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Sex differences and similarities in depressive- and anxiety-like behaviour in the Wistar-Kyoto rat

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HIGHLIGHTS

- WKY rats exhibit depressive- and anxiety-like behaviours in a test battery.
- These behaviours are expressed in a sex-dependent manner.
- This study supports the use of male and female WKY rats in preclinical research.

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ABSTRACT

Depression is a debilitating psychiatric disorder that is highly comorbid with anxiety. Depression is twice as prevalent in women as in men, however, females remain underrepresented in preclinical research. The stress hyperresponsive Wistar-Kyoto (WKY) rat displays hypolocomotion in a novel aversive environment and depressive- and anxiety-like behaviours, which have been mostly characterised in males. The current study characterised behaviour in male and female rats in a battery of behavioural paradigms. Adult male and female WKY rats were tested in the open field and forced swim tests (tests with a locomotor component); and the marble burying, novelty-induced hypophagia and sucrose preference tests (tests with a minimal locomotor component) and 24 h home-cage locomotor activity was also monitored. The tests were compared against the Sprague-Dawley (SD) strain, a commonly used "control" strain.

SD, but not WKY, females exhibited higher home-cage locomotor activity compared to males. In the open field, WKY rats of both sexes exhibited a significant reduction in locomotor activity and increased anxiety-like behaviour as demonstrated by reduced time in the aversive inner zone of the open field, compared to SD counterparts. In the marble burying test, WKY females, but not males, exhibited a trend towards increased burying, indicative of anxiety-like/neophobic behaviour. In comparison, WKY males, but not females, exhibited enhanced novelty-induced hypophagia, indicative of increased anxiety-like behaviour compared to SD rats. In the forced swim test, WKY rats of both sexes spent more time immobile compared with SD counterparts, indicating depressive-like behaviour. However, in comparison to SD rats, WKY males, but not females, exhibited anhedonic-like behaviour. In conclusion, WKY rats exhibit depressive- and anxiety-like behaviours that are complex and nuanced depending on the sex of the rat and testing conditions. This study supports the use of a varied test battery to fully characterise depression/anxiety-like behaviour in male and female rats.

1. Introduction

Depression and anxiety are leading causes of disability with a lifetime prevalence of 17% for depression [1] and almost 29% for anxiety disorders [2]. The co-morbidity of depression and anxiety is common, observed in over half of depressed patients assessed in a clinical setting [3]. Anxiety contributes to the exacerbation of depression, results in a greater suicidal risk and is associated with increased resistance to treatment [4]. Genetic vulnerability, as determined through twin studies, contributes to depression-anxiety comorbidity [5,6].

Animal models play an important role in understanding the neurobiology underlying anxiety and depression. Indeed, much of what we know of the neural substrates involved in these disorders arises from animal studies [7,8]. However, there are many pitfalls and limitations

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associated with preclinical research, including a lack of reproducibility/reliability of models and a failure of translation. As such, valid, robust and reproducible models are required. Research is now moving away from older, classical animal models towards assessing phenotypes and using genetic models which will help uncover the mechanisms underlying disorders, leading to improved translational models and better treatment targets [9]. Although complex psychiatric disorders can never be truly recapitulated in animal models, there is conservation of certain phenotypes throughout species allowing us to measure behaviour and neurobiological factors that have relevance from animals to humans. Specifically, depression is associated with a cluster of phenotypes that can be reliably measured in laboratory animals, including anhedonia, changes in weight/appetite, behavioural despair, psychomotor alterations, and cognitive deficits [10]. Regarding anxiety, there are a number of well-validated tests for its assessment in rodents, which are primarily based on the response to an aversive context or approach-avoidance conflict [11].

The Wistar-Kyoto (WKY) inbred rat strain was originally developed for use in preclinical cardiovascular research as the normotensive control to the spontaneously hypertensive line [12]. However, it was noted that WKY rats had an increased risk of developing stress-related ulcers [13]. Since then, it has been repeatedly shown that WKY rats are hyperresponsive to stress and display anxiety- and depressive-like behaviours when compared to other rat strains; most commonly, the Sprague-Dawley (SD) rat strain. Specifically, WKY rats reliably display increased immobility in the forced swim test (FST) [14–17], impaired social behaviour [18] and reduced time spent in the aversive zones of the open field and elevated plus maze [19–22]. Thus, this strain has been proposed as a putative genetic model of depression with co-morbid anxiety [23,24], which possesses high face and construct validity.

WKY rats also demonstrate hypoactivity when exposed to a novel aversive arena [25,26]. This behaviour may confound many classical behavioural tests which have a pronounced locomotor component, namely the aforementioned FST, open field test and elevated plus maze [27,28]. As such, it is essential to characterise behaviours using tests that do not possess an overt locomotor component. Paradigms such as the novelty-induced hypophagia, sucrose preference and marble burying tests employ ethologically-relevant contexts which may be more appropriate for detecting differences in behaviours in this strain. To this end, the first aim of the current study was to characterise locomotor activity and anxiety- and depressive-like behaviour of the WKY rat in a battery of behavioural tests.

