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

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.

1.1 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 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 oestrous 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.

1.2 Materials and Methods

1.2.1 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 12:12h light-dark cycle (lights on at 0800h). 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].

1.2.2 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, oestrous 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 – 14:00h). 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 24h.

1.2.3 Home Cage Activity Monitoring

Home cage activity monitoring was carried out as described previously [43], with some modifications. Briefly, each rat was placed into a cage with black bedding and a dark Perspex baseplate, to provide a contrast to aid in tracking the animal. A video camera (situated approximately 62cm above each cage) recorded footage to a hard-drive over a 24h period. For analysis, a codec was added by Super© software which allowed for playback by EthoVisionXT® (Version 8 Noldus, Netherlands) video tracking software, where distance moved (cm) was measured.

1.2.4 Open Field Test

Locomotor activity and anxiety-like behaviour in a brightly-lit, novel arena was assessed using the open field test. Animals were placed for 5 min in the brightly-lit (220 lux at base of the arena) open field apparatus, which consisted of a circular arena (diameter 75cm) with a white floor (plastic-covered wood flooring) and reflective walls. Animals were removed from the home cage and placed at the edge of the outer area. Locomotor activity (distance moved, cm) and time spent in the aversive inner zone (diameter 50cm) was assessed using a video tracking system (EthoVisionXT®) as previously described [20].

1.2.5 Marble Burying Test

In response to a novel or aversive stimuli, many rodents will bury the offending object as a defence mechanism [44, 45] and increased marble burying is interpreted as neophobia and a marker of fear/anxiety in rodents [46]. Fresh woodchip bedding was added to cage bottoms to depth of 3cm. Twenty dark, shiny marbles were evenly placed in rows on the surface of the bedding and rats were placed into this bedding with their home-cage lid for 20 minutes, under ambient lighting (lux 25-30). The number of buried marbles (those that were at least two-thirds covered) [47] was recorded.

1.2.6 Novelty-Induced Hypophagia

The novelty-induced hypophagia paradigm is an ethologically-relevant test based on the inhibition of feeding induced by novelty [48]. Rats are trained to consume a

palatable substance and then presented with this food in a novel environment, creating a conflict between the motivation to consume, and the aversion induced by the novel environment. Latency to consume and the amount eaten are measured to infer the anxiety levels of the animal. The following protocol was based on that reported previously [49], with some modifications. Rats were trained to consume a palatable substance for three days before testing. Ten Cheerios® cereal pieces (Nestlé) were placed in a ramekin and placed in the home-cage. On the fourth day, home-cage testing was carried out, to determine baseline consumption and latency levels to ensure that there were no sex/strain differences that would confound interpretation of the data. On the test day, animals were placed in a novel cage with no bedding containing a ramekin with 20 Cheerios®, in a brightly-lit (450 lux at base of cage) room for 20 min. Parameters recorded were the number of treats eaten and latency to consume.

1.2.7 Forced Swim Test

Behavioural despair was assessed using the modified FST [50]. In brief, rats were placed into 45 x 20 cm cylinders containing water (23-25^{o}C, 30cm depth) for 15 minutes. Rats were then re-exposed to the swim arena 24h later (day 2) for a 5 min period. Behaviours were assessed using the time-sampling method [50] and included time spent immobile (floating and movements necessary to keep its head above water), swimming (horizontal movements) and climbing (vigorous, upward directed movements).

1.2.8 Sucrose Preference Test

Animals were presented with 1% w/v sucrose solution or tap water placed on the right or left side of the home cage for 3h, for three days from 2-5 pm. Animals were not water deprived before testing. The bottle position was alternated each day and bottles were weighed before and after testing. Sucrose preference, calculated as an average over the three days, was measured as the percentage of sucrose solution intake out of the total liquid consumed during the 3h test.

