Mahsa Sherafatizangeneh

Analysis of genetic data to unravel the evolutionary history of hypothalamus neuropeptides and their role in cognition and behavior

Submitted as master's thesis at the Department of Psychology, University of Oslo

Autumn 2023

Analysis of genetic data to unravel the evolutionary history of hypothalamus neuropeptides and their role in cognition and behavior

Author: Mahsa Sherafatizangeneh

Supervisor: Daniel S. Quintana

Co-supervisor: Alina M. Sartorius

http://www.duo.uio.no/

Year: 2023

Abstract

The hypothalamus is a small but powerful part of the brain that controls many vital bodily functions. Its neurons and hormonal signaling network regulate body temperature, hunger, thirst, and sleep. These processes are essential for maintaining homeostasis and ensuring that the body functions properly. In addition to its role in physiological regulation, the hypothalamus also plays a crucial role in emotional processing, cognition, and behavior. It helps regulate mood, motivation, and decision-making by influencing the release of neurotransmitters like dopamine and serotonin. The hypothalamus also regulates the release and effects of neuropeptides, which are important signaling molecules that influence various physiological and behavioral processes. These include pain perception, stress response, immune function, and reproductive behavior. The hypothalamus is a complex and multifaceted part of the brain that plays a critical role in maintaining health and well-being. Many studies have revealed that comprehending the intricate connections between the hypothalamus and neuropeptides can offer valuable knowledge about the workings of different physiological and behavioral processes. The dN/dS ratio is a commonly used statistic in genetics. It compares the rate of non-synonymous substitutions (dN), which change amino acids and can be affected by natural selection, to the rate of synonymous substitutions (dS), considered neutral. A dN/dS ratio above 1 indicates positive selection, meaning changes in the protein are being favored. A ratio below 1 indicates adverse selection, meaning changes are being suppressed. A ratio around 1 indicates neutrality, meaning protein changes are neither favored nor disfavored.

This study analyzed the evolution rates of 13 hypothalamic neuropeptides and their receptor genes across eight human and non-human primate species. The findings indicate that, with the exception of *HCRTR1* in *Pan troglodytes* (chimpanzee), most neuropeptides have experienced negative selection. Variants under negative selection reveal which genetic areas are under pressure to stay the same because they are important for the organism to function correctly. The *HCRTR1* protein sequence in *Pan troglodytes* displayed a high dN/dS ratio (1.44), suggesting that positive selection may have occurred due to this species' unique behaviors and physiological responses. These results provide insight into the evolutionary history of neuropeptides and their receptors across species. The negative selection observed in most neuropeptides suggests that they have remained relatively conserved throughout evolution, likely due to their important roles in regulating various physiological and behavioral processes. However, the positive selection observed in *HCRTR1* in

Pan troglodytes highlights the potential for these molecules to evolve rapidly in response to specific environmental pressures and behavioral adaptations. These findings highlight the importance of studying the evolution of neuropeptides and their receptor genes, as they can provide valuable insights into the selective pressures that have shaped our biology and behavior over time. By understanding these evolutionary processes, we can better understand the complex interactions between genes and their environments, which may ultimately improve our ability to treat diseases and disorders that are influenced by these factors.

Acknowledgements

I would like to take a moment to express my utmost gratitude and appreciation towards my remarkable supervisors, Daniel Quintana, and Alina Marie Sartorius. I attribute the success of my project to their unwavering support, guidance, and mentorship. I am immensely grateful for their invaluable contributions, and I couldn't have succeeded without their encouragement and expertise. Furthermore, I would like to extend my heartfelt thanks to the exceptional psychology department at the University of Oslo, whose resources, facilities, and support have been integral to my research journey. Lastly, I would like to acknowledge my family for their constant love, encouragement, and prayers that have been my pillar of strength throughout my writing process. I am genuinely grateful for their unwavering support and consider myself fortunate to have such amazing people in my life.

Table of Contents

List of Tables

List of Figures

1: Introduction

1.1: Background

The hypothalamus is a brain region with highly conserved and functional structure across vertebrate species that affects basic aspects of physiological homeostasis and behavior (Xie & Dorsky, 2017). In mammals, the hypothalamus is a crucial regulator of homeostasis. It accomplishes this by combining internal and external sensory signals, processing them, and then releasing neuroendocrine peptides and regulatory autonomic signals to maintain homeostasis (Pearson & Placzek, 2013). The cortex and spinal cord, two major parts of the central nervous system (CNS), are made up of columnar structures, whereas the mammalian hypothalamus exhibits a distinct division into four levels, from front to back, and three zones emanating from side to side. These levels encompass the preoptic, anterior, tuberal, and mammillary hypothalamus, each containing a lateral, medial, and periventricular zone (Markakis, 2002). The hypothalamus mediates endocrine, autonomic, and behavioral activities to maintain homeostasis (Biran et al., 2015). It consists of a number of nuclei with distinct neuronal populations that produce neuropeptides and neurotransmitters that control essential bodily functions, such as temperature (Zhao et al., 2017) and metabolic rate (Waterson & Horvath, 2015), hunger and thirst (Bouret & Simerly, 2006), reproduction and sexual behavior (Stolzenberg & Numan, 2011), circadian rhythm (Saper et al., 2005), and emotional responses (Biran et al., 2015).

Therefore, hypothalamic dysfunction can substantially impact many aspects of health, such as energy imbalance, diabetes insipidus, and sleep disorders (Gao & Horvath, 2014; Maghnie et al., 2000; Shan et al., 2015). Although the hypothalamus is fairly small, it has numerous distinct cellular nuclei that express a range of neurotransmitters and peptide hormones. The same or/ comparable particular neuronal subtypes are present in animals as diverse as fish and humans, and their overall locations may be similar (Löhr & Hammerschmidt, 2011). The investigation of neuropeptide families and their receptors can give insights into phylogenetic links and evolutionary processes. In the case of neuropeptides, not only the receptor but also the peptide itself can evolve. Because neuropeptides are substantially smaller molecules than their receptors, the likelihood of mutation is lesser than that of the receptor (Hoyle, 1999). The peptides found in the hypothalamus are different from conventional neurotransmitters because they have longer half-lives, which enables them to spread to faraway areas (Leng & Ludwig, 2006).

Neuropeptides are a diverse class of signaling molecules that play important functions in animal physiology and behavior. They are peptides with small chain lengths that are produced through the enzymatic cleavage of bigger polypeptide precursors (Elphick et al., 2018). As neuropeptides are a very diverse group of brain and hypothalamus signaling molecules, their structures, functions, and locations are of great interest and importance (Jékely et al., 2018). Neuropeptides also influence social behavior, feeding behavior, stress, and anxiety. Numerous physiological systems and/or behaviors can be influenced and controlled by a single neuropeptide (Elphick et al., 2018; Jékely et al., 2018).

The ratio of non-synonymous to synonymous substitutions is a particularly helpful statistic for protein-coding genes. The ω ratio ($\omega = dN/dS$) describes gene evolutionary rates and can be a relevant feature, as it can determine which genes are the most conserved (these genes remain relatively unchanged throughout evolution) or least conserved (these genes have undergone more changes during evolution), as well as genes that may have undergone periods of adaptive evolution (Jeffares et al., 2007; Jeffares et al., 2015; Nielsen & Bustamante, 2005). dN/dS can be utilized to demonstrate how much selective pressure is working on a protein-coding gene (Spielman & Wilke, 2015). This research aims to study the genetic data for hypothalamus neuropeptides to clarify the evolutionary history and investigate the relationship between hypothalamus neuropeptides and cognition and behavior.

1.2: Oxytocin

The neuropeptide oxytocin (*OT*) has gained significant attention from various fields, such as neuroscience, psychology, and psychiatry, due to its positive effects on social behavior, anxiety reduction, and stress relief. It also shows potential for treating mental illnesses related to socio-emotional difficulties (Grinevich & Neumann, 2021).

A recent study by X and Y revealed that the genes oxytocin and vasopressin, which are paralogous, emerged from a local duplication caused by DNA transposable elements near the beginning of vertebrates. On the other hand, the genes for their receptors were developed through a combination of whole-genome duplication and segmental duplication. They conclude that the oxytocin and vasopressin receptor genes came about through whole-genome duplication and segmental duplication, leading to the creation of six receptors close to the start of jawed vertebrates, followed

by specific losses and gains in different lineages. The original *VTR1* and *VTR2* genes were formed via a tandem segmental duplication of the only *VTR* present in the vertebrate ancestor around 550 million years ago on the same chromosome. Finally, the combination of *VTR1A-VTR2A* on one chromosome paralogue and *OTR-VTR2B* on the other was brought about by a single round of whole-genome duplication in a gnathostome ancestor (Theofanopoulou et al., 2021).

Studies have shown that oxytocin, plays a significant role in social cognition and behavior. Administering oxytocin has been found to improve bonding behavior and peer recognition in various mammalian species (Yatawara et al., 2016). Oxytocin has been shown to facilitate positive social behaviors in mothers towards their offspring, such as feeding, nest building, and grooming. Conversely, such behaviors are less likely to occur when oxytocin receptors are blocked. These findings have important implications for our understanding of the neural mechanisms underlying social behavior and may pave the way for potential therapeutic interventions in the future (Jesso et al., 2011). Research has demonstrated the important role Oxytocin plays in initiating sexual behavior, arousal, and orgasm in both males and females (Tom & Assinder, 2010).

1.3: Vasopressin

Vasopressin, also called antidiuretic hormone (*ADH*) and arginine vasopressin (*AVP*), is created in the hypothalamus, and plays a role in regulating social behavior and fluid balance in the body (Arakawa & Higuchi, 2022).Magnocellular neurons in the hypothalamus's paraventricular nucleus and supra-ventricular nucleus produce this hormone from a 164 aa long pre-pro-hormone precursor (Sparapani et al., 2021). Additionally, lower levels of vasopressin are produced in the parvocellular cells of the paraventricular nucleus, also known as P-cells, which are neural elements located in the parvocellular layers of the lateral geniculate nucleus (*LGN*) in the thalamus (Morales-Medina et al., 2016; Sparapani et al., 2021).

Vasopressin actions are mediated by three receptor subtypes: *AVP1a, AVP1b*, and *AVP2. Avpr1a* and *Avpr1b* are expressed in the central nervous system, while *Avpr2* is limited to the periphery (Stevenson & Caldwell, 2012). It has been shown that vasopressin has an impact on socialization, territorial behavior, aggression, and social investigation, especially in males (Rigney et al., 2022). Experiencing stress in the early postnatal period, also known as early life stress, can affect brain function and alter social behavior in adulthood. Two important neuropeptides, arginine

vasopressin, and oxytocin, play a crucial role in shaping social behaviors such as aggression, social recognition, and social motivation (Kompier et al., 2019).

1.4: Corticotropin-releasing hormone

Corticotropin-releasing hormone (*CRH*) is critical in regulating the hypothalamic-pituitary-adrenal axis and various stress responses by interacting with the *CRH-R1* and *CRH-R2* receptors (Ketchesin et al., 2017). This peptide is synthesized in the hypothalamic paraventricular nucleus neurons and is triggered by both physical and psychological stressors from the limbic and brainstem centers (Sukhareva, 2021). In mammals, *CRH-R1* is predominantly present in the brain, anterior pituitary corticotropes, and lactotrophs, with lower expression levels observed in peripheral organs (Mano-Otagiri et al., 2016; Westphal et al., 2009). On the other hand, *CRH-R2* has a more widespread expression in the periphery, but its expression in the brain is relatively limited (Ketchesin et al., 2017).

Throughout extensive research, the role of *CRH* receptors and the availability of specific *CRH* agonists and antagonists have been identified as key factors in regulating numerous biological systems. *CRH* has been found to play a significant role in reproductive, neuropsychiatric, gastrointestinal, and immune disorders and tumor development (Caruso et al., 2022). Hillhouse and Grammatopoulos' (2006) research has also suggested that *CRH* has immunomodulatory effects and promotes inflammation in the body. Furthermore, it has been established that *CRH* plays an essential role in human pregnancy and parturition (Kim et al., 2018). Studies have also revealed that the *CRHR1* gene polymorphisms and impulsive personality traits are significant factors in aggressive behavior, particularly in male adolescents (Liu et al., 2020).

1.5: Dynorphin

Dynorphin (*DYN*) is a neuropeptide synthesized in various central nervous system regions, including the hypothalamus, striatum, hippocampus, and spinal cord. Its effects are mediated by binding to kappa opioid receptors *(KOR* or *KOPr*), regulated by G protein-coupled receptors (Schwarzer, 2009). *DYN* was first identified in 1975, and its receptor was subsequently cloned by Mansour et al (Mansour et al., 1995) and Le Merrer et al (Le Merrer et al., 2009). The

dynorphin/kappa opioid receptor system is widely distributed throughout the central nervous system and significantly regulates mood and emotional behaviors (Hang et al., 2015).

Butelman et al (Butelman et al., 2012) have identified that the *KOPr/DYN* system is crucial in regulating neuroendocrine activities and related behavioral, perceptual, reward, and mood processes, including those within the hypothalamic-pituitary-adrenal axis Additionally, Trezza (Trezza et al., 2011) has found that *KOP* within the nucleus accumbens is involved in social play behavior amongst rats and that *KOPr* agonism has the potential to inhibit social contact. Following exposure to drugs and subsequent withdrawal, dynorphin levels increase, which may seem paradoxical. This is likely due to the sensitivity of the *DYN/Kappa Opioid Receptor* system to positive and negative stressors, which can disrupt homeostasis in the body. Scientific research has indicated that this system plays a role in the development and symptoms of various neuropsychiatric disorders (Clark, 2020). In behavioral tests, activation of *KOR* has been demonstrated to result in cognitive disruptions that affect inhibitory control and decision-making processes that rely on working memory (Andrews, 2019).

