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On the Proximate and Ultimate Explanations of Sex Differences in Anxiety and Anxiety Disorders

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During their lifetime, women are twice as likely to meet the diagnostic criteria for an anxiety disorder compared to men (Baxter et al., 2013). However, studies investigating the mechanisms underlying these sex differences often focus on different levels of analysis, making it more difficult to incorporate findings from these different studies. This article synthesises and reviews a selection of these analyses to provide a better-integrated representation of the mechanisms underlying sex differences. It uses the framework of four questions developed by Tinbergen in 1963, disentangling the potential proximate and ultimate mechanisms involved. This article describes the evidence for different explanations, with a particular focus on testosterone, which is presented as a possible link between different correlates of anxiety. Analysis of ultimate mechanisms include the evaluation of the optimality approach of behavioural ecology as well as the approach of evolutionary psychology. The signal detection theory suggests that some sex differences in anxiety may be adaptive, whereas the theories of evolutionary psychology suggest a mismatch between current and past environments in our species history. Overall, it is not supported that there would be a single explanation at any level of analysis.

INTRODUCTION

Anxiety can be defined as an emotion associated with feelings of tension and worried thoughts, as well as the associated physiological response, which includes an increased heart rate and stress hormone secretion (American Psychological Association, 2022; Bateson et al., 2011). These mental and physiological changes are supposed to prepare the animal to successfully face or evade potential threats in the environment. However, excessive and persistent feelings of anxiety—characteristic of anxiety disorders—can significantly impact the quality of an individual's everyday life. The class of anxiety disorders constitutes one of the most common mental health issues in present society, with estimates of global prevalence ranging from 4.8% to 10.9% (Baxter et al., 2013).

Assessing prevalence of anxiety disorders is nevertheless difficult due to changes in their classification in newer versions of the Diagnostic and Statistical Manual of Mental Disorders, cultural variation in boundaries between non-pathological and pathological distress, and due to geographical differences in prevalence (Stein et al., 2017). Despite these barriers, there has been consistent evidence of sex differences in the prevalence and experience of anxiety disorders between men and women. National surveys of mental health in the US from 2002 and 2003 found a lifetime prevalence of any anxiety to be 33.3% in women and 22% in men, making anxiety 1.7 times more prevalent among women (McLean et al., 2011). Similarly, a systematic review of studies from 44 countries found that females were 1.9-2.3 times more likely to meet the diagnostic criteria of any anxiety disorder (Baxter et al., 2013). Analysis of 48 reviews confirmed a ratio of female-to-male prevalence of 1.9:1, which persisted over time (Remes et al., 2016). Subclinical levels of anxiety also seem to be more common among women than men (Hankin, 2009).

Apart from sex differences in prevalence, there seem to be certain differences in the experience of anxiety disorders. Among individuals suffering with generalised anxiety disorder, women tend to report more somatic complaints, fatigue, and muscle tension when compared to men (Jalnapurkar et al., 2018). On the other hand, men diagnosed with generalised anxiety disorder tend to report more arguments in their relationships as a result of excessive worry (Vesga-López et al., 2008). Furthermore, men with social anxiety disorder are more likely to fear dating compared to women with this diagnosis (Xu et al., 2012).

However, anxiety disorders among men and women still share many similarities. There seems to be no difference in age of onset of anxiety disorders, based on a recent meta-analysis of 8 studies with a total sample size of 3,256 males and 7,766 females (de Lijster et al., 2017). The age of onset varies based on particular type of anxiety disorder, where the earliest conditions manifest during childhood and early adolescence (before the age of 15), including separation anxiety disorder, specific phobias, and social anxiety disorder (ibid.; Hallers-Haalboom et al., 2020). On the other hand, disorders like generalised anxiety disorder or agoraphobia usually appear in young adulthood between 21 and 34 years of age (de Lijster et al., 2017).

AIMS AND OBJECTIVES

While these differences and similarities have been well-documented, there is still a considerable gap in our understanding of the mechanisms underlying them. As anxiety and anxiety disorders are currently some of the most common mental health problems, it is important to further investigate their aetiology to offer the most effective treatment possible that is well-suited to the individual. Furthermore, understanding the aetiology can serve to discover possible preventative measures in the future. Thus, this article aims to synthesise the current literature on

possible explanations of sex differences in anxiety, by using a framework developed by Niko Tinbergen (1963) in his seminal paper "On aims and methods of ethology". This framework is meant to provide a structure to the problems biologists are researching about a particular behaviour. It includes proximate and ultimate explanations. *Proximate explanations* encompass more mechanistic explanations (i.e., the factors that immediately lead to a trait), whereas the *ultimate explanations* address different aspects of the evolution of a behavioural pattern. While psychiatric research tends to focus on the proximate explanations including both ontogeny and mechanisms of control (Bateson et al., 2011), evolutionary psychology and behavioural ecology address the adaptive significance of a trait, in other words the way it relates to the overall fitness of an individual.

This article will begin by considering the proximate mechanisms, specifically the *causation* and *ontogeny* of sex differences in anxiety, drawing on evidence from human neuroimaging studies and correlational, as well as experimental, studies. The term *causation* here describes the anatomy and physiology that give rise to the particular trait (Nesse, 2013); in this case, the anatomical and physiological aspects that underlie sex differences in anxiety. The *ontogeny* of sex differences in anxiety covers the development of this trait over different life stages (Nesse, 2013).