1.2.9 Statistical Analysis

Kolmogorov and Levene tests were used to determine normality and homogeneity of variance, respectively. Data were analysed using IBM SPSS 21 statistical program

and for parametric data were analysed using two-way ANOVA (factors of sex and strain), followed, where appropriate, by the Student-Newman-Keuls *post-hoc* test. Non-parametric data were analysed with Kruskal-Wallis followed by Mann-Whitney U *post-hoc* test where appropriate. $P \leq 0.05$ was deemed significant. All data are presented as the mean + SEM.

1.3 Results

1.3.1 Reduced body weight gain in WKY males compared to SD controls

Two-way ANOVA revealed a significant effect of strain ($F_{(1,32)}$ = 10.96, P=0.001), sex ($F_{(1,32)}$ = 237.92, P<0.001), and a sex × strain interaction ($F_{(1,32)}$ = 26.91, P<0.001) on the amount of weight gained over the study period. As groups differed in their weights at the start of the experiment, the difference in weight *gain* was calculated. Body weight gain was significantly less in females of both strains compared to their male counterparts. WKY males gained less weight compared to SD males (Table 1), as previously reported [51].

	Males	**Females**
SD	154.7±6.3g*	71.7±0.7g^{++}
WKY	120.4±1.1g	79.2±1.0g^{+}

Table 1. Weight gain (grams) as measured by calculating the difference between weight at the end of the study and the weight on arrival. Data presented as mean ± SEM, *P<0.05 vs. SD counterparts, $^{+}$P<0.05, $^{++}$P<0.01 vs. males.

1.3.2 WKY rats of both sexes display context-dependent locomotor hypoactivity and anxiogenic behaviour in the open field

Two-way ANOVA revealed an effect of sex ($F_{(1, 32)}$ = 24.98, P<0.001) and a sex × strain interaction ($F_{(1, 32)}$ = 13.91, P<0.01) on total distance moved over 24h in the home-cage. SD females showed increased home-cage locomotor activity compared to their male counterparts, an effect not seen in WKY females (Fig. 1A). In contrast, there was no difference in activity between male SD and WKY rats.

Two-way ANOVA revealed an effect of strain ($F_{(1, 32)}$ = 79.10, P<0.001) and sex ($F_{(1, 32)}$ = 15.12, P<0.001) on total distance moved in the open field and an effect of strain ($F_{(1, 32)}$ = 32.82, P<0.001) and sex ($F_{(1, 32)}$ = 7.08, P<0.01) on time spent in the inner zone of the open field. Females showed increased activity in the open field when compared to males. WKY rats of both sexes displayed reduced locomotor activity and decreased time spent in the aversive inner zone of the open field, compared

with SD counterparts (Fig. 1B&C). Kruskal-Wallis revealed a significant difference between groups for defecation in the open field (K = 18.4, P<0.001). WKY rats of both sexes defecated more frequently during the test, compared with SD counterparts (Fig. 1D).

