Anxiety Disorders: Their role and a review of Glutamate and GABA Psychopharmacology. _Frontiers in Psychiatry_, _11_, 548505. https://doi.org/10.3389/fpsyt.2020.548505

46. Venkataraman, A., & Dias, B. G. (2022). Expanding the canon: An inclusive neurobiology of thalamic and subthalamic fear circuits. *Neuropharmacology*, *226*, 109380. https://doi.org/10.1016/j.neuropharm.2022.109380
47. He, Y., Jiang, L., Liu, H., Bu, Q., Kuang, W., Zhao, Y., Chen, Y., Zhang, N., Xiao, Y., Li, S., Chen, R., Han, S., Zhou, Y., Zhang, J., Wan, X., Xu, R., Wang, S., Zhang, H., Gu, H., . . . Cen, X. (2023). Hippocampal CIRCANK3 deficiency causes anxiety-like behaviors and social deficits by regulating the MIR-7080-3P/IQGAP1 pathway in mice. _Biological Psychiatry_, _95_(9), 896–908. https://doi.org/10.1016/j.biopsych.2023.10.017
48. Terbeck, S., Akkus, F., Chesterman, L. P., & Hasler, G. (2015). The role of metabotropic glutamate receptor 5 in the pathogenesis of mood disorders and addiction: combining preclinical evidence with human Positron Emission Tomography (PET) studies. _Frontiers in Neuroscience_, _9_, 86. https://doi.org/10.3389/fnins.2015.00086
49. Baldwin, D., Ajel, Nowak, Rafiq, & Masdrakis, V. (2013). Pregabalin for the treatment of generalized anxiety disorder: an update. _Neuropsychiatric Disease and Treatment_, 883. https://doi.org/10.2147/ndt.s36453
50. Fagan, H. A., & Baldwin, D. S. (2023). Pharmacological treatment of Generalised Anxiety Disorder: current practice and future directions. _Expert Review of Neurotherapeutics_, _23_(6), 535–548. https://doi.org/10.1080/14737175.2023.2211767
51. Generoso, M. B., Trevizol, A. P., Kasper, S., Cho, H. J., Cordeiro, Q., & Shiozawa, P. (2016). Pregabalin for generalized anxiety disorder. International Clinical Psychopharmacology, 32(1), 49–55. https://doi.org/10.1097/yic.0000000000000147
52. Nasir, M., Trujillo, D., Levine, J., Dwyer, J. B., Rupp, Z. W., & Bloch, M. H. (2020). Glutamate Systems in DSM-5 Anxiety Disorders: Their role and a review of Glutamate and GABA Psychopharmacology. _Frontiers in Psychiatry_, _11_, 548505. https://doi.org/10.3389/fpsyt.2020.548505
53. Tully, J. L., Dahlén, A. D., Haggarty, C. J., Schiöth, H. B., & Brooks, S. (2022). Ketamine treatment for refractory anxiety: A systematic review. _British Journal of Clinical Pharmacology_, _88_(10), 4412–4426. https://doi.org/10.1111/bcp.15374
54. Widman, A. J., & McMahon, L. L. (2018). Disinhibition of CA1 pyramidal cells by low-dose ketamine and other antagonists with rapid antidepressant efficacy. _Proceedings

of the National Academy of Sciences_, _115_(13), E3007–E3016. https://doi.org/10.1073/pnas.1718883115