Following the discussion on proximate mechanism, the text will address the ultimate mechanisms: the *function* and *phylogeny*. In this framework, the term *function* describes the contribution of the trait to an individual's fitness (Bateson & Laland, 2013). And the topic of *phylogeny* is concerned with the evolution of the trait over the history of the species (ibid.).

PROXIMATE CAUSES

Causes

Firstly, the causation, or mechanism of control, will be discussed with focus on the neurological and endocrinological explanations. The mechanisms underlying sex differences in anxiety and anxiety disorders are necessarily multifaceted, and seem to be influenced by the secretion and activity of different hormones (see Hallers-Haalboom et al., 2020). Prior to describing potential hormonal mechanisms, this article will briefly mention differences in brain structures that have been identified between males and females in the affective network. The affective network consists of multiple structures, including the prefrontal cortex, the hippocampus, and the extended amygdala complex (Jalnapurkar et al., 2018), and is involved in anxiety and anxiety disorders (Kaczkurkin et al., 2016). Using a dataset of magnetic resonance images, Kaczkurkin and colleagues (ibid.) found that elevated amygdala perfusion seems to mediate the developmental sex differences in anxiety. They used data from the Philadelphia Neurodevelopmental Cohort, which includes data on individuals between ages 12 and 23 years. Pubertal status interacted with gender, where females after puberty displayed higher levels of trait anxiety. Trait anxiety refers to stable individual differences in levels of anxiety (Tovilović et al., 2009). On the other hand, there were no sex differences between males and females in the pre- and midpubertal groups. Interestingly, state anxiety—i.e., the situational levels of anxiety (Kaczkurkin et al., 2016)—differed between the sexes not only in the post-pubertal group, but also in the mid-pubertal group, with females scoring higher. Regarding data from an MRI voxelwise analysis, higher levels of trait anxiety were associated with increased perfusion in the left amygdala. However, this study cannot help determine causality, as it is not experimental. Thus, while a possible direction of causality is that differences in brain structures that arise throughout adolescence influence behaviour and emotions, there may be alternative mechanisms involved. Nonetheless, the Philadelphia Neurodevelopmental Cohort dataset does not include endocrinological measures, and thus it is not possible to investigate how the differences in brain perfusion might have translated to levels of gonadal hormones to affect behaviour.

While it remains unknown how these differences in brain structures translate to other bodily systems, there has been interest in investigating the role of hormones, and particularly hormone fluctuations, in sex differences in anxiety. A key system regulating stress and anxiety is the Hypothalamic-Pituitary-Adrenal (HPA) axis (Hallers-Haalboom et al., 2020). The HPA axis has a regulatory function in production and secretion of corticosteroids under basal and stress conditions (Moisiadis & Matthews, 2014). Research on animal models suggests that the HPA axis is more sensitive in females compared to males (Heck & Handa, 2019). However, findings of human studies are less clear (Goel et al., 2014; Kudielka & Kirschbaum, 2005). The inconsistency in findings may be due to methodological differences, as there are more measures and paradigms available to assess HPA axis reactivity in humans than in animal models. The methodological aspect that seems particularly relevant to consider when comparing findings from different studies is the type of stressor used, which can be physical, psychological, or pharmacological (Goel et al., 2014). Nevertheless, differential sensitivity of the HPA axis may be a result of hormonal fluctuations during the female menstrual cycle (Hallers-Haalboom et al., 2020). Conversely, male HPA axis is not exposed to these hormonal fluctuations, which might make its response more stable and less vulnerable to stressors. These differences in HPA axis reactivity may contribute to sex differences in anxiety and anxiety disorders.

While fluctuations in gonadal hormones seem to destabilise the HPA system, testosterone appears to have a protective function against anxiety (McHenry et al., 2014). Evidence supporting this function of testosterone comes from patient studies on males with hypogonadism, a condition characterised by decreased levels of testosterone due to reduced functional activity of the gonads (ibid.). This deficiency manifests, among other symptoms, with increased levels of anxiety in affected males compared to males with typical levels of testosterone (Zarrouf et al., 2009). Furthermore, in cases where hypogonadism is treated by supplementing testosterone, anxiety symptoms are alleviated (Wang et al., 1996; Zarrouf et al., 2009). This suggests that sex differences in anxiety may be partially controlled by levels of testosterone, as testosterone levels in men are approximately ten times higher than those in women (McHenry et al., 2014). These anxiolytic effects of testosterone might be mediated by testosterone's effect on GABA neurotransmitters and receptors. Recent analysis of data from The Netherlands Study of Depression and Anxiety found that women diagnosed with anxiety disorder (generalised anxiety disorder, social anxiety disorder, or agoraphobia) or depressive disorder had lower levels of salivary testosterone compared to control females (Giltay et al., 2012). Overall, this evidence suggests a role of testosterone in protecting against anxiety disorder, which could partially explain sex differences in anxiety disorders and their prevalence, as men have typically higher levels of testosterone (McHenry et al., 2014). The potential selection mechanisms leading to this particular role of testosterone will be discussed below as part of the ultimate explanations.