1.6: Beta-endorphin

Beta-endorphins are peptides that have a range of effects on the body. They activate μ opioid receptors (*MOR*) in various body parts, including the brain and immune cells that regulate many systems (Pilozzi et al., 2020). These receptors are G-protein coupled receptors, so when beta-endorphin or other opioids bind to them, it triggers a signaling cascade in the cell (Livingston & Traynor, 2018). β-Endorphins have been identified as a critical player in regulating hormonal activity in response to stressful situations. This hormone is associated with the hypothalamic-pituitary-adrenal axis (*HPA*), which manages various bodily processes, such as metabolic and immunological responses. As noted by Pariante and Lightman in 2008 (Pariante & Lightman, 2008), the *HPA* plays a crucial role in coordinating the body's response to stress and maintaining homeostasis. (Smith & Vale, 2022) By regulating the release of hormones like β-Endorphins, the *HPA* ensures that the body can respond appropriately to stress and maintain its overall health and well-being (Pariante & Lightman, 2008).

Deficiency of central β-endorphins, which may be caused by genetic factors or adaptive changes. has been linked to an increased likelihood of increased alcohol consumption (Juárez & Molina-

Martínez, 2019). Among opioid receptors, the μ receptor mediates most of the analgesic and rewarding properties of opioids. Based on striking similarities between social distress, physical pain and opiate withdrawal, μ receptors have been proposed to play a critical role in modulating social behavior in humans and animals (Pellissier et al., 2018).

The μ opioid receptor is implicated in the reward, tolerance and withdrawal effects of alcohol and other drugs of abuse. This hypothesis is supported by the effects of alcohol on beta-endorphin release, of μ opioid receptor agonists and antagonists on alcohol consumption (Bergen et al., 1997). The role of μ receptor activation has also been extensively studied under conditions of social comfort .In such context, low to moderate μ receptor activation facilitates social behavior (Pellissier et al., 2018).

1.7: Gonadotropin-releasing hormone

In mammals, the initiation of reproductive activity is sparked by a hormone named Gonadotropin-releasing hormone (*GnRH).* Specialized neurons produce this hormone in a crucial part of the brain called the hypothalamus. Once released, *GnRH* interacts with a specific receptor called *GNRHR* located in the pituitary gland. This interaction produces two key hormones - *LH* and *FSH* - which are responsible for stimulating the gonads to produce both gametes and steroids. Interestingly, while *GNRHR* is primarily associated with reproduction, it has also been found in other areas of the brain and the adrenal gland, suggesting that *GnRH* may serve other vital purposes beyond simply facilitating reproduction (Millar, 2005; Sand et al., 2013). The hormone *GnRH*, which plays a role in reproduction, is mainly located in the hypothalamus. However, it also exists in various forms throughout the brain and can impact other vital functions such as feeding, reproductive behaviors, learning, and memory (Whitlock et al., 2019).

New research has shown that *GnRH* may significantly impact the regulation of aging and lifespan. As individuals age, inflammation in the hypothalamus is linked to the process, and there is a decrease in neurogenesis (Layman et al., 2011; Ottinger et al., 1997; Wang et al., 2020). Moreover, studies have indicated that *GnRH* could affect declarative memory and mood regulation, suggesting that it can contribute to cognitive, mood, and behavioral changes connected to sleep-disordered breathing (Nair et al., 2013).

1.8: Orexin/hypocretin

In 1998, two different research teams discovered two structurally similar neuropeptides that had an excitatory effect. One group named them "hypocretins," while the other group called them "orexins," which comes from the Greek word orexis, meaning hunger (Inutsuka & Yamanaka, 2013). Orexin comprises two components, orexin A and B, created from a single precursor peptide called prepro-orexin (Soya & Sakurai, 2020). Orexin neurons have strong connections to various brain parts, including the prefrontal cortex, limbic structures, hypothalamus, and brainstem. These connections control arousal, reward systems, and autonomic control. Orexin neurons have an excitatory effect on neuronal activity through the *OX1* and *OX2* receptors (Grimaldi et al., 2014).

Research shows that hypocretin/orexin-producing neurons connect to brain regions that control functions such as reward, learning, memory, emotion, attention, and arousal. As a result, scientists are actively investigating using hypocretin/orexin receptor antagonists to treat addiction, sleep disorders, obesity, mood, anxiety, and panic disorders (Soya & Sakurai, 2020). Orexins are also closely linked to motivated and active behavior in many species, with feeding behavior being a prime example. Orexin neurons respond to food-related cues and humoral signals, indicating low energy balance. Additionally, orexin neurons are activated by cues and contexts associated with rewards, such as food, sex, and drugs, which can trigger behaviors in response to these stimuli (Barson et al., 2013).

1.9: Kisspeptin

Kisspeptin and its receptor, *KISS1R*, help to initiate puberty and regulate the hypothalamic-pituitary-gonadal axis, making them crucial for mammalian reproduction (Cao et al., 2019).

Kisspeptin plays an essential role in reproduction by regulating the *HPG* axis, which initiates puberty and sexual maturation. In humans, kisspeptin neurons are located in the hypothalamus, particularly in the preoptic area and the infundibular nucleus. These areas are similar to the rostral periventricular region of the third ventricle and arcuate nucleus found in rodents and contain many GnRH cells that express *KISS1R* (López-Ojeda & Hurley, 2022).

Kisspeptin is an important hormone that helps regulate the menstrual cycle and promote mammal reproduction (Zhu et al., 2022). It also affects puberty, gonadal maturation, and the human

reproductive system. When kisspeptin activates specific neurons, they release a hormone (*GnRH*) that triggers the release of other hormones (luteinizing hormone and follicle-stimulating hormone) from the pituitary gland. These hormones then stimulate the release of sex hormones (like estrogens and progesterone) from the gonads. Kisspeptin also affects behavior, including emotions like anxiety and fear, by influencing various brain parts (López-Ojeda & Hurley, 2022). Studies have demonstrated that the administration of kisspeptin can have a beneficial effect on the emotional state of healthy men (Mills et al., 2019).

1.10: Neuropeptide Y

Neuropeptide Y (*NPY*) is a highly conserved neuropeptide found in the central nervous system and in peripheral tissues like the gut and cardiovascular system at high levels. Its effects are exerted through various receptor subtypes (Thorsell & Mathé, 2017). Vezzani and colleagues made a significant discovery in 1999 regarding the identification of five *NPY* receptors (*Y1, Y2, Y4, Y5,* and *Y6*) through cloning methods. These receptors belong to the G-protein-coupled receptor superfamily.(Vezzani et al., 1999). *NPY* regulates brain activity, stress coping, digestion, blood pressure, heart rate, body metabolism, and immune function. It is found throughout mammals' central and peripheral tissues, with a particular concentration in the nervous system (Li et al., 2019).

Food intake, blood vessel constriction, heart rate, anxiety, and bone homeostasis are all regulated by it *Y1* receptor (Pedragosa-Badia et al., 2013). The *Y2* receptor is mostly expressed in the thalamus, hypothalamus, and some peripheral nervous system regions as well as in hippocampal neurons There is evidence that both *Y1* and *Y2* receptors are relevant to emotional behavior. Intracerebroventricular injection of *NPY* reduces anxiety- and depression-related behavior in several animal models, this action being primarily mediated by *Y1* receptors (Pedragosa-Badia et al., 2013). The *Y1* receptor is a significant regulator of multiple bodily functions, including food intake, heart rate, anxiety levels, blood vessel constriction, and bone homeostasis, according to Pedragosa-Badia et al. (Pedragosa-Badia et al., 2013). The *Y2* receptor is primarily located in the hypothalamus thalamus, specific regions of the peripheral nervous system, and hippocampal neurons (Cattaneo et al., 2021). Research has indicated that both *Y1* and *Y2* receptors can impact emotional behavior. The administration of *NPY* via intracerebroventricular injection has been

observed to alleviate anxiety- and depression-related behavior in various animal models, with the *Y1* receptors serving as the primary mediators (Painsipp et al., 2008).

The *Y4* receptor is mainly found in the digestive tract, brain, pancreas, and prostate. It regulates food intake, energy balance, circadian rhythms, and intestinal transit while stimulating LH release. (Moriya et al., 2010; Pedragosa-Badia et al., 2013).On the other hand, the *Y5* protein is present in different parts of the brain, including the post-synaptic, hypothalamus, cortex, hippocampus, thalamus, and amygdala. It can also be found in other organs, such as the intestine, testis, ovary, prostate, pancreas, spleen, liver, skeletal muscle, kidney, heart, and placenta. The primary function of the *Y5* protein is to regulate food consumption and stimulates appetite (Shende & Desai, 2020).

1.11: Alpha-melanocyte stimulating hormone

The *MCR* system in the human consists of five receptors, namely *MC1R-MC5R*. Each of these receptors plays a crucial role in regulating various bodily functions, such as skin pigmentation, immune response modulation, glucose metabolism, energy balance, and feeding behavior (Zhou & Cai, 2017). Additionally, this system transmits signals that suppress appetite and is a crucial component in the neural circuits responsible for controlling central appetite (Wu et al., 2023).

Studies have linked *MCRs* in the brain to cognitive function. It has been observed that natural *MCR* agonists, such as melanocyte-stimulating hormones (*MSHs*), can improve learning, memory, and attention in both humans and rodents when used as treatment (Zhou et al., 2021). *MC1R* is a receptor in melanocytes and melanoma cells (*MCs*) responsible for driving melanogenesis. It is also associated with the Red Hair Color (*RHC*) phenotype and an increased risk of skin cancer due to specific variant alleles (Herraiz et al., 2021). The *MC4R* receptor in the hypothalamus plays a significant role in regulating hunger and energy balance. This receptor type is a G-protein-coupled receptor essential in developing obesity medication (Heyder et al., 2021).

1.12: Thyrotropin-releasing hormone

The hypothalamus and limbic regions of the brain produce Thyrotropin-releasing hormone (*TRH*) and its receptors (*TRHRs*). These receptors directly impact the brain and indirectly regulate

neurotransmitters such as glutamate, gamma-aminobutyric acid, acetylcholine, and dopamine. *TRH* plays a vital role in regulating mood and eating behavior, ultimately affecting an individual's overall well-being. Therefore, the production and regulation of *TRH* and its receptors are crucial for maintaining a healthy brain and body (Alvarez-Salas et al., 2022).

TRH is a small tripeptide consisting of the *pGlu-His-Pro-NH2* sequence. It was first discovered and studied due to its involvement in various neuroendocrine pathways (De La Cruz & Prokai-Tatrai, 2021). *TRH* also acts as a neurotransmitter and neuromodulator, influencing overall neurological effects throughout the *CNS*. It mediates effects on feeding behavior, thermogenesis, locomotor activation, and autonomic regulation. *TRH* shows excellent potential in treating neurological and psychological disorders by altering brain chemistry, behavior, and physiology (De La Cruz & Prokai-Tatrai, 2021).

When produced by the hypothalamus, *TRH* stimulates the pituitary's secretion of thyroid-stimulating hormone (*TSH*), and *TSH* then stimulates the thyroid gland to synthesize and secrete *THs*. The levels of THs are controlled by an endocrine axis negative feedback loop, with circulating THs controlling the production of *TRH* and *TSH*. High circulating *TH* levels decrease *TRH* and *TSH* production, thus returning *THs* to basal levels (Deal & Volkoff, 2021). Studies have indicated that with increasing age, *TRH's* generation, expression, and efficacy tend to decline. This observation raises the possibility that *TRH* might contribute to the onset of neurological disorders associated with aging, such as Alzheimer's disease and Parkinson's disease. (Daimon et al., 2013).

1.13: Prolactin

Prolactin (*PRL*) is a hormone made by the anterior pituitary gland and other tissues such as the mammary gland, prostate, skin, and brain. The prolactin receptor is present in many tissues, including the pituitary gland and brain regions such as the cerebral cortex, olfactory bulb, hypothalamus, hippocampus, and amygdala (Cabrera-Reyes et al., 2017). Prolactin plays a crucial role in milk production during lactation, and it also significantly impacts other bodily functions, including metabolism and energy balance. Without prolactin, the maternal body would struggle to adapt to the demands of pregnancy and lactation, which includes providing energy for the growing fetus and producing milk after birth (Lopez-Vicchi et al., 2020).

This hormone has many other functions, such as regulating the immune system, metabolism, brain function, and behavior. It can even protect against diabetes and has anti-inflammatory effects on the central nervous system. Moreover, *PRL* can enhance memory, cognition, and learning while reducing stress and anxiety (Byung et al., 2021). Studies suggest that individuals with a high risk of psychosis may experience decreased cognitive performance in reasoning, problem-solving, and general cognition tasks when their prolactin levels are low (Medina-Loera et al., 2020).