55. Lattie, D. T., Nehoff, H., Neehoff, S., Gray, A., & Glue, P. (2020). Anxiolytic effects of acute and maintenance ketamine, as assessed by the Fear Questionnaire subscales and the Spielberger State Anxiety Rating Scale. _Journal of Psychopharmacology_, _35_(2), 137–141. https://doi.org/10.1177/0269881120953991
56. Martens, M. a. G., Zghoul, T., Watson, E., Rieger, S. W., Capitão, L. P., & Harmer, C. J. (2024). Acute neural effects of the mood stabiliser lamotrigine on emotional processing in healthy volunteers: a randomised control trial. _Translational Psychiatry_, _14_(1), 211. https://doi.org/10.1038/s41398-024-02944-6
57. Costa, B., & Vale, N. (2023). Understanding lamotrigine's role in the CNS and possible future evolution. _International Journal of Molecular Sciences_, _24_(7), 6050. https://doi.org/10.3390/ijms24076050
58. Bowen, R. C., Balbuena, L., & Baetz, M. (2014). Lamotrigine reduces affective instability in depressed patients with mixed mood and anxiety disorders. _Journal of Clinical Psychopharmacology_, _34_(6), 747–749. https://doi.org/10.1097/jcp.0000000000000164
59. Coplan, J. D. (2015). Treating comorbid anxiety and depression: Psychosocial and pharmacological approaches. _World Journal of Psychiatry_, _5_(4), 366. https://doi.org/10.5498/wjp.v5.i4.366
60. Venkataraman, A., & Dias, B. G. (2022c). Expanding the canon: An inclusive neurobiology of thalamic and subthalamic fear circuits. _Neuropharmacology_, _226_, 109380. https://doi.org/10.1016/j.neuropharm.2022.109380
61. Papazisis, G., Dagklis, T., Pourzitaki, C., Melios, G., Kapoukranidou, D., & Kouvelas, D. (2009). P.2.18 Lamotrigine improves anxiety-like behaviour and memory in a rat model of hypoxic-ischaemic encephalopathy. _European Neuropsychopharmacology_, _19_, S48–S49. https://doi.org/10.1016/s0924-977x(09)70056-2
62. Saitoh, A., Yamada, M., Inagaki, M., & Yamada, M. (2012). O-50 - A glutamate-modulating drug, riluzole, is one of the candidate drugs for new generation antidepressants and/or anxiolytics. _European Psychiatry_, _27_, 1. https://doi.org/10.1016/s0924-9338(12)74150-0

63. Pittenger, C., Coric, V., Banasr, M., Bloch, M., Krystal, J. H., & Sanacora, G. (2008). Riluzole in the treatment of mood and anxiety disorders. CNS Drugs, 22(9), 761–786. https://doi.org/10.2165/00023210-200822090-00004
64. Kawashima, Y., Yamada, M., Furuie, H., Kuniishi, H., Akagi, K., Kawashima, T., Noda, T., & Yamada, M. (2023). Effects of riluzole on psychiatric disorders with anxiety or fear as primary symptoms: A systematic review. _Neuropsychopharmacology Reports_, _43_(3), 320–327. https://doi.org/10.1002/npr2.12364
65. Abdallah, C. G., Coplan, J. D., Jackowski, A., Sato, J. R., Mao, X., Shungu, D. C., & Mathew, S. J. (2012). A pilot study of hippocampal volume and N-acetylaspartate (NAA) as response biomarkers in riluzole-treated patients with GAD. _European Neuropsychopharmacology_, _23_(4), 276–284. https://doi.org/10.1016/j.euroneuro.2012.05.009
66. Petroff, O. a. C., Rothman, D. L., Behar, K. L., & Mattson, R. H. (1995). Initial observations on effect of vigabatrin on in vivo 1H spectroscopic measurements of Γ‐Aminobutyric acid, glutamate, and glutamine in human brain. Epilepsia, 36(5), 457–464. https://doi.org/10.1111/j.1528-1157.1995.tb00486.x
67. Möhler, H. (2011). The GABA system in anxiety and depression and its therapeutic potential. Neuropharmacology, 62(1), 42–53. https://doi.org/10.1016/j.neuropharm.2011.08.040
68. Zhang, Q., Zhu, L., Li, H., Chen, Q., Li, N., Li, J., Zhao, Z., Xiao, D., Tang, T., Bi, C., Zhang, Y., Zhang, H., Zhang, G., Li, M., Zhu, Y., Zhang, J., & Kong, J. (2024). Insights and progress on the biosynthesis, metabolism, and physiological functions of gamma-aminobutyric acid (GABA): a review. _PeerJ_, _12_, e18712. https://doi.org/10.7717/peerj.18712
69. Kim, S., & Kim, S. (2021). Functional dissection of glutamatergic and GABAergic neurons in the bed nucleus of the stria terminalis. _Molecules and Cells_, _44_(2), 63–67. https://doi.org/10.14348/molcells.2021.0006
70. Gauthier, I., & Nuss, P. (2015). Anxiety disorders and GABA neurotransmission: a disturbance of modulation. _Neuropsychiatric Disease and Treatment_, _11_, 165. https://doi.org/10.2147/ndt.s58841