Sex and gender are fundamental variables in research, but have been largely overlooked. Males complete suicide more often than females [29], but women are twice as likely to suffer from depression and have a higher risk of inheriting depression compared to men [30–32], and are more likely experience comorbid anxiety [33]. These statistics highlight the need for further research into the biological reasons for these sex differences. However, a literature review revealed that almost 90% of animal studies in neuroscience used only males [34,35]. As it is impossible to assume that data obtained from male animals also relates to females, the National Institute of Health recently issued a call to action to increase the number of female rodents used in preclinical research [36]. Undoubtedly, this serious bias has majorly hindered understanding of the mechanisms contributing to psychiatric disorders and translation of preclinical findings to the clinic. In fact, increasing evidence indicates sex differences in the response to antidepressant drugs (for review see [37]). For example, female rats show differential response to antidepressant drugs (see [38]) and the Flinders Sensitive Line, a genetic model of depression, displays sex differences in response to antidepressants [39]. As such, proper consideration of both sexes in research could lead to personalised gender-based medicine, which may go a long way to treating disorders predominantly found in either sex.

The exclusion of half of the population in the majority of preclinical research is due to the widely held assumption that females are inherently more variable than males due to the oestrus cycle. However, a recent meta-analysis has revealed that female rodents are not any more variable than males across a range of behavioural, neuroanatomical and immunological variables [40], supporting their inclusion in research. As such, comparative studies directly examining differences between male and female rodent models of psychiatric diseases are essential to help answer whether both sexes can be incorporated into preclinical investigations [37].

Therefore, a second aim of this study was to characterise sex differences in depressive- and anxiety-like behaviour in WKY rats. Using a test-battery approach to further expand the phenotype of male and female WKY rats, we examined locomotor activity (in the home-cage and open field), anxiety-like behaviour in tests based on exploration (the novel open field) and motivation (novelty-induced hypophagia), neophobia (marble-burying test), physiological measures (defecation, weight gain), and depressive-related behaviours including anhedonia (sucrose preference test), and behavioural despair (FST). Such an investigation will help in comprehensively addressing the extent to which sex differences exist in this important genetic model of depression/anxiety.

2. Materials and methods

2.1. Animal husbandry

Male and female Sprague–Dawley (SD) and Wistar-Kyoto (WKY/NHsd) rats aged 6 weeks old at the time of arrival (aged 7–11 weeks during testing) were obtained from Harlan, UK. Animals were singly-housed in plastic bottom cages (45 × 25 × 20 cm) containing woodchip bedding, in a temperature controlled room (20 ± 2 °C), relative humidity of 40–60%, with a 12:12 h light-dark cycle (lights on at 0800 h). Rats were fed a standard laboratory diet of rat chow pellets (2014 14% rodent diet, Harlan, UK); food and water were available ad libitum. Experimental protocols were carried out in accordance with the guidelines and approval of the Animal Care and Research Ethics Committee, National University of Ireland, Galway, under licence from the Health Products Regulatory Agency and in compliance with the European Union directive 2010/63/EU as well as the ARRIVE guidelines from the National Centre for the Replacement Refinement and Reduction of Animals in Research [41].

2.2. Experimental design

Animals were handled and weighed daily and allowed one week to habituate to the standard housing unit before testing. Rats were subjected to a battery of behavioural tests in the following order: home-cage monitoring, open field test, marble burying test, novelty-induced hypophagia test, FST and sucrose preferences tests. The order of testing of animals was pseudo-randomised and experimenters blind to group identity performed testing and behavioural analysis. The number of rats per group was as follows: SD-Male (n = 9), SD-Female (n = 9), WKY-Male (n = 9), WKY-Female (n = 9). To reduce the impact of stress on females, oestrus cycle was not monitored during the experiment. Instead, oestrus stage at the time of death was recorded as a rudimentary measure of the point at which female rats were at the end of the study (between 10:00–14:00 h). Oestrus cycle stages were determined by the relative frequency of leukocyte, cornified and nucleated epithelial cells as seen by cytological examination of vaginal smear under a microscope [42] The proportions are as follows: SD females (metoestrus n = 1, dioestrus n = 4, pro-oestrus n = 2, and oestrus n = 2; and WKY females (metoestrus n = 2, dioestrus n = 2, pro-oestrus n = 4, oestrus n = 1). Arenas were cleaned with warm water and detergent between animals to remove odour cues. Testing was carried out during the light-cycle, except home-cage tracking which was 24 h.