Ontogeny

Another level of proximate explanation concerns the ontogeny of the sex differences, in other words, the development over the lifetime of the individual and the key factors that influence it. Starting with the genetic factors, a large twin study (n = 45,850 participants) set in Australia and the United States examined the genetic and environmental factors contributing to neuroticism, a trait characterised by anxiety, among other emotions (Lake et al., 2000). Authors found an overall greater heritability of neuroticism in women (41%) compared to men (35%). These findings provide evidence that sex differences in anxiety may arise due to a differential genetic predisposition. Alternatively, the increased heritability among women could be explained by differences in socialisation of boys and girls. If girls experience higher levels of reinforcement of their neurotic traits, the heritability would be higher when compared to boys who might be discouraged from expressing anxiety-related traits as they are often considered inconsistent with the male gender role. Moreover, men are on average less likely to seek help from mental health professionals (Addis & Mahalik, 2003), which may widen the sex differences in the likelihood of being diagnosed with an anxiety disorder. Alternatively, diagnostic criteria in use may be overrepresenting issues in female population and underrepresenting those in males (Addis & Cohane, 2005). There is some evidence that gender roles are related to symptoms of anxiety and fear (Chambless & Mason, 1986; Ollendick et al., 2002).

Chambless and Mason (1986) found that in men, inventory measures of masculinity were strongly inversely associated with avoidance behaviour (r = –0.45) and scores on the State-Trait Anxiety Inventory (r = –0.62). However, as gender roles in society are changing, these effect sizes are likely not reliable anymore. Campaigns such as *Man Therapy* or *Real Men, Real Depression* have aimed to destigmatize male mental health and to encourage men to seek help when struggling (Seidler et al., 2018). This shift in public attitude towards male expression of emotions likely has had some impact on male anxiety as well, making men more willing to show symptoms of anxiety.

Postnatally, sex differences in the risk of developing anxiety disorders seem to first appear in childhood. Lewinsohn and colleagues (1998) explored the cumulative incidence estimates for female and male adolescents. Based on retrospective data, they found that at the age of 6, the likelihood of girls experiencing an anxiety disorder is twice as high compared to boys. However, the use of retrospective data may be less reliable and may limit the strength and accuracy of this evidence, as the data gathered on age of onset were based solely on participants' memory.

The average age of onset, as mentioned above, does not seem to differ between sexes. Nevertheless, there is some evidence suggesting a decrease in sex differences in the prevalence of anxiety disorders after the age of 65 (Altemus et al., 2014; Krasucki et al., 1998), and an overall decline in the prevalence of anxiety disorders with age (Byers et al., 2010). These trends may be due to anxiety-related mortality or as a consequence of menopause (Krasucki et al., 1998). However, it may also be due to diagnostic matters; in particular, difficulties distinguishing between cognitive impairment and anxiety disorders (Jalnapurkar et al., 2018). This also likely contributes to the inconsistency in research findings in this area.

The exact age-dependent pattern of sex differences depends on the specific anxiety disorder (Kessler et al., 2012). For example, among elderly individuals with panic disorder, smaller gender differences seem to be on the account of a disproportionate decrease in prevalence of female anxiety compared to the decline in prevalence of male anxiety (ibid.).

Overall, the ontogeny of sex differences in anxiety seems to include different heritability of anxiety-related vulnerability between sexes, which may be due to different socialization of boys and girls. The sex differences seem to be stable throughout adulthood and narrow among the elderly population.

In summary, proximate explanations analyse behaviour from the short-term, mechanistic level. However, they do not consider the evolutionary processes that lead to the appearance and preservation of a particular trait. These evolutionary processes are addressed as ultimate mechanisms.

ULTIMATE MECHANISMS

Function

Ultimate explanations address how the trait relates to an individual's fitness—i.e., survival and successful reproduction. If a heritable trait increases an individual's likelihood of survival and reproduction compared to other conspecifics, this trait will increase in frequency within a population. Conversely, a trait that leads to a reduced likelihood of survival and reproduction should decrease in frequency over time, in other words be selected against. Anxiety-like response is supposed to prepare the individual to survive when faced with a threat such as a predator. Hyperventilating increases oxygenation of blood and diverting blood to the muscles prepares them for rapid action (Bateson et al., 2011). If this type of response increases the chances of an individual's survival, it persists and spreads in the population over time.