71. Roth, F. C., & Draguhn, A. (2012). GABA Metabolism and Transport: Effects on synaptic efficacy. _Neural Plasticity_, _2012_, 1–12. https://doi.org/10.1155/2012/805830
72. Zafar, S., & Jabeen, I. (2018). Structure, function, and modulation of Γ-Aminobutyric acid transporter 1 (GAT1) in neurological disorders: A Pharmacoinformatic prospective. _Frontiers in Chemistry_, _6_, 397. https://doi.org/10.3389/fchem.2018.00397
73. Hou, X., Rong, C., Wang, F., Liu, X., Sun, Y., & Zhang, H. (2020). GABAergic System in stress: Implications of GABAergic neuron subpopulations and the Gut-Vagus-Brain pathway. _Neural Plasticity_, _2020_, 1–11. https://doi.org/10.1155/2020/8858415
74. Kujala, J., Ciumas, C., Jung, J., Bouvard, S., Lecaignard, F., Lothe, A., Bouet, R., Ryvlin, P., & Jerbi, K. (2024). GABAergic inhibition shapes behavior and neural dynamics in human visual working memory. _Cerebral Cortex_, _34_(2). https://doi.org/10.1093/cercor/bhad522
75. Maguire, J. L., & Mennerick, S. (2023). Neurosteroids: mechanistic considerations and clinical prospects. _Neuropsychopharmacology_, _49_(1), 73–82. https://doi.org/10.1038/s41386-023-01626-z
76. Gauthier, I., & Nuss, P. (2015). Anxiety disorders and GABA neurotransmission: a disturbance of modulation. _Neuropsychiatric Disease and Treatment_, _11_, 165. https://doi.org/10.2147/ndt.s58841
77. Sigel, E., & Ernst, M. (2018). The benzodiazepine binding sites of GABAA receptors. _Trends in Pharmacological Sciences_, _39_(7), 659–671. https://doi.org/10.1016/j.tips.2018.03.006
78. Penninx, B. W. J. H., Pine, D. S., Holmes, E. A., & Reif, A. (2021). Benzodiazepines for the long-term treatment of anxiety disorders? – Authors' reply. _The Lancet_, _398_(10295), 120. https://doi.org/10.1016/s0140-6736(21)00931-4
79. Breilmann, J., Girlanda, F., Guaiana, G., Barbui, C., Cipriani, A., Castellazzi, M., Bighelli, I., Davies, S. J., Furukawa, T. A., & Koesters, M. (2019). Benzodiazepines versus placebo for panic disorder in adults. _Cochrane Database of Systematic Reviews_, _3_, CD010677. https://doi.org/10.1002/14651858.cd010677.pub2
80. Beyer, C., Currin, C. B., Williams, T., & Stein, D. J. (2024). Meta-analysis of the comparative efficacy of benzodiazepines and antidepressants for psychic versus somatic

symptoms of generalized anxiety disorder. _Comprehensive Psychiatry_, _132_, 152479. https://doi.org/10.1016/j.comppsych.2024.152479