1.14: Dopamine

Dopamine (*DA*) is a neurotransmitter broadly distributed throughout the central nervous system and several peripheral regions, including the cardiovascular and renal systems (Rangel-Barajas et al., 2015). Five types of dopamine receptors, namely *D1, D2, D3, D4*, and *D5*, belong to the large G-protein coupled receptor superfamily (Ayano, 2016; Seeman, 2010). These dopamine receptor subtypes are classified into two major subclasses: types 1 and 5 are similar in structure and drug sensitivity and are known as the "*D1like*" group or class of receptors. On the other hand, dopamine receptor types 2, 3, and 4 are called the "*D2like*" group. Dopamine is a crucial neurotransmitter that plays a central role in various bodily functions, including pleasurable reward behavior, prolactin production inhibition (involved in lactation), sleep, mood, attention, learning, behavior, control of nausea and vomiting, and pain processing. Moreover, dopamine is also involved in regulating movement, emotion, and cognition (Ayano, 2016; Romanelli et al., 2010).

The brain's dopaminergic system has four main pathways, detailed in Table 1, and their functions have been demonstrated such as the Nigro-Striatal Pathway, in which fibers originate from the substantia nigra and extend rostrally to become extensively dispersed throughout the basal ganglia (caudate nucleus and the putamen) (Ayano, 2016). Degeneration of the nigrostriatal system causes Parkinson's disease (Hughes et al., 2002). The Mesolimbic Pathway begins in the ventral tegmental area and spreads to several other areas, including the amygdala, pyriform cortex, lateral septal nuclei, and the nucleus accumbens (Ayano, 2016; Malenka et al., 2009; Paulus & Schomburg, 2006). The Mesocortical Pathway is a pathway in the brain that involves dopaminergic fibers. These fibers originate from the ventral tegmental area and project to the frontal cortex and septohippocampal regions. Dopamine levels in the prefrontal cortex have been shown to enhance

working memory and attention. The Tuberoinfundibular Pathway originates from the arcuate and paraventricular nuclei and terminates at the pituitary gland, specifically the median eminence. Within this pathway, dopamine effectively inhibits the release of prolactin (Ben-Jonathan & Hnasko, 2001; Malenka et al., 2009; Paulus & Schomburg, 2006).

Table 1.

A summary of dopamine pathways and their important functions

Pathway	Function
Nigrostriatal	Movement and sensory stimuli
Mesolimbic	Pleasure and reward seeking behaviors, addiction, emotion, perception.
Mesocortical	Cognition, memory, attention, emotional behavior, and learning
Tuberoinfundibular	Control of the hypothalamic pituitary endocrine system, inhibition of prolactin secretions

1.15: dN/dS ratio

Over time, mutations in a population can either be lost or fixed based on their impact on the organisms. Natural selection and random genetic drift (the change in frequency of an existing gene variant in the population due to random chance) influence the rate at which mutations occur. As populations age, neutral mutations accumulate at the same rate as genomic mutations. A common approach for evaluating selection pressure in protein-coding genes is calculating the evolutionary rate ratio (Spielman & Wilke, 2015). In 1994, Goldman and Yang, as well as Muse and Gaut, suggested using a codon substitution model to estimate the ratio of nonsynonymous to synonymous substitution rates (ω) in a maximum likelihood context (Goldman & Yang, 1994; Muse & Gaut, 1994).

By analyzing synonymous and nonsynonymous rates in genes across different evolutionary lineages, we can obtain valuable insights into the underlying mechanisms of molecular evolution.

Such an approach is particularly useful in understanding the distinct effects of mutation and selection on these substitutions (Yang & Nielsen, 1998). When a nucleotide substitution alters the amino acid in a protein, it's known as a nonsynonymous substitution. On the other hand, if a nucleotide substitution doesn't change the amino acid in the protein, it's referred to as a synonymous substitution. According to the neutral theory, purifying selection will remove nonsynonymous substitutions but permit synonymous substitutions, so there will be fewer nonsynonymous substitutions than synonymous substitutions (Choudhuri, 2014).

DN/dS ratio is used to identify protein sequences that are undergoing purifying selection ($dN/dS < 1$), evolving neutrally ($dN/dS \approx 1$), or experiencing positive, diversifying selection ($dN/dS > 1$) by demonstrating how rapidly the amino acids in a protein are evolving in comparison to synonymous changes (Spielman & Wilke, 2015). Over the past two decades, dN/dS-based models have made significant progress and boast impressive sophistication (Spielman & Wilke, 2015). These models are a popular choice for analysis due to their adaptability and easy software implementation. They can also accommodate various evolutionary scenarios, including synonymous rate variation (meaning changes in DNA sequences that code for amino acids in a protein sequence but do not change the encoded amino acid) and episodic and/or lineage-specific selection (Spielman & Wilke, 2015).

Episodic selection is a term used to describe sudden environmental disruptions that can significantly impact a species' population structure. These disruptions may include alterations in geography, substrate availability, exposure to new hosts or vectors, the effects of climate change, and pollution stress (Brasier, 1995). Lineage selection happens when one biological lineage's frequency changes compared to another. This type of selection is a generalization of natural selection individually. Favorable alleles, equivalent to favored lineages in natural selection, play a crucial role in this process; lineage and individual-based selections are equivalent for alleles with simple positive or negative fitness effects (Akçay & Van Cleve, 2016).

1.16: Aim of the study

The hypothalamus is a critical component of human physiology, regulating essential bodily processes, behaviors, and cognitive functions. This structure plays a crucial role in maintaining overall health and wellness by controlling the intake of food and electrolytes, maintaining energy balance, regulating body temperature, managing sleep-wake cycles, responding to stress, facilitating reproductive and parenting behaviors, sexual behavior, and controlling automatic functions. The release of hormones and neuropeptides by distinct neuronal populations helps to modulate these processes, making the hypothalamus an essential mechanism that is conserved across many vertebrate species (Benevento et al., 2022).

Recent advances in neuroscience have led to significant progress in our understanding of the nervous system. Scientists are now able to examine the relationship between genes and their expression and determine a gene's level of evolutionary selection through the dN/dS ratio. This approach allows for a comparative analysis of ratios across various species, enabling us to gain valuable insights into the positive and negative changes taking place within the nervous system (Miller et al., 2019).

The dN/dS ratio (ω) is widely used to measure selection pressure in protein-coding genes. It compares the number of nonsynonymous substitutions to synonymous substitutions in a gene. This ratio is valuable in understanding gene selection trends in various organisms, from viruses and bacteria to large eukaryotic genomes. An ω value greater than 1 indicates positive selection, an ω value equal to 1 indicates neutral evolution, and an ω value less than 1 shows negative selection. Characterizing gene sets, ω can help explain genome function and measure selection pressure and adaptations throughout evolutionary history (Kaczanowska et al., 2022).

Multiple studies have delved into the development and function of hypothalamic neuropeptides. An intriguing aspect is the varying rates at which these neuropeptides develop in different species and how they are associated with behavior and cognition. Despite the dissimilarities among species, significant genetic similarities exist that have persisted over millions of years This research analyzed the evolution rates of 13 hypothalamic neuropeptides and their receptor genes across eight species. The analysis of sequence data provided by Dumas et al. (2021) was used to determine the dN/dS ratio for each hormone. Furthermore, an investigation was conducted into the potential impact of these hormone alterations on behavior and cognition.

2: Materials and Methods

2.1: Gene Selection for Hypothalamic Hormone Receptors:

The hypothalamus facilitates the control of processes essential to an organism's or a species' survival, such as eating, sleeping, regulating body temperature, and responding to stress. Hypothalamic structure and function are exceptionally well preserved in all vertebrate species. This research aims to map the evolution of hypothalamic neuropeptides in human and non-human primate species. Table 2 shows 14 selected hormones and 24 of their receptors included in our study. The following is a description of all of the data that was used:

The following is a description of all of the data that was used:

i) Oxytocin

- ***OXTR:*** The peptide hormone oxytocin acts on the centrally expressed oxytocin receptor (*OXTR*), which regulates numerous species' social and reproductive behaviors. During labor, the uterus relies heavily on the oxytocin-oxytocin receptor system. The human oxytocin receptor is 389 amino acids long and has seven transmembrane domains, making it a G-protein-coupled receptor (Waltenspühl et al., 2020).

ii) **Vasopressin**

- ***AVPR1***: This gene produces a protein that serves as an arginine vasopressin receptor. This receptor is a member of the G-protein-coupled receptor subfamily containing the *AVPR1B, AVPR2*, and *OXT* receptors. The receptor controls the release of coagulation factors, platelet aggregation, cell proliferation, and glycogenolysis (Hernández-Pérez et al., 2018).
- ***AVPR2***: The vasopressin receptor type 2, or V2 receptor, is part of the seven-transmembrane-domain G protein-coupled receptor (*GPCR*) superfamily. The pituitary hormone arginine vasopressin (*AVP*) stimulates the *V2* receptor in the kidney tubule, mostly in the distal convoluted tubule and collecting ducts, to concentrate urine and maintain water homeostasis. Despite its tissue location, the

V2 receptor is expressed outside the kidney. In fetal lung tissue and lung cancer, alternative splicing affects gene expression (Holmes et al., 2003).

iii) **Corticotropin-releasing hormone**

- ***CRHR1***: This gene codes for a G-protein-coupled receptor that binds to neuropeptides from the corticotropin-releasing hormone family. These neuropeptides are important regulators of the hypothalamic-pituitary-adrenal system. The encoded protein is crucial for activating signal transduction pathways that control several physiological functions, such as stress, reproduction, immunological response, and obesity (Hernandez-Diaz et al., 2021; Sannes et al., 2021).
- ***CRHR2***: This gene produces a protein member of the corticotropin-releasing hormone receptor subfamily and the G-protein-coupled receptor 2 family. The hypothalamus produces *CRH*, which is crucial for coordinating the endocrine, autonomic, and behavioral reactions to stress and immunological assault (Amin et al., 2022) (Bruce et al., 2022).

iv) **Dynorphin**

- ***OPRK1***: The genetic sequence under consideration encodes for an opioid receptor belonging to the class of G protein-coupled receptors with seven transmembrane domains. The entity in question functions as a receptor for endogenous ligands and a diverse range of exogenous opioids. The opioid receptor is responsible for mediating the analgesic, hypo locomotor, and negative effects of synthetic opioids and the perception of pain. Genetic variations have been associated with increased susceptibility to alcoholism and opiate addiction (Guerrero et al., 2014; Lee et al., 2022).

v) **Beta-endorphin**

- ***OPRM1:*** This gene encodes the mu-opioid receptor (*MOR*) and is one of at least three different types of opioid receptors found in humans. Beta-endorphin and enkephalin are two examples of endogenous opioid peptides and opioid

analgesics that predominantly target the *MOR*. The *MOR* has a crucial role in dependency on nicotine, cocaine, and alcohol through its modulation of the dopamine system (Tour et al., 2022).

vi) **Gonadotropin-releasing hormone**

- ***GNRHR1:*** This gene is responsible for producing the type 1 gonadotropin-releasing hormone receptor. This receptor belongs to a family of seven transmembrane G-protein-coupled receptors (*GPCRs*). It can be found on the surfaces of various tissues, such as pituitary gonadotrope cells, lymphocytes, the breast, the ovary, and the prostate. When activated, the receptor releases gonadotropic luteinizing hormone (*LH*) and follicle-stimulating hormone (*FSH*) (Ciaramella et al., 2015; Li et al., 2022).
- ***GNRHR2:*** The gonadotropin-releasing hormone 2 receptor gene (*GnRHR2*) encodes a seven-transmembrane G-protein-coupled receptor. The prevalence of *GnRH-II* is significantly higher in extracerebral regions compared to *GnRH-I*. It is particularly observed in the prostate, bone marrow, and kidney (Cheng & Leung, 2005).

vii) **Orexin/hypocretin**

The orexin system, composed of two G protein-coupled receptors known as *OX1* and *OX2*, has been extensively studied over the past two decades. These receptors control behavioral arousal, sleep, and alertness because the neuropeptides *OX-A* and *OX-B* activate them (Scammell & Winrow, 2011).

- ***HCRT1***: The *HCRT1* gene produces a G-protein-coupled receptor that plays a role in regulating food intake. This receptor specifically targets the neuropeptide Orexin A, which is produced in the hypothalamus. Another gene called *HCRTR2* also encodes a G-protein-coupled receptor that can bind to both Orexin A and Orexin B (Thompson et al., 2014).
- ***HCRTR2***: This gene is responsible for encoding a G-protein-coupled receptor that is believed to play a role in regulating food consumption. The protein it produces

is able to identify and attach to two neuropeptides, orexin A and orexin B, which are found in the hypothalamus (Wang et al., 2018).

vii) **Kisspeptin**

- ***KISS1***: The metastasis suppressor gene *KISS1* generates a protein from this gene that serves as a G protein-coupled receptor that binds to the peptide metastin. The fact that this gene appears to be involved in the start of puberty supports the hypothesis that it controls endocrine function based on the tissue distribution of the expressed gene. Central precocious puberty and hypogonadotropic hypogonadism have both been linked to mutations in this gene (Gottsch et al., 2009; Smith et al., 2005).

viii) **Neuropeptide Y**

- ***NPY1R***: This gene is a member of the superfamily of G-protein-coupled receptors. The *Y1* receptor, which comprises 384 amino acids, is primarily activated by *NPY* and the peptide *YY (PYY)*. Along with the hypothalamus, hippocampus, neocortex, and thalamus, the receptor is also expressed in adipose tissue, blood vessels, the colon, kidneys, adrenal glands, hearts, and the placenta. It controls hunger, blood pressure, heart rate, anxiety, and bone homeostasis (Bhat et al., 2022; Yang et al., 2018).