In addition to the response itself, another important factor impacting fitness is the type or intensity of stimulus that triggers this response. This is modelled in the signal detection theory (Bateson et al., 2011). In this model, there is a threshold beyond which the stimulus triggers an anxiety-like response, otherwise no response is produced. Thus, individuals with anxiety disorders can be modelled as having a particularly low threshold (ibid.). Similarly, the sex differences in anxiety could be modelled as females having lower threshold for triggering a response compared to males. The authors then use the model to provide explanations for differences in anxiety via the concept of vulnerability. Vulnerability is defined as the fraction of fitness cost of false alarm (i.e., when there is no threat, but a response is nonetheless triggered) and fitness cost of a miss (when there is a threat present, but no response is triggered). If an animal lives in a more dangerous environment—where the cost of missing a threat may be death, whereas the cost of fleeing is only some insignificant energy expenditure—a lower optimal threshold would be expected. Conversely, if females are more vulnerable to threats (e.g., as a result of lower physical strength, necessity to look after offspring, etc.), they would have a lower threshold for anxiety, and thus would be more prone to developing anxiety disorders. This model is supported by evidence from studies investigating the relationship between physical strength and anxiety. Kerry and Murray (2021) found that grip strength, which was used as a proxy for overall strength, was negatively associated with anxiety (r = –0.26), and that grip strength mediated sex differences in anxiety in a sample of over 800 US students. This is consistent with a similar study conducted on five samples of US students (n = 1405), which also found a negative association between strength and anxiety (Manson et al., 2022). Using a composite score of grip and chest strength measurements, they found a weaker, yet significant association between strength and anxiety (r = –0.12). However, the character of these studies limits the strength of the evidence, as direction of causality cannot be reliably determined using a cross-sectional observational design. While a possible explanation consistent with the theory of the model is that women should be more prone to anxiety disorders because their optimal threshold for detecting threats is lower due to their smaller physical strength, it cannot be reliably inferred.

Nevertheless, there is evidence that grip strength is associated with testosterone, which presents a potential mechanism that connects the potential proximate and ultimate explanations. This evidence comes from a study by Chiu and colleagues (2020), which used data from the National Health and Nutrition Examination Survey in the US. Grip strength was positively associated with serum testosterone levels.

This explanation assumes that sex differences in non-clinical anxiety are an optimal solution to different challenges posed by the environment. This assumption, made by behavioural ecology, stands in some opposition to the premise of evolutionary psychology. Evolutionary psychologists posit that human traits evolved in response to selection pressures faced by our ancestors in the Pleistocene, who lived as hunter-gatherers (Cosmides & Tooby, 2016). These selection pressures and the related environment is called the Environment of Evolutionary Adaptedness (EEA), a concept first developed by John Bowlby (Irons, 1998). Hunter-gatherers in the EEA supposedly faced many threats from the environment, and thus increased vigilance due to anxiety likely was beneficial, but in the present, it is no longer adaptive.

Altemus (2006) postulated that the increased prevalence of anxiety disorders in females may be a result of the demographic transition, after which families have fewer children and females thus spend less time of their lives being pregnant or lactating. This explanation proposes that sex differences in anxiety might be due to a mismatch between the EEA, where increased levels of female anxiety were partially compensated for by the action of gonadal hormones secreted during gestation and lactation, and the present, where women wean their offspring earlier and have longer inter-birth intervals, and thus are less protected by these hormones (Altemus, 2006). The gonadal hormones secreted during pregnancy and lactation have the ability to suppress the HPA axis and the stress response system (ibid.). This is partially supported by a study looking at breast- and bottle-feeding mothers in the US, which found lower levels of perceived stress in breast- as opposed to bottle-feeding mothers (Mezzacappa & Katkin, 2002). However, there may be other confounding variables, such as social stigma associated with bottle-feeding. Hence, authors also looked at mood changes throughout a feeding session, and found that after breast-feeding, mothers reported decreased levels of negative mood and increased levels of positive mood. Still, this study assessed overall negative mood and did not focus on anxiety measures, thus it provides only limited support for this argument. However, the effect of pregnancy and lactation on anxiety is likely restricted as pregnancy is simultaneously a stressful period where future mothers face many challenges. This

is shown in the inconsistency in results of studies examining anxiety levels during pregnancy. For instance, in a review of 8 studies, Hertzberg and Wahlbeck (1999) found that panic symptoms were alleviated in about 41% of women in the sample during pregnancy, while also being aggravated in 38% of women. Furthermore, we do not have evidence on the anxiety levels of hunter-gatherers in the Pleistocene. Nevertheless, this explanation suggests a protective effect of gonadal hormones secreted during pregnancy and lactation on female anxiety levels, otherwise leaving the female HPA axis and stress response system destabilised and less able to deal with stressors effectively. Male stress response systems, in contrast, have not been subjected to these hormonal changes as a result of changes in reproductive behaviour. However, this explanation is valid only in explaining sex differences in anxiety in human societies that have undergone the demographic transition. Sex differences in anxiety beyond this scope would need to be addressed by other evolutionary explanations, such as the signal detection theory.

Lastly, this article considers the ultimate explanations behind the potential protective function of testosterone against anxiety. In the Pleistocene, as well as in current hunter-gatherer societies, males have specialised in hunting large game, as well as extracting hard-to-acquire resources, such as honey, which are very dangerous and require a high level of skill (Kaplan et al., 2000; Marlowe, 2007). Thus, males might have to have lower levels of anxiety to be able to persevere in the face of threat, as opposed to females, who need to look after vulnerable offspring that are not able to resist threats from the environment. Here, males should optimally be willing to face more risks, which is also related to their decreased vulnerability given physical strength, as discussed above. Thus, some support for this argument can be drawn from studies on risk-taking. A meta-analysis of 69 studies found a small positive association between testosterone levels and measures of risk-taking ($r = 0.12$) and such finding was robust when controlling for sex (Kurath & Mata, 2018). However, in many of the included studies, risk-taking was measured via self-report questionnaires or using tasks which would have low ecological validity when addressing evolutionary mechanisms. Tasks of high ecological validity are necessarily restricted by ethical guidelines; however, they could include some real, albeit minor, losses or happen outside the laboratory (Bran & Vaidis, 2020).