81. Gomez, A. F., Barthel, A. L., & Hofmann, S. G. (2018). Comparing the efficacy of benzodiazepines and serotonergic anti-depressants for adults with generalized anxiety disorder: a meta-analytic review. _Expert Opinion on Pharmacotherapy_, _19_(8), 883–894. https://doi.org/10.1080/14656566.2018.1472767
82. Gomez, A. F., Barthel, A. L., & Hofmann, S. G. (2018). Comparing the efficacy of benzodiazepines and serotonergic anti-depressants for adults with generalized anxiety disorder: a meta-analytic review. *Expert Opinion on Pharmacotherapy*, *19*(8), 883–894. https://doi.org/10.1080/14656566.2018.1472767
83. Jacob, T. C., Michels, G., Silayeva, L., Haydon, J., Succol, F., & Moss, S. J. (2012). Benzodiazepine treatment induces subtype-specific changes in GABAAreceptor trafficking and decreases synaptic inhibition. _Proceedings of the National Academy of Sciences_, _109_(45), 18595–18600. https://doi.org/10.1073/pnas.1204994109
84. Rupprecht, R., Schüle, C., & Gudermann, T. (2026). Neuroactive steroids for the treatment of depression and anxiety: gaps and promises. _European Archives of Psychiatry and Clinical Neuroscience_, _276_(2), 405–406. https://doi.org/10.1007/s00406-026-02196-z
85. Zorumski, C. F., Paul, S. M., Covey, D. F., & Mennerick, S. (2019). Neurosteroids as novel antidepressants and anxiolytics: GABA-A receptors and beyond. Neurobiology of Stress, 11, 100196. https://doi.org/10.1016/j.ynstr.2019.100196
86. Walton, N. L., & Maguire, J. L. (2025). Neurosteroids: a lifelong impact on brain health. _Frontiers in Behavioral Neuroscience_, _19_, 1644615. https://doi.org/10.3389/fnbeh.2025.1644615
87. Maguire, J. L., & Mennerick, S. (2023b). Neurosteroids: mechanistic considerations and clinical prospects. _Neuropsychopharmacology_, _49_(1), 73–82. https://doi.org/10.1038/s41386-023-01626-z
88. Walton, N. L., Antonoudiou, P., Barros, L., Dargan, T., DiLeo, A., Evans-Strong, A., Gabby, J., Howard, S., Paracha, R., Sánchez, E. J., Weiss, G. L., Kong, D., & Maguire, J. L. (2023). Impaired endogenous neurosteroid signaling contributes to behavioral deficits

associated with chronic stress. Biological Psychiatry, 94(3), 249–261. https://doi.org/10.1016/j.biopsych.2023.01.022

Chapter 5.0: General Discussion and Future Directions

Anxiety has accompanied life since its earliest form, from the solitary depths of the ocean to vast lands. Each chapter approached anxiety from a different perspective. The historical review traced the concept from *angor* to the modern concept of generalised anxiety. The anatomical level examined the projections constituting the anxiety network. Circuit-level analysis explored how brain rhythm coordinates these regions, while the pharmacology chapter addressed the neurochemical imbalance underlying anxiety disorders. Together, these perspectives converge on a single conclusion: anxiety is not exclusively a human affective state, but shared across social and solitary organisms alike.

Here, we propose a deliberate conceptual separation between anxiety as a physiological affective state and anxiety disorders as a pathological condition. Anxiety is an evolutionary, preparatory, adaptive and transient response. It drives coping behaviour aimed at facing potential dangers with a future-oriented attentional focus, which is non-pathological and governed by an active somatic state. In contrast, when anxiety becomes chronic, hampering personal or social functioning, and impairing physiological mechanisms, it manifests as an anxiety disorder. The fundamental limitation across the literature is the absence of experimental differences between physiological anxiety and pathological anxiety. While this thesis argues for a conceptual separation, most studies do not explicitly distinguish between a transient adaptive response and a persistent pathological state.