- ***NPY2R***: This gene plays a role in developing the heart's left ventricle and outflow tract. It is believed to improve the functions of the neuropeptide *Y* receptor and calcium channel regulator. However, it has also been associated with medical conditions such as obesity, morbid obesity, and Huntington's disease (Treutlein et al., 2017) (Mittapalli & Roberts, 2014).
- ***NPY4R:*** The protein in question is comprised of a sequence of 375 amino acids. It is primarily detected in the gastrointestinal system, central nervous system, endocrine pancreas, and prostate gland. The control of food intake, circadian rhythms, energy balance, and the stimulation of *LH* release all influence the aforementioned physiological processes (Pedragosa-Badia et al., 2013).

- ***NPY5R***: This gene produces a protein that is a receptor for the neuropeptide *Y* and peptide *YY*. Defects in this gene are linked to eating disorders, and the encoded protein appears vital in controlling food intake. The *Y5* receptor may regulate circadian rhythm and spermatogenesis by inhibiting *LH* secretion (Clark et al., 2018; Raposinho et al., 2004).

viii) **Alpha-melanocyte stimulating hormone**

- ***MC1R*:** This gene produces the *MSH* receptors, which stand for melanocyte-stimulating hormone. The protein produced by this gene functions as a G-protein-coupled receptor that spans seven transmembrane domains and controls melanogenesis. There are red and black forms of melanin. Mutations in genes with lost functions increase the production of pheomelanin, which lightens the skin and hair. A malfunction in the *MC1R* causes melanoma and other forms of skin cancer (Cheng et al., 2007; Gantz et al., 1994).
- ***MC4R***: The protein that is the product of this gene is a melanocortin receptor, which means it is a membrane-bound receptor. The melanocortin-4 receptor (*MC4R*) is an important therapeutic target for syndromic obesity because of its role in energy balance. This gene has been linked to autosomal dominant obesity due to mutations (Kim et al., 2000; Lasaga et al., 2008).

ix) **Thyrotropin-releasing hormone**

- ***TRHR***: This gene codes for a G protein-coupled receptor for thyrotropin-releasing hormone, generally known as *TRH*. It stimulates the release of thyroid-stimulating hormone (*TSH*) and prolactin (*PRL*). Two distinct subtypes of *TRH* receptors, namely *TRH1* and *TRH2*, have been identified in rats. However, in humans, only the *TRH1* subtype has been detected (Jackson, 1982; Xu et al., 2022; Yang et al., 2022).

x) **Dopamine**

- ***DRD1***: This gene is responsible for encoding dopamine receptor type 1 (*D1*). The majority of the brain's dopamine receptors are of the *D1* subtype. Dopamine *D1* receptors have a role in neural development, mediate various behavioral responses, and control the actions of *D2* dopamine receptors (Kern et al., 2015).
- ***DRD2***: The G-protein-coupled receptor can facilitate the inhibition of adenylyl cyclase function. The missense mutation of this gene has been identified as the cause of myoclonus dystonia, while other mutations in the same gene have been linked to schizophrenia (Jocham et al., 2009).
- ***DRD3***: Receptors of the *D3* subtype mediate their activity through G proteins that block adenylyl cyclase. The limbic system, which regulates thinking, feeling, and hormone production, is where this receptor is found in the brain (Le Foll et al., 2005).
- ***DRD4*****:** The G-protein-coupled receptor of the *D4* subtype prevents adenylyl cyclase from being produced. It is a target for medications that treat Parkinson's disease and schizophrenia. Attention deficit/hyperactivity disorder, autonomic nervous system dysfunction, and the personality attribute of novelty seeking have all been linked to mutations in this gene (Rowe et al., 1998) (Munafò et al., 2008).
- ***DRD5***: Neurons in the limbic areas of the brain express this receptor. Compared to the *D1* subtype, it has a ten-fold greater affinity for dopamine. On chromosomes 1 and 2, this gene's pseudogenes are located (Müller et al., 2012; Pornour et al., 2015).

xi) **Prolactin**

- ***PRLR***: The prolactin receptor encoded by this gene is a type I cytokine receptor family member. It is a receptor for the anterior pituitary hormone. This gene's alternatively spliced transcripts have been characterized as encoding various membrane-bound and soluble isoforms that may be used to control the endocrine and autocrine effects of prolactin in healthy tissue and malignancy (Wilkanowska et al., 2014).

Table 2.

The origin, structure, and receptor gene for the selected hormones are shown in this table.

Selected Hormones	**Receptors Gene**	**Region of production**
Oxytocin	OXTR	Hypothalamus, Posterior pituitary gland
Vasopressin	AVPR1A AVPR2	Hypothalamus, posterior pituitary gland
Corticotropin-releasing hormone	CRHR1 CRHR2	Hypothalamus, Also synthesized in Peripheral tissues, such as T lymphocytes and Placenta
Dynorphin	μ-opioid receptor (MOR) in brain and mu- and kappa-receptors (KOR) in spinal cord	Hypothalamus, the Striatum, the Hippocampus, and the Spinal cord
Beta-endorphin	μ-opioid receptor (MOR)	Hypothalamus, Pituitary gland
Gonadotropin-releasing hormone	GNRHR1 GNRHR2	Hypothalamus, Anterior pituitary
Orexin/hypocretin	HCRTR1 HCRTR2	Lateral Hypothalamic nucleus
Kisspeptin	KISS1R	Hypothalamus and Hippocampal dentate gyrus
Neuropeptide Y	NPY1R NPY2R NPY4R NPY5R	Hypothalamus, Arcuate nucleus (ARC)
Alpha-melanocyte stimulating hormone	MC4R, MC1R	Hypothalamus, Anterior lobe of the pituitary gland and skin cells
Thyrotropin-releasing hormone	TRHR1	Paraventricular nucleus of the hypothalamus
Prolactin	PRLR	Hypothalamus, Anterior pituitary gland
Dopamine	DRD1, DRD2, DRD3, DRD4, DRD5	Hypothalamus, Substantia nigra, Ventral tegmental area

M. *mulatta*, and C. *jacchus*) as references. The dN/dS ratios were computed for each of these alignments, and orthologous protein-coding genes or *DNA* sequences were found.

Data on gene variability across primate evolution, including the human lineage, is made available via this application. The outcomes are provided for the gene's most extended consensus coding sequence (*CCDS*). A collection of linked and aligned *DNA, RNA*, or protein sequences is known as a consensus sequence. The consensus sequence among related sequences is determined by the nucleotide(s) or amino acid residue(s) that are most often encountered at each place. There are several analysis options that can be selected regarding the gene level quality of coverage: Low (genes having a *CDS* coverage of more than 20%), medium (genes that span more than 50% of the *CDS*), or high (genes covering more than 80% of the *CDS*). There are also several analysis options that can be selected regarding allele frequency (i.e., the proportion of rare to common alleles is determined for a variety of different single nucleotide polymorphisms allele frequencies): *MAF* < 1% (present in fewer than one percent of the population), *MAF* < 5% (less than 5% of the population carries the mutation), or all that shows all allele frequencies and no filter applied (https://github.com/GHFC/genevo/wiki/Search-form).

The ratio of dN/dS for each gene is plotted in dN/dS by taxon. Eight different species including *Altai* (Altai Neandertal), *Homo sapiens* (modern human), *Denisovan, Callithrix jacchus* (common marmoset), *Macaca Mulatta* (rhesus macaque), *Gorilla gorilla* (western gorilla), *Pongo abelii* (Sumatran orangutan), and *Pan troglodytes* (common chimpanzee) can be compared using this graph. An interesting figure shows the left hemisphere of the human brain. The illustrated brain map (as seen in Figure 1) illustrates the regional uniqueness of gene expression. A chromatic scale is used to represent expression levels, with red denoting strong expression, green denoting low expression, and black denoting expression that is near the mean. The color scale used to depict specificity runs from yellow (signifying that the gene is particularly expressed in the area) to blue (showing no particular specificity), with the absence of specificity being signified by the color black and a value of zero (https://genevo.pasteur.fr/)

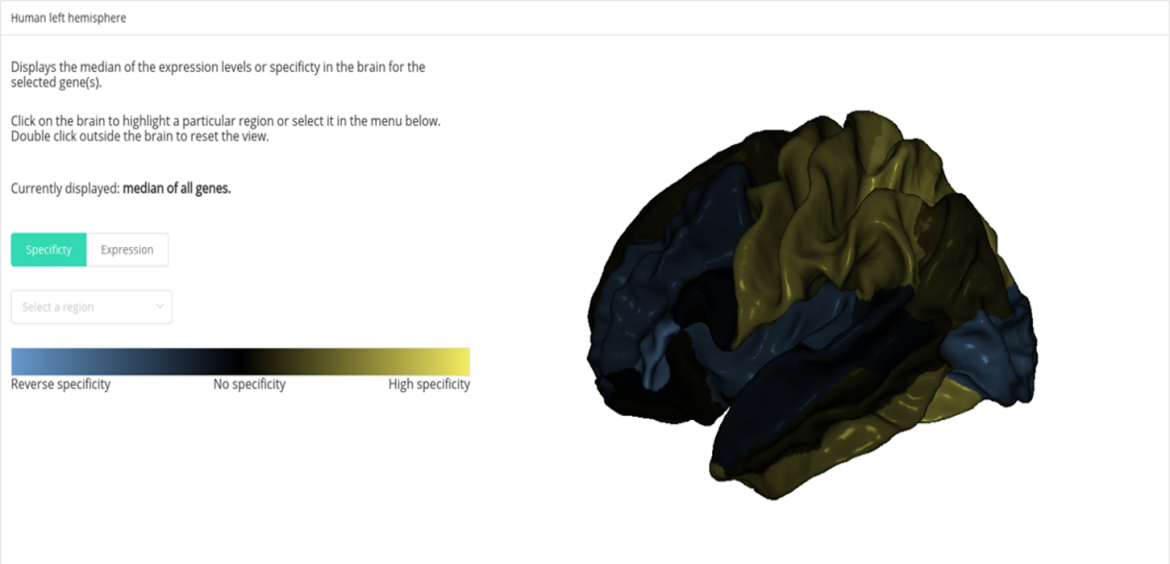

Figure 1. Human left hemisphere graph

2.3: Analyzing the dN/dS ratio

The dN/dS ratio denoted as "ω" is a well-known and reliable measure for estimating the level of selection pressure applied to sections of proteins that encode genetic information. This evaluation is based on Yang and Bielawski's (2000) dN/dS ratio formula. The mentioned ratio is a metric for quantifying the degree of selective pressure exerted on regions of the *DNA* or nucleotide sequences. This analysis compares the frequency of synonymous and nonsynonymous mutations in protein-coding nucleotide sequences. A ratio greater than one indicates positive or Darwinian selection; a ratio less than one implies purifying selection; and a ratio of one suggests neutral selection. In this study, the dN/dS ratios for the evolution of hypothalamic receptor genes in *Homo sapiens*, two archaic humans (*Altai* and *Denisovan)*, and five primate species (Including *Callithrix jacchus, troglodytes, Gorilla gorilla, Pongo abelii and Macaca mulatta*) was gathered using the GenEvo tool that was established and supplied to the user by Dumas and colleagues (Dumas et al., 2021).

To ensure the accuracy of the analysis, a meticulous selection process implemented that exclusively considered 1-to-1 orthologs and exact matches. As a result, 11 genes eliminated from the investigation, ultimately leaving us with 13 that warranted further examination. Additionally,

all relevant allele frequencies was considered to facilitate a comprehensive analysis. The primary data was obtained using the approach implemented by Dumas and colleagues in 2021, which involved utilizing a medium-quality coverage of diverse primate genomes. This approach only included genes that met specific quality requirements, such as at least 50% of their sequence being incorporated into the consensus coding sequence assembly. Consequently, certain coding regions may have been excluded from our analysis.

The ggtree R package, created by Yu et al. in 2017, was used to produce a condensed and easy-to-understand version of the phylogenetic tree. This package is particularly effective at graphically presenting tree-related data within an evolutionary framework. It can also annotate and visualize the tree with relevant information and variables, making it a valuable tool for researchers. For further details regarding this package, please refer to: https://github.com/YuLab-SMU/ggtree.

Table 3.

presents the dN/dS ratio, as provided by GenEvo, for each gene of selection across eight species. Each gene's corresponding ratio is listed alongside its name. A ratio below one indicates negative selection, whereas a ratio above one indicates positive selection. A ratio of one denotes neutrality.