Phylogeny

The other aspect of ultimate explanations addresses the phylogeny of the trait, in other words, how the trait has evolved over the history of the species (Bateson & Laland, 2013). It achieves this goal by mapping the trait's presence in related species. However, little is known about the phylogeny of sex differences in anxiety, as such comparative approach requires data on many species. By comparing different species based on the presence or absence of sex differences in anxiety, it is possible to hypothesize the trait's evolution, as well as gain complementary data to support or refute hypotheses of its function. However, behaviour and anxiety do not fossilise, and thus phylogenetic data can be only based on extant species. Still, sex differences in fear and anxiety-like responses have been demonstrated in animal models, most commonly in rats (Kokras & Dalla, 2014). A comparative analysis is needed because it might provide more evidence on the potential function or utility of sex differences in anxiety, as explanations posited by evolutionary psychology could potentially be called into question if the trait is present within primates and other taxa. For instance, the demographic-transition explanation put forward by Altemus (2006) is specific to human societies, and is unable to account for sex differences in other species. If a similar pattern of sex differences in anxiety was found among non-human primates with short interbirth intervals, it would be possible that a different explanation is more valid in addressing these sex differences in humans, as well as non-human primates.

Conclusion

In brief, sex differences in anxiety and anxiety disorders can be analysed at different levels. The structure used to order these different levels is the framework developed by Tinbergen (1963), which distinguishes between proximate and ultimate explanations. The possible proximate mechanisms discussed include differences in the affective network in the brain, as well as differences in HPA axis reactivity and role of different hormones. Testosterone in particular seems to impact anxiety levels in men as well as women. Furthermore, testosterone levels have been found to explain some variance in grip strength and risk-taking behaviours. As males have higher levels of testosterone on average, testosterone thus could serve as a proximate mediator of signal detection theory and the risk-taking explanation of sex differences in anxiety.

Regarding ontogeny, there seem to be different relative contributions of genetic factors to anxiety between the sexes. Sex differences in anxiety-related vulnerability then emerge in childhood; however, the average age of onset is typically similar for both sexes.

The ultimate explanations discussed in this paper include the signal detection theory, which postulates that females should have a lower threshold for anxiety-like responses because they are on average more vulnerable to threats. This was supported by correlations between strength and anxiety.

Alternative explanations from evolutionary psychology point to the demographic transition and the role of gonadal hormones in anxiety during pregnancy and lactation. However, this explanation would only explain sex differences in human anxiety, as other animals have not undergone this demographic transition. This is where explanations of function would benefit from data on the phylogeny of sex differences in anxiety, as explanations such as these are specific to selection pressures faced by hunter-gatherers as opposed to non-human primates or other mammals. However, data on the sex differences in anxiety are currently lacking across many species. Nevertheless, sex differences were found in rats, which are commonly used as animal models of anxiety.

Another proposed explanation discusses the potential of risk-taking in males to be evolutionary adaptive and mediated by testosterone. However, this is based on correlational evidence, often using measures of low ecological validity, which limits the support for this explanation.

This article integrates literature on sex differences in anxiety and anxiety disorders, and outlines possible links between different levels of analysis. These sex differences might already be embedded prenatally, via differences in contribution of genetic factors, and mediated postnatally by gonadal hormones. Not much is known about their phylogeny; however, several explanations of function have been proposed, including signal detection theory, role of demographic transition, and risk-taking behaviour.

Interdisciplinary Commentary

NATURAL SCIENCES & ETHOLOGY

Sex difference in anxiety and anxiety-related behaviour: Insights from animal studies

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The current male bias in psychiatric preclinical studies significantly deviates from the female-biased susceptibility to anxiety in humans, and the discontinuity of such sex differences across species will determine the translatability of animal studies. This article examines behavioural, anatomical, and functional continuity and discrepancies across rodent, non-human primates (NHP) and human studies, and finds the translational validity of rodent studies rather limited by various discrepancies. Conversely, NHP models are phylogenetically, behaviourally, and functionally more closely related to the human model especially in sex differences, which highlights their importance in upcoming preclinical research.

Introduction

Across decades, anxiety disorders have consistently shown a remarkable sex difference in prevalence, with females about twice as likely as males to be affected (Pigott, 1999; Santomauro et al., 2021; Seedat et al., 2009). The COVID-19 outbreak has aggravated the health burden of anxiety disorders especially on women, accounting for 6.11 million additional disability-adjusted life-years (DALYs) for women worldwide, more than twice the burden on men (2.94 million additional DALYs; Santomauro et al., 2021). In contrast, preclinical studies, from rodent models to non-human primates (NHPs), have primarily focused on male animals or sex-pooled samples, questioning their translatability to human clinical practice. Recent behavioural, anatomical, and functional animal studies may shed some light on the cross-species (dis-)continuity of sex difference in anxiety.