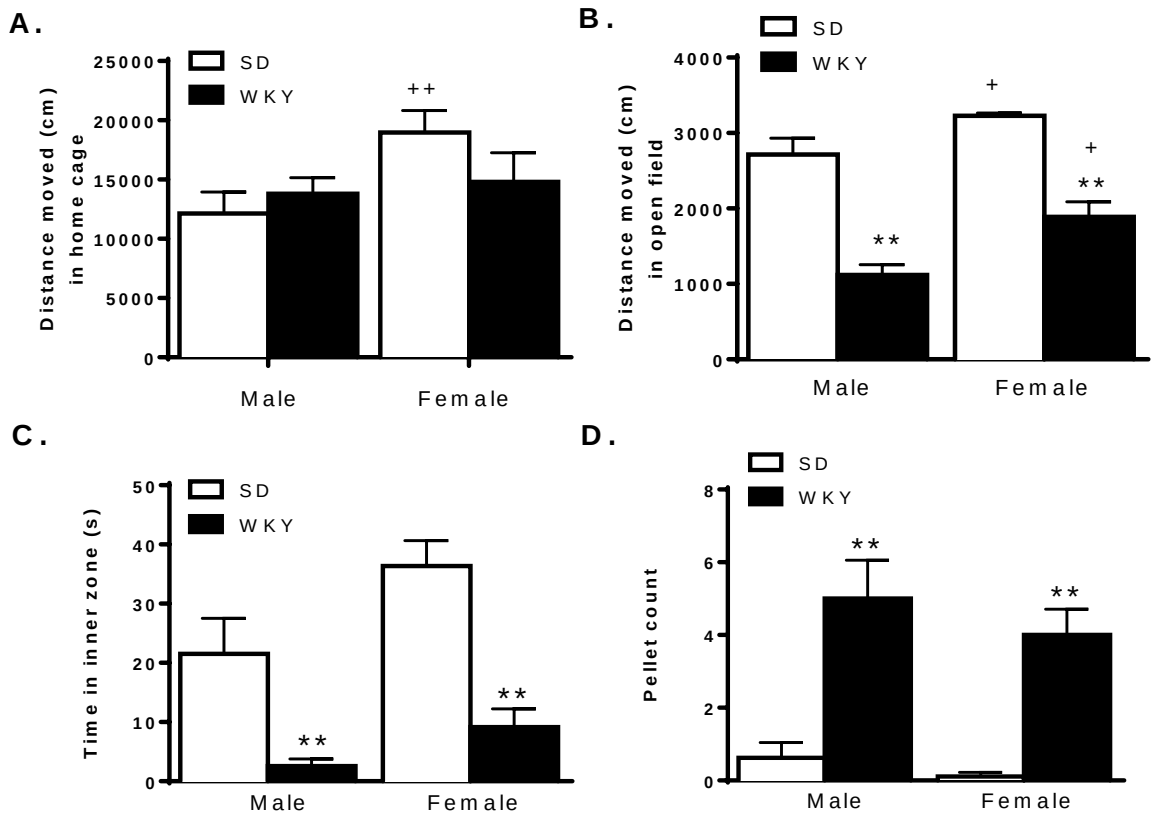

Fig. 1 Locomotor activity and anxiety-like behaviour of SD and WKY rats. (A) 24h home-cage locomotor activity (distance moved, cm). (B) Distance moved during 5 min in the open field (cm). (C) Time spent in the aversive inner zone of the open field. (D) Faecal pellet count at the end of the 5 min open field test. Data presented as mean ± SEM, n=9/group, **P<0.01 vs. SD counterparts, $^{+}$P<0.05, $^{++}$P<0.01 vs. male counterparts.

1.3.3 Burying of a novel object

Two-way ANOVA revealed an effect of sex ($F_{(1, 31)}$ = 4.59, P=0.04) and strain ($F_{(1, 31)}$ = 7.63, P=0.01) on the number of objects buried during the test. There was no

significant difference in burying behaviour between WKY males and their SD counterparts. Although it failed to reach statistical significance, WKY females exhibited a strong tendency to bury more marbles than SD female controls (Fig. 2).

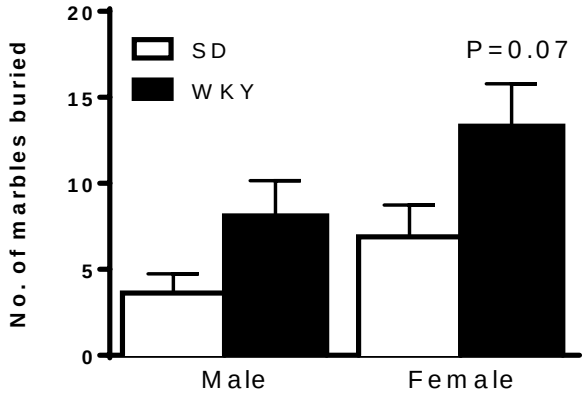

Figure 2. Number of objects buried during the marble burying test. Data presented as mean ± SEM, n=9/group. P=0.07 vs. SD.