Gene	dN/dS - Homo sapiens	dN/dS - Altaï	dN/dS - Denisovan	dN/dS - Pan troglodytes	dN/dS - Gorilla gorilla	dN/dS - Pongo abelii	dN/dS - Macaca mulatta	dN/dS - Callithrix jacchus
AVPR1A	0.146	0.219	0.223	0.136	0.072	0.074	0.121	0.117
CRHR1	0.101	0.101	0.200	0.203	0.039	0.014	0.039	0.056
DRD1	0.344	0.339	0.325	0.345	0.067	0.115	0.048	0.053
DRD2	0.182	0.182	0.188	0.092	0.036	0.038	0.027	0.052
DRD3	0.081	0.163	0.108	0.065	0.075	0.159	0.234	0.091
DRD4	0.126	0.126		0.290				
HCRTR1	0.062	0.125	0.064	1.443	0.061	0.059	0.116	
HCRTR2	0.201	0.200	0.203	0.809	0.404	0.166	0.087	0.606
MC4R	0.391	0.784	0.784	0.391	0.170	0.097	0.180	0.174
NPY2R	0.122	0.122	0.122	0.122	0.122	0.122	0.122	0.122
NPY5R	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350
OXTR	0.050	0.096	0.119	0.132	0.029	0.067	0.050	0.060
PRLR	0.209	0.208	0.209	0.138	0.422	0.218		0.682

3: Results

3.1: Analyzing the natural selection patterns among various species

To study the development of the 14 hypothalamic neuropeptides observed in the primate lineage, the GenEvo program and the underlying algorithm created by Dumas and colleagues (Dumas et al., 2021) were used. This provided a glimpse into the evolutionary history of these neuropeptides. While selection patterns in 13 receptor genes and their orthologs were identified (i.e., the "same" gene in several species), 11 genes had to be removed since no 1-to-1 orthologs existed. Then a comparison of the dN/dS ratio across eight species, *including the pan troglodytes, Callithrix jacchus, Gorilla gorilla, Pongo abelii, Macaca mulatta, Denisovans, Altai, and Homo sapiens*, was conducted. When the ratio of dN to dS equals 1, there is no selective pressure on the protein

sequence, allowing for neutral, nonsynonymous mutations. However, when the dN/dS ratio is above 1, it indicates changes in the protein sequence's function or the adaptive quality of nonsynonymous mutations, known as Darwinian selection (Kryazhimskiy & Plotkin, 2008). On the other hand, if the ratio of nonsynonymous to synonymous nucleotide substitutions, dN/dS, is less than 1, it suggests that there are functional constraints on the protein sequence, making nonsynonymous mutations harmful (Wolf et al., 2009). By using the ggtree R package that Yu et al. provided in 2017 phylogenetic tree was clearly and informally presented. This package proved a handy tool, enabling us to efficiently visualize and annotate phylogenetic trees with pertinent data and variables. For further details on this package, please visit the URL: (https://github.com/YuLab-SMU/ggtree). Figure 2 illustrates the results; it has been found that most of these genes underwent negative selection across all eight primate species compared to their common primate ancestor. Specifically, the gene group's median value was 0.14, with the bootstrap confidence interval ranging from 0.18 to 0.40. This leads me to conclude that these hypothalamic hormone genes were conserved during the evolution of modern primates ranging from 0.18 to 0.40. This suggest that these hypothalamic hormone genes were conserved during the evolution of modern primates.

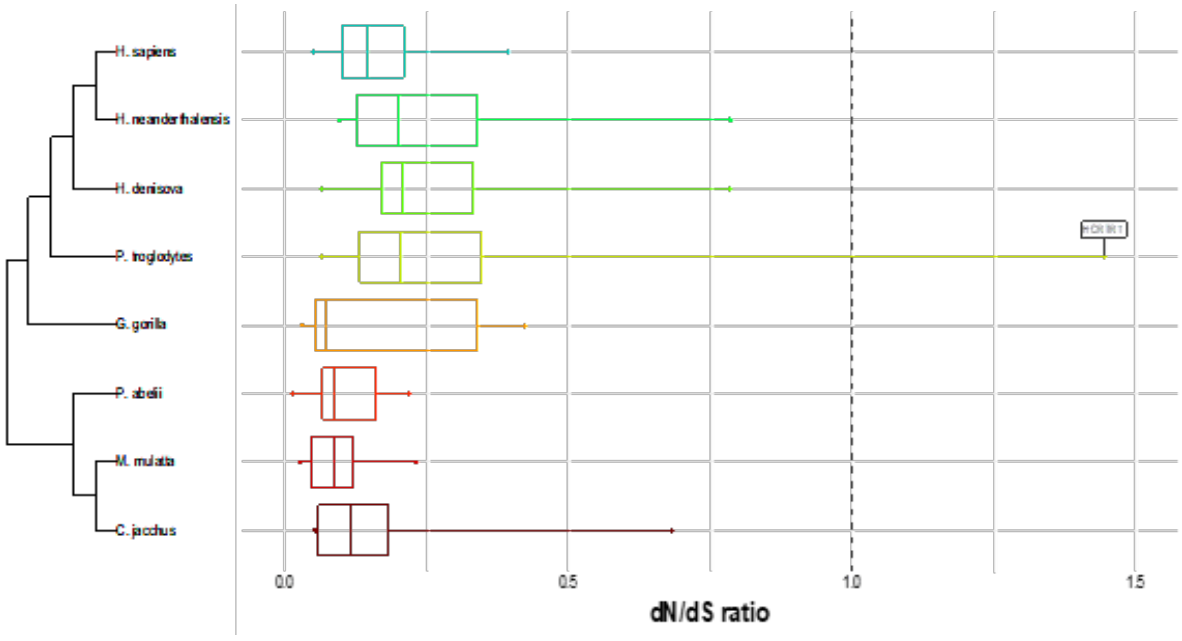

Figure 2. Displaying the dN/dS ratios for specific genes of interest observed across eight distinct species.

According to the data presented in Figure 2, it can be observed that among the *Homo sapiens* genes analyzed*, MC4R* and *DRD1* exhibit the highest dN/dS ratios, with rates of 0.39 and 0.34, respectively. Conversely, *DRD4* has the lowest ratio in this species, with a value of 0.12. It is essential to acknowledge that the specific genes under examination exhibit ratios below one, a clear indication of having undergone purifying selection That is how harmful genetic variants (alleles) are removed from a population.The results of the data analysis indicate that *Altaii* displays the highest ratio for the *MC4R* gene and the lowest ratio for the *OXTR* gene, with scores of 0.78 and 0.09, respectively. In addition, the *DRD4* and *HCRTR1* genes have a similar ω value of 0.12. Figure 2 shows a slight deviation towards a value of 1 for *Altaii, Pan troglodytes*, and *Denisovan*, with corresponding values of 0.78, 0.80, and 0.78. Notably, there is no available data for the dN/dS ratio of *DRD4* in *Denisovan, Gorilla gorilla, Pongo abelii, Macca mulata*, and *Callithrix jacchus*. The fact that ω is less than 1 for all other genes supports negative selection.

It is worth noting that neuropeptide Y receptors, *NPY2R* and *NPY5R*, in all of the selected species have remained unchanged throughout evolution that suggests a significant level of conservation across various species. Figure 2 also shows that among all species, only Pan troglodytes have a dN/dS ratio greater than 1 in the *HCRTR1* protein sequence, precisely at 1.44. This indicates that mutations in the protein sequence that alter its function benefit this species. Furthermore, my observations reveal that the *HCRTR2* gene has the highest ratio at 0.8, coming in second place. However, *DRD3* and *DRD2* have the lowest ratios at 0.06 and 0.09, respectively. All genes, except for *HCRTR1*, have a ratio less than 1, indicating an adverse selection effect on the hypothalamic receptor genes in Pan troglodytes. The genes *AVPR1, OXTR*, and *PRLR* had the lowest change observed after *DRD2*, with a value of 0.12. The gene set of *Gorilla gorilla* shows that seven genes have a ratio below 0.1, with *OXTR* having the lowest quantity at 0.02 and *AVPR1* having a ratio of 0.07. *PRLR* and *HCRTR1* have levels of 0.4, which makes them practically identical. Meanwhile, *NPY2R* and *NPY5R* have values of 0.3, placing them in second place.

With a ratio of 0.20 in *DRD2* and *DRD3* and 0.03 in *CRHR1, Macaca mulatta* also has a low ratio. The study found that negative selection has an impact on the selected genes in *Pongo abelii*. The small and narrow ratio, which ranges from 0.01 in *CRHR1* to 0.2 in *PRLR*, with the majority showing 0.1, illustrates this. According to the collected information, GenEvo does not give a ratio

for *PRLR*, and this is analogous to the fact that *Callithrix jacchus* does not provide a ratio for *DRD4* and *HCRTR1.Macaca mulatta* similarly exhibits a small ratio, with a ratio of 0.20 in *DRD2* and *DRD3* and 0.03 in *CRHR1*. The tiny and close ratio, which ranges from o.1 in *CRHR1* to 0.2 in *PRLR*, while most display 0.1, has interestingly demonstrated that the selected genes in *Pongo abelii* is subject to negative selection. As can be seen from the data, GenEvo does not supply us with any ratio for *PRLR*; the same happened for Callithrix Jacchus. In the latter species, the mean ratio was found to be 0.18. The *DRD1*, *DRD2*, and *CRHR1* were similar at 0.05, while *PRLR* and *HCRTR2* had a ratio of 0.6. our understanding of behavior and cognition and will be further explored in the next chapter.

4: Discussion

The hypothalamus produces and secretes hormones and neuropeptides that play a crucial role in various physiological processes (Shafer et al., 2022). This region is highly conserved across species and regulates functions such as appetite, hormone secretion, stress response, body temperature, sexual behavior, eating behavior, and movement initiation. Recent research has suggested that the hypothalamus may also serve as an interface for diverse cognitive and behavior functions (Aitta-Aho et al., 2016; Burdakov & Peleg-Raibstein, 2020; Kosse & Burdakov, 2019; Petrovich, 2018). The hypothalamic neuropeptides modulate motivated behaviors to acquire natural reinforces like food and water to maintain homeostasis. These neuropeptides can function within the hypothalamus or extend to other regions to engage with other systems that regulate behavior and physiology (Thiele, 2017). Overall, the hypothalamus is a crucial component of the brain that plays a vital role in regulating various physiological and cognitive processes, making it an essential area of study in neuroscience. Additionally, understanding the role of the hypothalamus in regulating mood and emotions could lead to new treatments for depression and anxiety disorders. The present study analyzed the dN/dS ratio in eight primate species to gain insights into the evolutionary history and selection patterns of 14 hypothalamic neuropeptides and 13 receptor genes. Notably, 11 genes were removed from analysis due to the lack of 1-to-1 orthologs, underscoring the importance of conducting more extensive research in this area.

In terms of evolution, genes that are essential for an organism's survival are more likely to be passed down over time. This means that organisms with these genes have a better chance of living and reproducing, passing on these beneficial genes to their offspring. As a result, these genes become more prevalent in the population, leading to the development of new characteristics and adaptations, ultimately contributing to the variety of life on earth. This process is called natural selection and is a basic principle of evolutionary biology. Studies conducted by Ish-Am et al. and Bergmiller et al. (Bergmiller et al., 2012; Ish-Am et al., 2015) have further supported this concept by examining the genetic composition of different organisms. They discovered that genes that are crucial for vital biological processes are more commonly found across species than those that are less important.

According to this study, natural selection played a big part in the evolution of neuropeptides in eight different species. The research found that most of the neuropeptides studied, except for *HCRTR1* in common chimpanzee (*Pan troglodytes*), had a dN/dS ratio of less than 1, meaning they were subject to adverse selection. This suggests that these neuropeptides are essential for biological functions and have been conserved throughout the evolutionary process. Also, It appears that the majority of genes related to hypothalamic neuropeptide function have remained largely unchanged throughout primate evolutionary history. This suggests a strong level of conservation, even though these genes are known to impact cognition and behavior.

This finding provides insight into the intricate mechanisms of natural selection and the intricate development of primate species. Among the species investigated, it was found that only Common chimpanzee exhibited a dN/dS ratio greater than one for the protein sequence of *HCRTR1*. *HCRTR1* is a G protein-coupled receptor that binds to orexins and exerts an impact on the body. Orexins, or hypocretins, are neuropeptides present in the hypothalamus that contribute to various physiological functions, including the regulation of sleep and wakefulness, food intake, and coordination of the stress response (Pulver et al., 2020). The orexin system is crucial for mammalian survival, as it keeps them alert during activities that require heightened attention, such as avoiding danger or searching for food. Hence, orexins play a crucial role in maintaining healthy sleep patterns and regulating essential life-sustaining behaviors (Yamanaka, 2003).

The dN/dS ratio of 1.44 that observed in the *HCRTR1* protein sequence of chimpanzees suggests that positive selection may have occurred in this species, potentially related to its unique behaviors

and physiological responses. This may be associated with their sleep patterns, as *HCRTR1* regulates sleep and wakefulness and Studies in knockout animals indicate that both *HcrtR1* and *HcrtR2* signaling affect sleep/wake stability (Mieda et al., 2011).This implies that *HCRTR1* may play a critical and distinct role in the physiology or behavior of chimpanzee*s* compared to other primates. The survival and health of all living organisms largely depend on their genes. Understanding the positive selection of this gene in chimpanzees is worth examining if unique environmental or social factors affect them. Further studies are necessary to comprehend the function of *HCRTR1* in chimpanzee physiology and behavior.

Studies have suggested that human sleep patterns have evolved from those of our primate ancestors, as noted by Coolidge and Wynn in 2006 (Coolidge & Wynn, 2006). One example is the neurological disorder called narcolepsy, which affects how we regulate sleep and wakefulness and has been linked to orexin system (Nepovimova et al., 2019). Narcolepsy is a rare brain disorder that reflects a selective loss or dysfunction of orexin (also known as hypocretin) neurons of the lateral hypothalamus. Narcolepsy type 1 is characterized by excessive daytime sleepiness and cataplexy, accompanied by sleep–wake symptoms, such as hallucinations, sleep paralysis and disturbed sleep (Bassetti et al., 2019). Studies suggest that both orexin receptors, *HcrtR1* and *HcrtR2*, play a role in generating narcolepsy symptoms, with *HcrtR2* possibly being more involved. It's important not to overlook the contribution of *HcrtR1* to the narcolepsy phenotype, as the two receptors may have some overlapping or reciprocal functions (Tisdale et al., 2021).