Sex difference in anxiety-related behaviour across species

Rodents and NHPs are the two commonly used models to study anxiety-related behaviour. For rodent models, there exist tried-and-tested standard methods to characterise anxiety-related behaviour, including open field tests (where rodents are put in the centre of an open field and the less anxious individuals spend more time exploring the peripheral areas and move around more in the field), and the elevated plus-maze (EPM, consisting of four arms elevated above ground, two of which are protected with walls, connected by a central area; the less anxious individuals spend more time in the unprotected open arms) (**Figure 1**). Performance in the EPM test is highly variable across different strains of mice, with wild, Swiss CD-1 (Holmes et al., 2000), KK/HIJ (Inglis et al., 2019) and BALB/cJ strains (An et al., 2011) showing minimal variation between sexes, whereas for the most commonly used C57BL/6 strain, females display a significantly higher level of anxiety-related behaviour than males, but measurements in time spent in open arms and arm transitions are highly inconsistent across studies (ibid.; Inglis et al., 2019). Similarly, base-line performance of rats in EPM tests also fails to show consistent sex differences, studies concluding females displaying significantly more (Gogokhia et al., 2021), significantly less (Knight et al., 2021) anxiety-related behaviour, or showing no significant sex difference (Keeley et al., 2015). On the other hand, open field tests also fail to provide conclusive evidence for sex difference in anxiety amongst

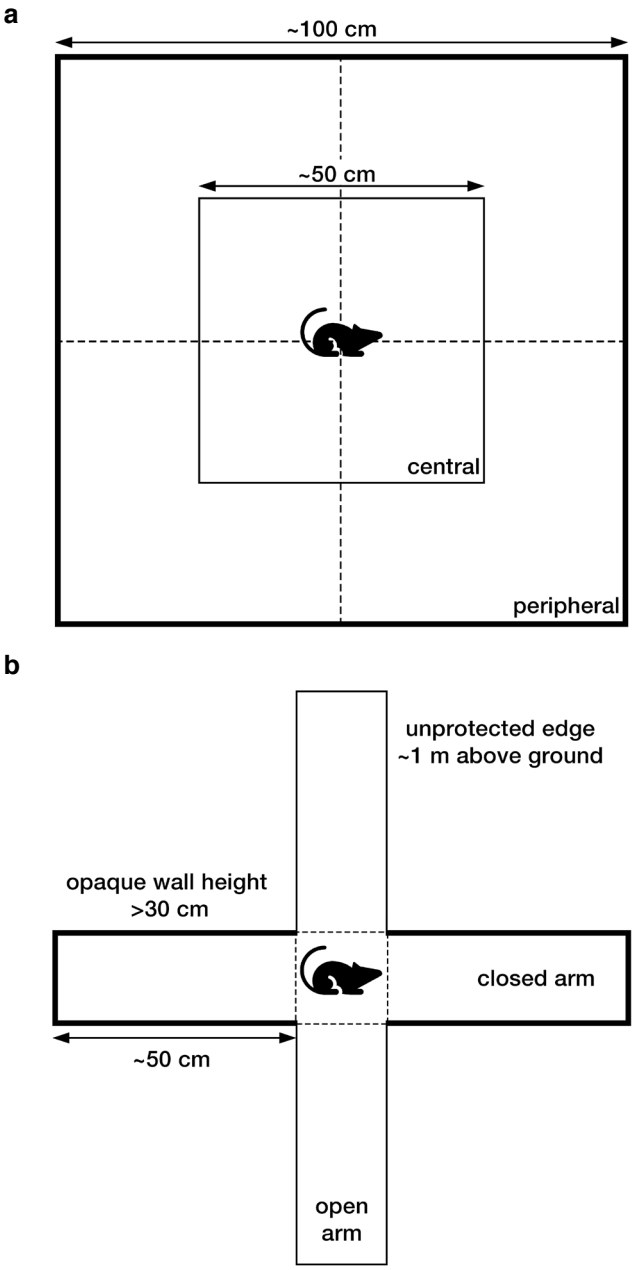

Figure 1 | Conventional rodent anxiety tests. Starting position of animals is indicated with a mouse illustration and each animal is allowed a fixed amount of time in the apparatus. (**a**) Open field test, measuring duration in peripheral region and frequency of quadrant crossing; (**b**) Elevated plus-maze, measuring duration in open arm and frequency of transitions between arms. For both tests, higher frequency / duration represents lower anxiety.

either mice or rats, results being largely non-significant (An et al., 2011; Gogokhia et al., 2021; Inglis et al., 2019). From one study that obtained significant results, contrasting conclusions can be reached focusing on different measurements: female rats enter the peripheral zone in the open field more often, and cover a longer distance than males, possibly pointing towards male-biased anxiety in rats; but they spend less time in the peripheral zone than males, suggesting the exact opposite (Keeley et al., 2015). It can thus be concluded that conventional rodent behavioural testing paradigms cannot establish a stable sex difference in anxiety-related behaviour. Noteworthy are, however, the various confounding factors underlying the testing schemes, including potential sex difference in the natural exploratory tendency of rodents to novel venues and