Chapters 2 and 3 demonstrate a tightly integrated network, establishing that anxiety is regulated by a network rather than a single brain centre. Every brain region, connected through short and long projections and synchronised brain waves, operates in concert. Chapter 4 further demonstrates that no single neurotransmitter explains anxiety. SSRIs reconfigure circuit dynamics over weeks rather than acting simply through acute serotonin elevation. Critically, the circuits underlying physiological and pathological anxiety are the same. The difference lies in the duration of activation, the weighing of threat, and the failure of the prefrontal extinction. Many earlier models have underrepresented deeper subcortical structures such as the lateral habenula,

locus coeruleus, and nucleus accumbens; recent findings indicate these structures play a significant role in anxiety regulation and warrant further integration into existing circuit models.

A significant limitation of the current evidence base is its species imbalance. Rodent models have dominated anxiety research, from neural circuits mapping to pharmacological development. In rodents and canines, the olfactory system is predominant in contrast to primates, and olfactory stimuli for anxiety may follow a distinct underlying pathway, as olfactory input innervates brain centres directly via the olfactory nerve rather than through the thalamus. This structural difference alone suggests that rodent-derived circuit models cannot be directly transposed to species with different primary sensory architecture. For non-human primates and canines, the literature remains scarce, which not only indicates the research gap but also reflects an incomplete understanding of the neural processes of these sentient organisms. Anxiety disorders manifest in different forms, from generalised anxiety to social anxiety disorders in primates and separation anxiety in canines. Yet no studies have systematically mapped the distinct neural pathways underlying each disorder. From the circuit reviewed, different brain regions are recruited during activation, depending on the specific stimulus and disorder type, and, in humans, a given disorder can manifest differently across individuals.

Two brain structures in particular deserve attention as emerging research directions. First, the role of the cerebellum and the spinal cord in anxiety remains underexplored; both are likely to be involved in the somatic regulation of anxiety, yet existing circuit models have yet to integrate their contribution. Second, most neural pathway models have failed to account for episodic memory as an anxiety trigger, without the involvement of primary sensory regions. Unravelling the underlying memory-anxiety pathway, how anxiety is initiated from stored experiences without sensory input, represents one of the most important conceptual and mechanistic gaps remaining in the field.

The pharmacological evidence adds a further layer of complexity. Drug action predicts distinct circuit dynamics across species, even for the same molecular target. Broader cross-species drug-interaction studies spanning multiple neurotransmitter systems would aid in developing a deeper mechanistic understanding and may identify points of divergence that current single-species models obscure.

Given the ongoing limitations of ethical experimentation, non-invasive neuroimaging approaches, including functional MRI, structural MRI, and PET/SPECT, offer significant potential for closing these gaps. Functional MRI, via blood-oxygen-level-dependent (BOLD) contrast, would allow direct mapping of the memory-anxiety pathway in humans without reliance on rodent circuit inference. PET/SPECT through regional cerebral blood flow measurement would enable real-time assessment of circuit activation across species. Voxel-based morphometric analysis could further quantify structural differences across species and disorder subtypes. More importantly, studying anxiety in parallel to genetics and epigenetics will yield significant results, not only in understanding how psychiatric disorders develop, but in developing a precise line of treatment.

From a translational and veterinary psychiatry perspective, the absence of a species-adapted diagnostic framework equivalent to the Diagnostic and Statistical Manual of Mental Disorders (DSM) remains a substantive gap. Developing such a framework for veterinary psychiatry would significantly advance both clinical and research practice in the field.

5.1 Conclusion

Anxiety is a physiological process innate to sentient organisms, a feature of their biological composition. Anxiety disorders emerge when that same inherent survival system fails to disengage, persisting beyond its functional purpose. The circuit evidence across species is promising but incomplete, and it would be methodologically inappropriate to apply rodent-derived models universally, given the fundamental differences in sensory architecture, social attachment systems, and cognitive complexity among species. What this thesis establishes is a conceptual and empirical foundation: that anxiety and anxiety disorders are biologically continuous but functionally distinct, that this shared circuit permits rigorous cross-species study with the right tools; and that closing the current research gaps will not only deepen our understanding of one of the most ancient affective states, but directly improve the quality of life for patients across species in whom that system has failed to find its resolution.