Researching the genetic causes of sleep disorders in chimpanzees may lead to improved treatments for both humans and chimpanzees suffering from similar conditions. It is worth noting that only *HCRTR2* agonists have been tested in clinical trials thus far. There has been no investigation into the use of *HCRTR1* agonists for treating excessive daytime sleepiness (*EDS*), a complex symptom characterized by a strong desire to sleep during the day along with issues such as attention deficits, anxiety, and reduced cognitive function (Justinussen et al., 2022).

Additionally, studying the genetic differences between chimpanzees and humans can provide valuable insights into how sleep regulation has evolved and its impact on cognitive function as getting enough sleep is crucial for our cognitive function and overall health. Various experiments have shown that sleep is essential for working memory, decision-making, attention, and visual-

motor performance (Dinges et al., 1997; Durmer & Dinges, 2005). However, it's essential to note that this study represents only a small step towards understanding the complex evolution of species' chimpanzee behavior and cognition. To gain a deeper understanding, further research is required to investigate the evolutionary function of neuropeptides in other parts of the brain and explore a broader range of species. For example, the higher dN/dS ratio in chimpanzees could also be attributed to genetic drift or other non-adaptive processes rather than positive selection which could be studied in future research. Another possible avenue of investigation could be to examine the expression patterns of *HCRTR1* in different tissues and under different conditions in chimpanzees and other primates. This could shed light on the gene's potential physiological or behavioral roles selection, in chimpanzees and help explain why positive selection has acted on it. Another approach could be to use genetic engineering techniques to introduce specific mutations into the *HCRTR1* gene and observe the resulting changes in function. Overall, the discovery of positive selection acting on the *HCRTR1* gene in chimpanzees highlights the importance of studying the evolution of genes across different species.

5. Conclusion

Recent research found that almost all neuropeptides, except for *HCRTR1* in common chimpanzees, have played a crucial role in biological functions and have remained unchanged throughout evolution. This finding has provided insight into the complex mechanisms of natural selection and the development of primate species. The *HCRTR1* protein sequence in chimpanzees has a dN/dS ratio of 1.44, indicating that positive selection may have occurred due to the unique behaviors and physiological responses associated with it. Further research is necessary to investigate the evolutionary function of neuropeptides in other brain regions and a more comprehensive range of species to better understand their behavior and cognition. It is feasible to use genetic engineering techniques to introduce specific mutations into the *HCRTR1* gene and observe the resulting changes in function.

References

Aitta-Aho, T., Pappa, E., Burdakov, D., & Apergis-Schoute, J. (2016). Cellular activation of hypothalamic hypocretin/orexin neurons facilitates short-term spatial memory in mice. *Neurobiology of learning and memory*, *136*, 183-188.

Akçay, E., & Van Cleve, J. (2016). There is no fitness but fitness, and the lineage is its bearer. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1687), 20150085.

Alvarez-Salas, E., García-Luna, C., Soberanes-Chávez, P., & de Gortari, P. (2022). Role of the thyrotropin-releasing hormone of the limbic system in mood and eating regulation. *Journal of Integrative Neuroscience*, *21*(2), 47.

Amin, M., Ott, J., Gordon, D., Wu, R., Postolache, T. T., Vergare, M., & Gragnoli, C. (2022). Comorbidity of Novel CRHR2 Gene Variants in Type 2 Diabetes and Depression. *International journal of molecular sciences*, *23*(17), 9819.

Andrews, M. M. (2019). *Physical and computational methods of investigating the relationship between stress, cognition, and behavior in the context of the KOR-dynorphin system*

Arakawa, H., & Higuchi, Y. (2022). Exocrine scent marking: coordinative role of arginine vasopressin in the systemic regulation of social signaling behaviors. *Neuroscience & Biobehavioral Reviews*, 104597.

Ayano, G. (2016). Dopamine: receptors, functions, synthesis, pathways, locations and mental disorders: review of literatures. *J Ment Disord Treat*, *2*(120), 2.

Barson, J. R., Morganstern, I., & Leibowitz, S. F. (2013). Complementary roles of orexin and melanin-concentrating hormone in feeding behavior. *International Journal of Endocrinology*, *2013*.

Bassetti, C. L., Adamantidis, A., Burdakov, D., Han, F., Gay, S., Kallweit, U., Khatami, R., Koning, F., Kornum, B. R., & Lammers, G. J. (2019). Narcolepsy—clinical spectrum, aetiopathophysiology, diagnosis and treatment. *Nature reviews Neurology*, *15*(9), 519-539.

Ben-Jonathan, N., & Hnasko, R. (2001). Dopamine as a prolactin (PRL) inhibitor. *Endocrine reviews*, *22*(6), 724-763.

Benevento, M., Hökfelt, T., & Harkany, T. (2022). Ontogenetic rules for the molecular diversification of hypothalamic neurons. *Nature reviews neuroscience*, *23*(10), 611-627.

Bergen, A., Kokoszka, J., Peterson, R., Long, J., Virkkunen, M., Linnoila, M., & Goldman, D. (1997). μ opioid receptor gene variants: lack of association with alcohol dependence. *Molecular psychiatry*, *2*(6), 490-494.

Bergmiller, T., Ackermann, M., & Silander, O. K. (2012). Patterns of evolutionary conservation of essential genes correlate with their compensability. *PLoS genetics*, *8*(6), e1002803.

Bhat, R., Thangavel, H., Abdulkareem, N. M., Vasaikar, S., De Angelis, C., Bae, L., Cataldo, M. L., Nanda, S., Fu, X., & Zhang, B. (2022). NPY1R exerts inhibitory action on estradiol-stimulated growth and predicts endocrine sensitivity and better survival in ER-positive breast cancer. *Scientific Reports*, *12*(1), 1972.

Biran, J., Tahor, M., Wircer, E., & Levkowitz, G. (2015). Role of developmental factors in hypothalamic function. *Frontiers in neuroanatomy*, *9*, 47.

Bouret, S., & Simerly, R. (2006). Developmental programming of hypothalamic feeding circuits. *Clinical genetics*, *70*(4), 295-301.

Brasier, C. (1995). Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. *Canadian Journal of Botany*, *73*(S1), 1213-1221.

Bruce, J. K., Burns, G. L., Soh, W. S., Nair, P. M., Sherwin, S., Fan, K., Dowling, L. R., Goggins, B. J., Koloski, N., & Potter, M. (2022). Defects in NLRP6, autophagy and goblet cell homeostasis are associated with reduced duodenal CRH receptor 2 expression in patients with functional dyspepsia. *Brain, Behavior, and Immunity*, *101*, 335-345.

Burdakov, D., & Peleg-Raibstein, D. (2020). The hypothalamus as a primary coordinator of memory updating. *Physiology & behavior*, *223*, 112988.

Butelman, E. R., Yuferov, V., & Kreek, M. J. (2012). κ-opioid receptor/dynorphin system: genetic and pharmacotherapeutic implications for addiction. *Trends in neurosciences*, *35*(10), 587-596.

Byung, H. D. N. H. O., Hoanga, P. Y. N. M. H., Chungc, W. H. J. H. Y., & Kima, M.-S. (2021). Associations between Prolactin, Diabetes, and Cognitive Impairment: A Literature Review.

Cabrera-Reyes, E. A., Limón-Morales, O., Rivero-Segura, N. A., Camacho-Arroyo, I., & Cerbón, M. (2017). Prolactin function and putative expression in the brain. *Endocrine*, *57*, 199-213.

Cao, Y., Li, Z., Jiang, W., Ling, Y., & Kuang, H. (2019). Reproductive functions of Kisspeptin/KISS1R Systems in the Periphery. *Reproductive Biology and Endocrinology*, *17*, 1-9.

Caruso, A., Gaetano, A., & Scaccianoce, S. (2022). Corticotropin-Releasing Hormone: Biology and Therapeutic Opportunities. *Biology*, *11*(12), 1785.

Cattaneo, S., Verlengia, G., Marino, P., Simonato, M., & Bettegazzi, B. (2021). NPY and gene therapy for epilepsy: how, when,... and Y. *Frontiers in Molecular Neuroscience*, *13*, 608001.

Cheng, C. K., & Leung, P. C. (2005). Molecular biology of gonadotropin-releasing hormone (GnRH)-I, GnRH-II, and their receptors in humans. *Endocrine reviews*, *26*(2), 283-306.

Cheng, Z., Xiong, Z., Subbarayan, M., Chen, X., & Gambhir, S. S. (2007). 64Cu-labeled alpha-melanocyte-stimulating hormone analog for microPET imaging of melanocortin 1 receptor expression. *Bioconjugate chemistry*, *18*(3), 765-772.

[Record #264 is using a reference type undefined in this output style.]

Ciaramella, V., Chianese, R., Pariante, P., Fasano, S., Pierantoni, R., & Meccariello, R. (2015). Expression analysis of Gnrh1 and Gnrhr1 in spermatogenic cells of rat. *International Journal of Endocrinology*, *2015*.

Clark, D. L., McCormick, J. L., & Velleman, S. G. (2018). Effect of incubation temperature on neuropeptide Y and neuropeptide Y receptors in turkey and chicken satellite cells. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *219*, 58-66.

Clark, S. D. (2020). The role of dynorphin and the kappa opioid receptor in schizophrenia and major depressive disorder: a translational approach. *The Kappa Opioid Receptor*, 525-546.

Coolidge, F., & Wynn, T. (2006). The effects of the tree-to-ground sleep transition in the evolution of cognition in early Homo. *Before Farming*.

Daimon, C. M., Chirdon, P., Maudsley, S., & Martin, B. (2013). The role of Thyrotropin Releasing Hormone in aging and neurodegenerative diseases. *American journal of Alzheimer's disease (Columbia, Mo.)*, *1*(1).

De La Cruz, D., & Prokai-Tatrai, K. (2021). Advancing the utility of thyrotropin-releasing hormone (TRH) as a CNS agent.

Deal, C. K., & Volkoff, H. (2021). Effects of thyroxine and propylthiouracil on feeding behavior and the expression of hypothalamic appetite-regulating peptides and thyroid function in goldfish (Carassius auratus). *Peptides*, *142*, 170578.

Dinges, D. F., Pack, F., Williams, K., Gillen, K. A., Powell, J. W., Ott, G. E., Aptowicz, C., & Pack, A. I. (1997). Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4–5 hours per night. *Sleep*, *20*(4), 267-277.

Dumas, G., Malesys, S., & Bourgeron, T. (2021). Systematic detection of brain protein-coding genes under positive selection during primate evolution and their roles in cognition. *Genome Research*, *31*(3), 484-496.

Durmer, J. S., & Dinges, D. F. (2005). Neurocognitive consequences of sleep deprivation. Seminars in neurology,

Elphick, M. R., Mirabeau, O., & Larhammar, D. (2018). Evolution of neuropeptide signalling systems. *Journal of Experimental Biology*, *221*(3), jeb151092.

Gantz, I., Yamada, T., Tashiro, T., Konda, Y., Shimoto, Y., Miwa, H., & Trent, J. M. (1994). Mapping of the gene encoding the melanocortin-1 ([alpha]-melanocyte stimulating hormone) receptor (MC1R) to human chromosome 16q24. 3 by fluorescence in situ hybridization. *Genomics;(United States)*, *19*(2).

Gao, X.-B., & Horvath, T. (2014). Function and dysfunction of hypocretin/orexin: an energetics point of view. *Annual review of neuroscience*, *37*, 101-116.

Goldman, N., & Yang, Z. (1994). A codon-based model of nucleotide substitution for protein-coding DNA sequences. *Molecular biology and evolution*, *11*(5), 725-736.

Gottsch, M. L., Clifton, D. K., & Steiner, R. A. (2009). From KISS1 to kisspeptins: an historical perspective and suggested nomenclature. *Peptides*, *30*(1), 4-9.

Grimaldi, D., Silvani, A., Benarroch, E. E., & Cortelli, P. (2014). Orexin/hypocretin system and autonomic control: new insights and clinical correlations. *Neurology*, *82*(3), 271-278.

Grinevich, V., & Neumann, I. D. (2021). Brain oxytocin: how puzzle stones from animal studies translate into psychiatry. *Molecular psychiatry*, *26*(1), 265-279.

Guerrero, M., Urbano, M., Brown, S. J., Cayanan, C., Ferguson, J., Cameron, M., Devi, L. A., Roberts, E., & Rosen, H. (2014). Optimization and characterization of an opioid kappa receptor (OPRK1) antagonist. *Probe Reports from the NIH Molecular Libraries Program [Internet]*.

Hang, A., Wang, Y.-J., He, L., & Liu, J.-G. (2015). The role of the dynorphin/κ opioid receptor system in anxiety. *Acta Pharmacologica Sinica*, *36*(7), 783-790.

Hernandez-Diaz, Y., Gonzalez-Castro, T. B., Juarez-Rojop, I. E., Tovilla-Zarate, C. A., Lopez-Narvaez, M. L., Genis-Mendoza, A. D., Fresan, A., & Nicolini, H. (2021). The role of rs242941, rs1876828, rs242939 and rs110402 polymorphisms of CRHR1 gene and the depression: systematic review and meta-analysis. *Genes & Genomics*, *43*, 1339-1349.

Hernández-Pérez, O. R., Crespo-Ramírez, M., Cuza-Ferrer, Y., Anias-Calderón, J., Zhang, L., Roldan-Roldan, G., Aguilar-Roblero, R., Borroto-Escuela, D. O., Fuxe, K., & Perez de la Mora, M. (2018). Differential activation of arginine-vasopressin receptor subtypes in the amygdaloid modulation of anxiety in the rat by arginine-vasopressin. *Psychopharmacology*, *235*, 1015-1027.