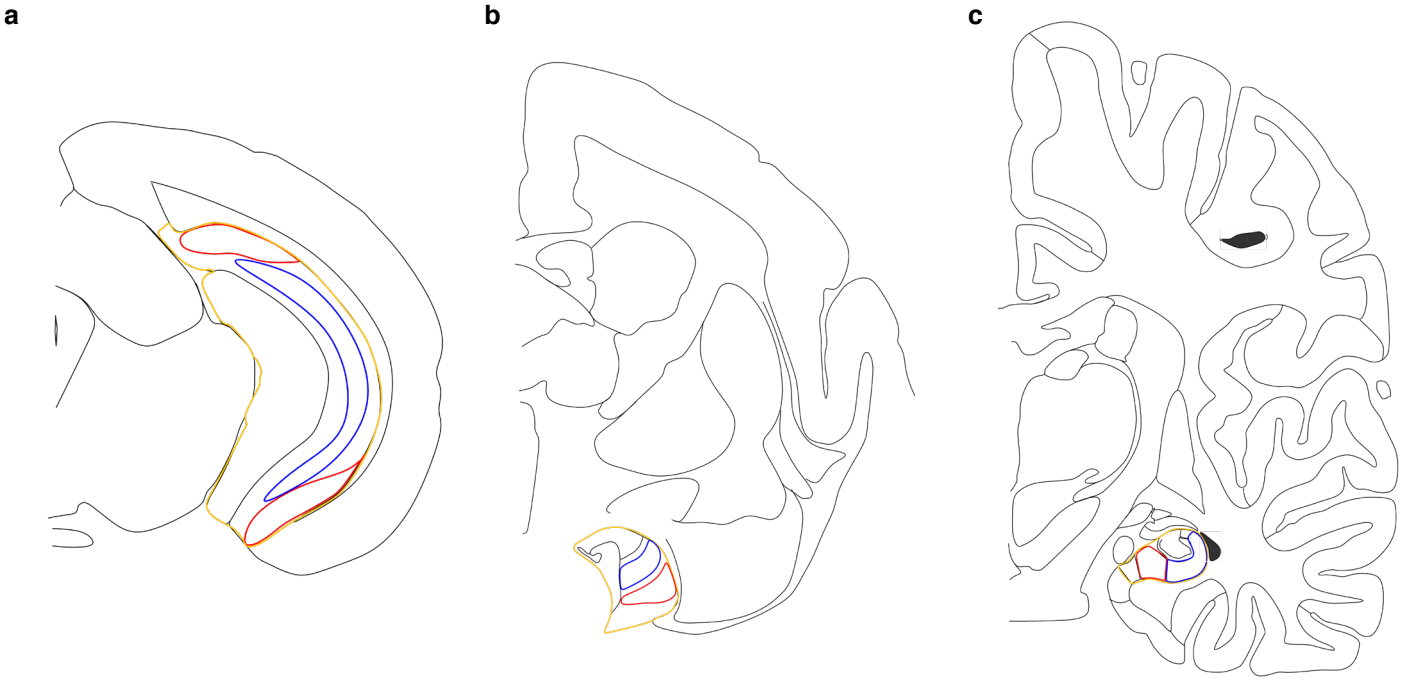

Figure 2 | Hippocampus (yellow), CA1 of hippocampus (red) and subiculum (blue) in: (**a**) mice *Mus musculus* (Paxinos & Franklin, 2001, redrawn from Plate 58); (**b**) common marmoset *Callithrix jacchus* (Paxinos et al., 2012, redrawn from fig. 77a, p. 146); (**c**) *Homo sapiens* (Mai et al., 2015, redrawn from fig. 47, p. 250)

unfamiliar objects and base-line locomotion activity, which may buffer any sex difference in anxious affective states. Another possible hypothesis is that these anxiety tests corroborated on male rodents cannot completely reflect anxiety in females, as suggested by the contrasting results from EPM and a novel acoustic startle testing protocol (Börchers et al., 2022). Hence, sex differences in the mechanisms underlying anxiety in rodents cannot be ruled out.

NHPs have only recently been established as an animal model for human anxiety, but conventional behavioural indicators of anxiety have been established (including scratching, yawning, freezing, and self-grooming), as have testing protocols examining animals' response to potentially threatening novel objects or human intruders. However, only two studies, to the author's knowledge, explicitly examined sex differences in the base-line anxiety level amongst NHPs. Macaques of different sexes are reported to show different anxiety-related behaviour in response to a human intruder, males yawning more often whilst females refraining to the back of the home-cage and freezing more often than males (Hamel et al., 2017). Another observational study scored the grand total of body shaking, scratching, grooming, and yawning in absence of any external stimuli and found that male macaques displayed these anxiety-related behaviours much more often than females, whether in single or paired housing (Baker et al., 2012). Although the measurement of grand-total is questionable, these initial results indicate a potential sex difference in the mechanism eliciting anxiety, reflected by differential behavioural presentations in different conditions.

Neurological continuity and discrepancy

There is a long line of evidence supporting the causal role of the limbic system, in particular the hippocampus, hypothalamic-pituitary-adrenal (HPA) axis, and amygdala, in anxiety-related behaviours and the anxious affective state. The homologous systems, as suggested by overwhelming evidence, are also functionally related to anxiety in rats (Flandreau et al., 2012), mice (Paretkar & Dimitrov, 2018) and NHPs (Roseboom et al., 2021). Moreover, the general structure of the cortical-subcortical regulatory network controlling anxiety, including medial and dorsolateral prefrontal cortex (mPFC/dlPFC), anterior cingulate cortex (ACC), caudate, habenular nuclei, and periaqueductal grey (PAG) is conserved across rodents (de Oliveira et al., 2019; Hou et al., 2022; McIlwrath et al., 2020), NHPs (Misslin, 2003; Pincus et al., 2021) and human (Awasthi et al., 2020; Galgano et al., 2019). In addition, orbitofrontal cortex (OFC) and bed nucleus of stria terminalis (BNST) are identified as upstream regulatory organs of the HPA axis and amygdala, respectively, in primate species but not in rodents (Awasthi et al., 2020; Berry et al., 2022; Pincus et al., 2021), potentially suggesting a unique role of primate studies in anxiety. Overall, the highly conserved general structure of neural networks governing anxiety provides a basis for any mechanistic continuity that might explain sex differences.

Sex differences in anxiety can be attributed to both global, histological, and genetic factors, and the different functional connection of specific regions. Of particular interest is the difference in the 5-hydroxytryptamine (5-HT) system, especially receptor binding and re-uptake of 5-HT from synaptic clefts via the 5-HT transporter (SERT/Slc6a4). Female rodents are reported to have a higher 5-HT content in the key anxiety-related regions including vmPFC and amygdala, whereas males have a higher 5-hydroxyindolacetate (5-HIAA)/5-HT ratio in these regions (Duchesne et al., 2009), concordant with female NHPs showing a much higher binding affinity of 5HT to SERT which indicates a much faster 5-HT turnover in females (Christian et al., 2009), but contrasting the finding that women synthesise less 5-HT than men (Sakai et al., 2006). The higher 5-HT turnover in female NHPs compounded with lower level of 5-HT synthesis in primate species coincide with the clinical presentation of highly anxious major depressive patients (Hou et al., 2006), providing a feasible explanation for female-biased susceptibility to anxiety in humans. Conversely, inhibition of SERT-mediated 5-HT re-uptake in rodents results in an increase in anxiety-related behaviour (Olivier et al., 2008), which contrasts the high 5-HT turnover in human affective disorders. It is evident that, whilst several physiological characters of the 5-HT system are conserved, its function is greatly altered during evolution from rodents to human, whereas sex differences in NHPs may be continuous with those of human in the 5-HT system. This is consistent with the closer phylogenetic relationship between human and NHP, as compared to rodents.

Sex difference is also manifest in the circuitry in specific brain circuits governing anxiety. One potential target is identified as the ventral hippocampus (including subiculum and field CA1) where lesions produced anxiolytic effects for male rodents selectively (Wang et al., 2019). One potential explanation for this is the higher abundance of GABAergic neurons projecting from BNST to ventral hippocampus in

male rodents, which are activated after fear conditioning, as evidenced by the expression of Fos (an immediate early gene in stress response) in these GABAergic neurons (Urien et al., 2022). This in turn agrees with the higher development rate of neurons in CA1 in male rodents facilitated by androgen (Zhang et al., 2008), and the later transition of these GABAergic neurons from excitatory to inhibitory function (Galanopoulou, 2008) which explain the higher connectivity of ventral hippocampus to anxiety-related systems. The more advanced development of ventral hippocampus is also implicated in humans, with higher branching and dendritic density in CA1 in male young adults (Markham et al., 2005), indicating a highly conserved developmental course of this region. However, the functional correlate of this similar ontogeny may have been altered: Studies with depressed female macaques show a reduced density of pre- and post-synaptic markers in CA1 (Willard et al., 2014), possibly suggesting a novel affective function of this region in primates. A reasonable hypothesis to explore is that new connections evolved in female primates in ventral hippocampus, which is likely given the remarkable change in hippocampal anatomy from rodents to primate species (**Figure 2**). Exploring the nature of these synapses as well the function of this region in male primates would cast more light on the inter-species continuity of this hot-spot in sex difference.

Can non-human primates bridge the gap?

From the above analysis it is clear that conventional rodent models have limited translatability to humans in investigating sex differences in anxiety. Whilst the overall structure of the neuronal circuitry governing anxiety is conserved, discrepancies still exist at genetic, anatomical, and functional levels. The conventional rodent behavioural tests also lack consistency, further limiting the predictive validity of rodent models in investigating sex difference in anxiety. On the other hand, despite the limited number of studies, NHP models have shown an early indication on their superiority, in that behavioural tests highly mimic the human response to potentially threatening scenarios, and in the greater correspondence of neural networks. However, it should be noted that anxiety-related behaviours may be affected by individual factors such as housing conditions, food and social interaction (other animals in the room; Baker et al., 2012). With the sample size innately limited for NHP studies compared to the vast clinical sample amongst humans, the sex versus individual factors effect may constitute a hurdle to the validity of results from NHPs. Nevertheless, as the functional (dis-)continuity between rodent models and humans can only be more fully understood by investigating NHP models, a transitional stage in evolution from rodents to humans, directly comparing the sexes in NHP behavioural studies is still a broad, open field worth exploring.

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