Herraiz, C., Martínez-Vicente, I., & Maresca, V. (2021). The α-melanocyte-stimulating hormone/melanocortin-1 receptor interaction: A driver of pleiotropic effects beyond pigmentation. *Pigment cell & melanoma research*, *34*(4), 748-761.

Heyder, N. A., Kleinau, G., Speck, D., Schmidt, A., Paisdzior, S., Szczepek, M., Bauer, B., Koch, A., Gallandi, M., & Kwiatkowski, D. (2021). Structures of active melanocortin-4 receptor–Gs-protein complexes with NDP-α-MSH and setmelanotide. *Cell Research*, *31*(11), 1176-1189.

Holmes, C. L., Landry, D. W., & Granton, J. T. (2003). Science review: vasopressin and the cardiovascular system part 1–receptor physiology. *Critical care*, *7*(6), 1-8.

Hoyle, C. H. (1999). Neuropeptide families and their receptors: evolutionary perspectives. *Brain research*, *848*(1-2), 1-25.

Hughes, A. J., Daniel, S. E., Ben-Shlomo, Y., & Lees, A. J. (2002). The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. *Brain*, *125*(4), 861-870.

Inutsuka, A., & Yamanaka, A. (2013). The regulation of sleep and wakefulness by the hypothalamic neuropeptide orexin/hypocretin. *Nagoya journal of medical science*, *75*(1-2), 29.

Ish-Am, O., Kristensen, D. M., & Ruppin, E. (2015). Evolutionary conservation of bacterial essential metabolic genes across all bacterial culture media. *Plos one*, *10*(4), e0123785.

Jackson, I. M. (1982). Thyrotropin-releasing hormone. *New England Journal of Medicine*, *306*(3), 145-155.

Jeffares, D. C., Pain, A., Berry, A., Cox, A. V., Stalker, J., Ingle, C. E., Thomas, A., Quail, M. A., Siebenthall, K., & Uhlemann, A.-C. (2007). Genome variation and evolution of the malaria parasite Plasmodium falciparum. *Nature genetics*, *39*(1), 120-125.

Jeffares, D. C., Tomiczek, B., Sojo, V., & dos Reis, M. (2015). A beginners guide to estimating the non-synonymous to synonymous rate ratio of all protein-coding genes in a genome. *Parasite genomics protocols*, 65-90.

Jékely, G., Melzer, S., Beets, I., Kadow, I. C. G., Koene, J., Haddad, S., & Holden-Dye, L. (2018). The long and the short of it–a perspective on peptidergic regulation of circuits and behaviour. *Journal of Experimental Biology*, *221*(3), jeb166710.

Jesso, S., Morlog, D., Ross, S., Pell, M. D., Pasternak, S. H., Mitchell, D. G., Kertesz, A., & Finger, E. C. (2011). The effects of oxytocin on social cognition and behaviour in frontotemporal dementia. *Brain*, *134*(9), 2493-2501.

Jocham, G., Klein, T. A., Neumann, J., von Cramon, D. Y., Reuter, M., & Ullsperger, M. (2009). Dopamine DRD2 polymorphism alters reversal learning and associated neural activity. *Journal of Neuroscience*, *29*(12), 3695-3704.

Juárez, J., & Molina-Martínez, L. M. (2019). Opioid System and Alcohol Consumption. In *Neuroscience of Alcohol* (pp. 435-442). Elsevier.

Justinussen, J. L., Egebjerg, C., & Kornum, B. R. (2022). How hypocretin agonists may improve the quality of wake in narcolepsy. *Trends in Molecular Medicine*.

Kaczanowska, J., Ganglberger, F., Chernomor, O., Kargl, D., Galik, B., Hess, A., Moodley, Y., von Haeseler, A., Bühler, K., & Haubensak, W. (2022). Molecular archaeology of human cognitive traits. *Cell Reports*, *40*(9), 111287.

Kern, A., Mavrikaki, M., Ullrich, C., Albarran-Zeckler, R., Brantley, A. F., & Smith, R. G. (2015). Hippocampal dopamine/DRD1 signaling dependent on the ghrelin receptor. *Cell*, *163*(5), 1176-1190.

Ketchesin, K. D., Stinnett, G. S., & Seasholtz, A. F. (2017). Corticotropin-releasing hormone-binding protein and stress: from invertebrates to humans. *Stress*, *20*(5), 449-464.

Kim, J. Y., Wu, W. H., Jun, J. H., Sohn, J., & Seo, Y. S. (2018). Effects of corticotropin-releasing hormone on the expression of adenosine triphosphate-sensitive potassium channels (Kir6. 1/SUR2B) in human term pregnant myometrium. *Obstetrics & Gynecology Science*, *61*(1), 14-22.

Kim, M. S., Rossi, M., Abusnana, S., Sunter, D., Morgan, D., Small, C. J., Edwards, C., Heath, M. M., Stanley, S. A., & Seal, L. J. (2000). Hypothalamic localization of the feeding effect of agouti-related peptide and alpha-melanocyte-stimulating hormone. *Diabetes*, *49*(2), 177-182.

Kompier, N. F., Keysers, C., Gazzola, V., Lucassen, P. J., & Krugers, H. J. (2019). Early life adversity and adult social behavior: focus on arginine vasopressin and oxytocin as potential mediators. *Frontiers in behavioral neuroscience*, *13*, 143.

Kosse, C., & Burdakov, D. (2019). Natural hypothalamic circuit dynamics underlying object memorization. *Nature communications*, *10*(1), 2505.

Kryazhimskiy, S., & Plotkin, J. B. (2008). The population genetics of dN/dS. *PLoS genetics*, *4*(12), e1000304.

Lasaga, M., Debeljuk, L., Durand, D., Scimonelli, T. N., & Caruso, C. (2008). Role of α-melanocyte stimulating hormone and melanocortin 4 receptor in brain inflammation. *Peptides*, *29*(10), 1825-1835.

Layman, W. S., Hurd, E. A., & Martin, D. M. (2011). Reproductive dysfunction and decreased GnRH neurogenesis in a mouse model of CHARGE syndrome. *Human molecular genetics*, *20*(16), 3138-3150.

Le Foll, B., Goldberg, S. R., & Sokoloff, P. (2005). The dopamine D3 receptor and drug dependence: effects on reward or beyond? *Neuropharmacology*, *49*(4), 525-541.

Le Merrer, J., Becker, J. A., Befort, K., & Kieffer, B. L. (2009). Reward processing by the opioid system in the brain. *Physiological reviews*.

Lee, S. J., Logsdon, A. F., Yagi, M., Baskin, B. M., Peskind, E. R., Raskind, M. M., Cook, D. G., & Schindler, A. G. (2022). The dynorphin/kappa opioid receptor mediates adverse immunological and behavioral outcomes induced by repetitive blast trauma. *Journal of Neuroinflammation*, *19*(1), 288.

Leng, G., & Ludwig, M. (2006). Information processing in the hypothalamus: peptides and analogue computation. *Journal of neuroendocrinology*, *18*(6), 379-392.

Li, C., Wu, X., Liu, S., Zhao, Y., Zhu, J., & Liu, K. (2019). Roles of neuropeptide Y in neurodegenerative and neuroimmune diseases. *Frontiers in Neuroscience*, *13*, 869.

Li, X., Zhang, X., Shen, Z., Chen, Z., Wang, H., & Zhang, X. (2022). GnRH receptor mediates lipid storage in female adipocytes via AMPK pathway. *International Journal of Medical Sciences*, *19*(9), 1442.

Liu, L., Qiao, Y., Shao, Y., Yu, S.-Y., Zhang, C., Zhang, R., Wang, D.-X., Zhao, M., & Xie, B. (2020). Association of corticotropin-releasing hormone receptor-1 gene polymorphisms and personality traits with violent aggression in male adolescents. *Journal of molecular neuroscience*, *70*, 145-154.

Livingston, K. E., & Traynor, J. R. (2018). Allostery at opioid receptors: modulation with small molecule ligands. *British journal of pharmacology*, *175*(14), 2846-2856.

Löhr, H., & Hammerschmidt, M. (2011). Zebrafish in endocrine systems: recent advances and implications for human disease. *Annual review of physiology*, *73*, 183-211.

López-Ojeda, W., & Hurley, R. A. (2022). Kisspeptin in the Limbic System: New Insights Into Its Neuromodulatory Roles. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *34*(3), 190-195.

Lopez-Vicchi, F., De Winne, C., Brie, B., Sorianello, E., Ladyman, S. R., & Becu-Villalobos, D. (2020). Metabolic functions of prolactin: Physiological and pathological aspects. *Journal of neuroendocrinology*, *32*(11), e12888.

Maghnie, M., Cosi, G., Genovese, E., Manca-Bitti, M. L., Cohen, A., Zecca, S., Tinelli, C., Gallucci, M., Bernasconi, S., & Boscherini, B. (2000). Central diabetes insipidus in children and young adults. *New England Journal of Medicine*, *343*(14), 998-1007.

Malenka, R., Nestler, E., & Hyman, S. (2009). Chapter 6: widely projecting systems: monoamines, acetylcholine, and orexin. *Molecular neuropharmacology: A foundation for clinical neuroscience*, 147-157.

Mano-Otagiri, A., Nemoto, T., Yamauchi, N., Kakinuma, Y., & Shibasaki, T. (2016). Distribution of Corticotrophin-Releasing Factor Type 1 Receptor-Like Immunoreactivity in the Rat Pituitary. *Journal of neuroendocrinology*, *28*(12).

Mansour, A., Fox, C. A., Akil, H., & Watson, S. J. (1995). Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends in neurosciences*, *18*(1), 22-29.

Markakis, E. A. (2002). Development of the neuroendocrine hypothalamus. *Frontiers in neuroendocrinology*, *23*(3), 257-291.

Medina-Loera, S., Flores-Medina, Y., Escamilla-Orozco, R. I., Saracco-Álvarez, R. A., Rosel-Vales, M., Flores-Ramos, M., & Mondragón-Maya, A. (2020). Association between prolactin serum levels and cognitive function in chronic schizophrenia patients. *Salud mental*, *43*(1), 21-25.

Mieda, M., Hasegawa, E., Kisanuki, Y. Y., Sinton, C. M., Yanagisawa, M., & Sakurai, T. (2011). Differential roles of orexin receptor-1 and-2 in the regulation of non-REM and REM sleep. *Journal of Neuroscience*, *31*(17), 6518-6526.

Millar, R. P. (2005). GnRHs and GnRH receptors. *Animal reproduction science*, *88*(1-2), 5-28.

Miller, C. T., Hale, M. E., Okano, H., Okabe, S., & Mitra, P. (2019). Comparative principles for next-generation neuroscience. *Frontiers in behavioral neuroscience*, *13*, 12.

Mills, E. G., O'Byrne, K. T., & Comninos, A. N. (2019). Kisspeptin as a behavioral hormone. Seminars in Reproductive Medicine,

Mittapalli, G. K., & Roberts, E. (2014). Ligands of the neuropeptide Y Y2 receptor. *Bioorganic & medicinal chemistry letters*, *24*(2), 430-441.

Morales-Medina, J. C., Witchey, S. K., & Caldwell, H. K. (2016). The role of vasopressin in anxiety and depression. *Melatonin, Neuroprotective Agents and Antidepressant Therapy*, 667-685.

Moriya, R., Fujikawa, T., Ito, J., Shirakura, T., Hirose, H., Suzuki, J., Fukuroda, T., MacNeil, D. J., & Kanatani, A. (2010). Pancreatic polypeptide enhances colonic muscle contraction and fecal output through neuropeptide Y Y4 receptor in mice. *European journal of pharmacology*, *627*(1-3), 258-264.

Müller, D., Zai, C., Sicard, M., Remington, E., Souza, R., Tiwari, A., Hwang, R., Likhodi, O., Shaikh, S., & Freeman, N. (2012). Systematic analysis of dopamine receptor genes (DRD1–DRD5) in antipsychotic-induced weight gain. *The pharmacogenomics journal*, *12*(2), 156-164.

Munafò, M. R., Yalcin, B., Willis-Owen, S. A., & Flint, J. (2008). Association of the dopamine D4 receptor (DRD4) gene and approach-related personality traits: meta-analysis and new data. *Biological psychiatry*, *63*(2), 197-206.

Muse, S. V., & Gaut, B. S. (1994). A likelihood approach for comparing synonymous and nonsynonymous nucleotide substitution rates, with application to the chloroplast genome. *Molecular biology and evolution*, *11*(5), 715-724.

Nair, D., Ramesh, V., Li, R. C., Schally, A. V., & Gozal, D. (2013). Growth hormone releasing hormone (GHRH) signaling modulates intermittent hypoxia-induced oxidative stress and cognitive deficits in mouse. *Journal of neurochemistry*, *127*(4), 531-540.

Nepovimova, E., Janockova, J., Misik, J., Kubik, S., Stuchlik, A., Vales, K., Korabecny, J., Mezeiova, E., Dolezal, R., & Soukup, O. (2019). Orexin supplementation in narcolepsy treatment: a review. *Medicinal Research Reviews*, *39*(3), 961-975.

Nielsen, R., & Bustamante, C. D. (2005). Population genetics of molecular evolution. *Statistical methods in molecular evolution*, 63-99.

Ottinger, M., Thompson, N., Viglietti–Panzica, C., & Panzica, G. (1997). Neuroendocrine regulation of GnRH and behavior during aging in birds. *Brain Research Bulletin*, *44*(4), 471-477.

Painsipp, E., Wultsch, T., Edelsbrunner, M. E., Tasan, R. O., Singewald, N., Herzog, H., & Holzer, P. (2008). Reduced anxiety-like and depression-related behavior in neuropeptide Y Y4 receptor knockout mice. *Genes, Brain and Behavior*, *7*(5), 532-542.

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends in neurosciences*, *31*(9), 464-468.

Paulus, W., & Schomburg, E. D. (2006). Dopamine and the spinal cord in restless legs syndrome: does spinal cord physiology reveal a basis for augmentation? *Sleep medicine reviews*, *10*(3), 185-196.

Pearson, C. A., & Placzek, M. (2013). Development of the medial hypothalamus: forming a functional hypothalamic-neurohypophyseal interface. *Current Topics in Developmental Biology*, *106*, 49-88.

Pedragosa-Badia, X., Stichel, J., & Beck-Sickinger, A. G. (2013). Neuropeptide Y receptors: how to get subtype selectivity. *Frontiers in endocrinology*, *4*, 5.

Pellissier, L. P., Gandía, J., Laboute, T., Becker, J. A., & Le Merrer, J. (2018). μ opioid receptor, social behaviour and autism spectrum disorder: reward matters. *British journal of pharmacology*, *175*(14), 2750-2769.

Petrovich, G. D. (2018). Lateral hypothalamus as a motivation-cognition interface in the control of feeding behavior. *Frontiers in systems neuroscience*, *12*, 14.

Pilozzi, A., Carro, C., & Huang, X. (2020). Roles of β-endorphin in stress, behavior, neuroinflammation, and brain energy metabolism. *International journal of molecular sciences*, *22*(1), 338.

Pornour, M., Ahangari, G., Hejazi, S. H., Ahmadkhaniha, H. R., & Akbari, M. E. (2015). Dopamine receptor gene (DRD1-DRD5) expression changes as stress factors associated with breast cancer. *Asian Pacific Journal of Cancer Prevention*, *15*(23), 10339-10343.

Rangel-Barajas, C., Coronel, I., & Florán, B. (2015). Dopamine receptors and neurodegeneration. *Aging and disease*, *6*(5), 349.

Raposinho, P. D., Pedrazzini, T., White, R. B., Palmiter, R. D., & Aubert, M. L. (2004). Chronic neuropeptide Y infusion into the lateral ventricle induces sustained feeding and obesity in mice lacking either Npy1r or Npy5r expression. *Endocrinology*, *145*(1), 304-310.

Rigney, N., de Vries, G. J., Petrulis, A., & Young, L. J. (2022). Oxytocin, vasopressin, and social behavior: from neural circuits to clinical opportunities. *Endocrinology*, *163*(9), bqac111.

Romanelli, R. J., Williams, J. T., & Neve, K. A. (2010). Dopamine receptor signaling: intracellular pathways to behavior. *The dopamine receptors*, 137-173.

Rowe, D., Stever, C., Giedinghagen, L., Gard, J., Cleveland, H., Terris, S., Mohr, J., Sherman, S., Abramowitz, A., & Waldman, I. (1998). Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Molecular psychiatry*, *3*(5), 419-426.

Sand, E., Bergvall, M., Ekblad, E., D'Amato, M., & Ohlsson, B. (2013). Expression and distribution of GnRH, LH, and FSH and their receptors in gastrointestinal tract of man and rat. *Regulatory peptides*, *187*, 24-28.

Sannes, A.-C., Risøy, A., Christensen, J. O., Nielsen, M. B., & Gjerstad, J. (2021). Spinal pain in employees exposed to abusive supervision: Evidence of a sex and CRHR1 CTC haplotype interaction. *Molecular Pain*, *17*, 17448069211042123.

Saper, C. B., Lu, J., Chou, T. C., & Gooley, J. (2005). The hypothalamic integrator for circadian rhythms. *Trends in neurosciences*, *28*(3), 152-157.

Scammell, T. E., & Winrow, C. J. (2011). Orexin receptors: pharmacology and therapeutic opportunities. *Annual review of pharmacology and toxicology*, *51*, 243-266.

Schwarzer, C. (2009). 30 years of dynorphins—new insights on their functions in neuropsychiatric diseases. *Pharmacology & therapeutics*, *123*(3), 353-370.

Seeman, P. (2010). Historical overview: introduction to the dopamine receptors. *The dopamine receptors*, 1-21.

Shafer, M. E., Sawh, A. N., & Schier, A. F. (2022). Gene family evolution underlies cell-type diversification in the hypothalamus of teleosts. *Nature Ecology & Evolution*, *6*(1), 63-76.

Shan, L., Dauvilliers, Y., & Siegel, J. M. (2015). Interactions of the histamine and hypocretin systems in CNS disorders. *Nature reviews Neurology*, *11*(7), 401-413.

Shende, P., & Desai, D. (2020). Physiological and therapeutic roles of neuropeptide Y on biological functions. *Cell Biology and Translational Medicine, Volume 7: Stem Cells and Therapy: Emerging Approaches*, 37-47.

Smith, J. T., Cunningham, M. J., Rissman, E. F., Clifton, D. K., & Steiner, R. A. (2005). Regulation of Kiss1 gene expression in the brain of the female mouse. *Endocrinology*, *146*(9), 3686-3692.

Smith, S. M., & Vale, W. W. (2022). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in clinical neuroscience*.

Soya, S., & Sakurai, T. (2020). Evolution of orexin neuropeptide system: structure and function. *Frontiers in Neuroscience*, 691.

Sparapani, S., Millet-Boureima, C., Oliver, J., Mu, K., Hadavi, P., Kalostian, T., Ali, N., Avelar, C. M., Bardies, M., & Barrow, B. (2021). The biology of vasopressin. *Biomedicines*, *9*(1), 89.

Spielman, S. J., & Wilke, C. O. (2015). The relationship between dN/dS and scaled selection coefficients. *Molecular biology and evolution*, *32*(4), 1097-1108.

Stevenson, E. L., & Caldwell, H. K. (2012). The vasopressin 1b receptor and the neural regulation of social behavior. *Hormones and behavior*, *61*(3), 277-282.

Stolzenberg, D. S., & Numan, M. (2011). Hypothalamic interaction with the mesolimbic DA system in the control of the maternal and sexual behaviors in rats. *Neuroscience & Biobehavioral Reviews*, *35*(3), 826-847.

Sukhareva, E. (2021). The role of the corticotropin-releasing hormone and its receptors in the regulation of stress response. *Vavilov Journal of Genetics and Breeding*, *25*(2), 216.

Theofanopoulou, C., Gedman, G., Cahill, J. A., Boeckx, C., & Jarvis, E. D. (2021). Universal nomenclature for oxytocin–vasotocin ligand and receptor families. *Nature*, *592*(7856), 747-755.

Thiele, T. E. (2017). Neuropeptides and addiction: an introduction. *International review of neurobiology*, *136*, 1-3.

Thompson, M. D., Xhaard, H., Sakurai, T., Rainero, I., & Kukkonen, J. P. (2014). OX1 and OX2 orexin/hypocretin receptor pharmacogenetics. *Frontiers in Neuroscience*, *8*, 57.

Thorsell, A., & Mathé, A. A. (2017). Neuropeptide Y in alcohol addiction and affective disorders. *Frontiers in endocrinology*, *8*, 178.

Tisdale, R. K., Yamanaka, A., & Kilduff, T. S. (2021). Animal models of narcolepsy and the hypocretin/orexin system: Past, present, and future. *Sleep*, *44*(6), zsaa278.

Tom, N., & Assinder, S. J. (2010). Oxytocin in health and disease. *The international journal of biochemistry & cell biology*, *42*(2), 202-205.

Tour, J., Sandström, A., Kadetoff, D., Schalling, M., & Kosek, E. (2022). The OPRM1 gene and interactions with the 5-HT1a gene regulate conditioned pain modulation in fibromyalgia patients and healthy controls. *Plos one*, *17*(11), e0277427.

Treutlein, J., Strohmaier, J., Frank, J., Witt, S. H., Rietschel, L., Forstner, A. J., Lang, M., Degenhardt, F., Dukal, H., & Herms, S. (2017). Association between neuropeptide Y receptor Y2 promoter variant rs6857715 and major depressive disorder. *Psychiatric genetics*, *27*(1), 34-37.

Trezza, V., Damsteegt, R., Achterberg, E. M., & Vanderschuren, L. J. (2011). Nucleus accumbens μ-opioid receptors mediate social reward. *Journal of Neuroscience*, *31*(17), 6362-6370.

Vezzani, A., Sperk, G., & Colmers, W. F. (1999). Neuropeptide Y: emerging evidence for a functional role in seizure modulation. *Trends in neurosciences*, *22*(1), 25-30.

Waltenspühl, Y., Schöppe, J., Ehrenmann, J., Kummer, L., & Plückthun, A. (2020). Crystal structure of the human oxytocin receptor. *Science Advances*, *6*(29), eabb5419.

Wang, C., Wang, Q., Ji, B., Pan, Y., Xu, C., Cheng, B., Bai, B., & Chen, J. (2018). The orexin/receptor system: molecular mechanism and therapeutic potential for neurological diseases. *Frontiers in Molecular Neuroscience*, *11*, 220.

Wang, X., Yang, J., Lu, T., Zhan, Z., Wei, W., Lyu, X., Jiang, Y., & Xue, X. (2020). The effect of swimming exercise and diet on the hypothalamic inflammation of ApoE-/-mice based on SIRT1-NF-κB-GnRH expression. *Aging (Albany NY)*, *12*(11), 11085.

Waterson, M. J., & Horvath, T. L. (2015). Neuronal regulation of energy homeostasis: beyond the hypothalamus and feeding. *Cell metabolism*, *22*(6), 962-970.

Westphal, N. J., Evans, R. T., & Seasholtz, A. F. (2009). Novel expression of type 1 corticotropin-releasing hormone receptor in multiple endocrine cell types in the murine anterior pituitary. *Endocrinology*, *150*(1), 260-267.

Whitlock, K. E., Postlethwait, J., & Ewer, J. (2019). Neuroendocrinology of reproduction: Is gonadotropin-releasing hormone (GnRH) dispensable? *Frontiers in neuroendocrinology*, *53*, 100738.

Wilkanowska, A., Mazurowski, A., Mroczkowski, S., & Kokoszyński, D. (2014). Prolactin (PRL) and prolactin receptor (PRLR) genes and their role in poultry production traits. *Folia Biologica (Kraków)*, *62*(1), 1-8.

Wolf, J. B., Künstner, A., Nam, K., Jakobsson, M., & Ellegren, H. (2009). Nonlinear dynamics of nonsynonymous (dN) and synonymous (dS) substitution rates affects inference of selection. *Genome biology and evolution*, *1*, 308-319.

Wu, Q., Chen, J., Hua, T., & Cai, J. (2023). Alpha-melanocyte-stimulating hormone-mediated appetite regulation in the central nervous system. *Neuroendocrinology*, 1-1.

Xie, Y., & Dorsky, R. I. (2017). Development of the hypothalamus: conservation, modification and innovation. *Development*, *144*(9), 1588-1599.
Xu, Y., Cai, H., You, C., He, X., Yuan, Q., Jiang, H., Cheng, X., Jiang, Y., & Xu, H. E. (2022). Structural insights into ligand binding and activation of the human thyrotropin-releasing hormone receptor. *Cell Research*, *32*(9), 855-857.
Yang, F., Zhang, H., Meng, X., Li, Y., Zhou, Y., Ling, S., Sun, D., Lv, P., Liu, L., & Shi, P. (2022). Structural insights into thyrotropin-releasing hormone receptor activation by an endogenous peptide agonist or its orally administered analogue. *Cell Research*, *32*(9), 858-861.
Yang, Z., Han, S., Keller, M., Kaiser, A., Bender, B. J., Bosse, M., Burkert, K., Kögler, L. M., Wifling, D., & Bernhardt, G. (2018). Structural basis of ligand binding modes at the neuropeptide Y Y1 receptor. *Nature*, *556*(7702), 520-524.
Yang, Z., & Nielsen, R. (1998). Synonymous and nonsynonymous rate variation in nuclear genes of mammals. *Journal of molecular evolution*, *46*, 409-418.
Yatawara, C., Einfeld, S., Hickie, I., Davenport, T., & Guastella, A. (2016). The effect of oxytocin nasal spray on social interaction deficits observed in young children with autism: a randomized clinical crossover trial. *Molecular psychiatry*, *21*(9), 1225-1231.
Zhao, Z.-D., Yang, W. Z., Gao, C., Fu, X., Zhang, W., Zhou, Q., Chen, W., Ni, X., Lin, J.-K., & Yang, J. (2017). A hypothalamic circuit that controls body temperature. *Proceedings of the National Academy of Sciences*, *114*(8), 2042-2047.
Zhou, Y., & Cai, M. (2017). Novel approaches to the design of bioavailable melanotropins. *Expert opinion on drug discovery*, *12*(10), 1023-1030.
Zhou, Y., Chawla, M. K., Rios-Monterrosa, J. L., Wang, L., Zempare, M. A., Hruby, V. J., Barnes, C. A., & Cai, M. (2021). Aged brains express less melanocortin receptors, which correlates with age-related decline of cognitive functions. *Molecules*, *26*(20), 6266.
Zhu, N., Zhao, M., Song, Y., Ding, L., & Ni, Y. (2022). The KiSS-1/GPR54 system: Essential roles in physiological homeostasis and cancer biology. *Genes & Diseases*, *9*(1), 28-40.