1.3.4 WKY males, but not females, exhibited enhanced novelty-induced hypophagia when compared to SD counterparts

There was no significant effect of strain or sex on latency to consume in the home cage (SD males 9.1±4.1s; WKY males 14.6±5.1s; SD females 19.3±3.4s; WKY females 22.0±8.5s) or amount eaten (all animals consumed all treats during the home-cage session) and all animals exhibited hypophagia in the novel cage as demonstrated by increased latency to consume in the novel cage (Fig. 3a) vs. the home cage (see above data).

Two-way ANOVA revealed an effect of strain ($F_{(1, 31)} = 7.01$, $P=0.013$) and sex ($F_{(1, 31)} = 5.73$, $P>0.05$) and a sex × strain interaction ($F_{(1, 31)} = 12.97$, $P=0.001$) for latency to consume in the novel cage, and a sex × strain interaction for amount consumed ($F_{(1,32)} = 8.18$, $P=0.007$). SD females exhibited increased novelty-induced hypophagia when compared to SD male counterparts, an effect not seen in WKY rats. Male WKY rats exhibited increased latency to consume and reduced number of treats eaten when compared to SD counterparts, indicating augmented novelty-induced hypophagia (Fig. 3A&B). However, although WKY females did not have a

longer latency to consume in the novel cage when compared to WKY male counterparts, this group did consume more treats than their male counterparts.

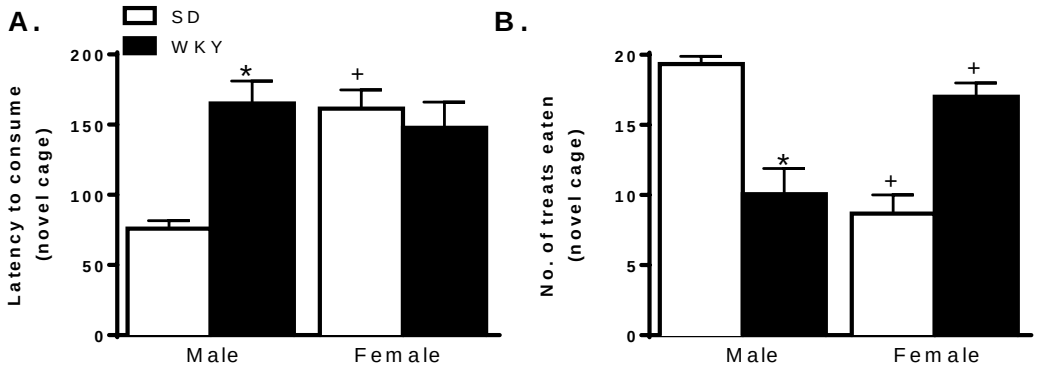

Figure 3. Novelty-induced hypophagia is enhanced in male WKY rats (A) Latency to consume treats in the novel cage. (B) Number of treats eaten in the novel cage. *P<0.05 vs. SD, $^{+}$P<0.05 vs. male.

1.3.5 Test-specific and sex-dependent depressive-like behaviour in WKY rats

In the FST, two-way ANOVA revealed a significant effect of strain ($F_{(1, 28)}$ = 12.55, P=0.001) on immobility time. Both male and female WKY rats exhibited increased time spent immobile when compared to their SD counterparts (Fig. 4A). Analysis of climbing behaviour in the FST revealed an effect of strain ($F_{(1,27)}$ = 18.49, P<0.001) and sex ($F_{(1,27)}$ = 3.78, P=0.03). WKY rats exhibited significantly less climbing compared to SD counterparts (SD vs. WKY males 23.0±2.9 vs. 8.5±1.9; SD vs. WKY females 13.3±2.5 vs. 7.89±1.9). There was no effect of strain or sex on swimming behaviour (data not shown).

In the SPT, two-way ANOVA revealed an effect of strain ($F_{(1,30)}$ = 4.37, P=0.045) and sex ($F_{(1,30)}$ = 4.67, P=0.039). WKY males showed less sucrose preference compared to SD males, an effect not seen in WKY females (Fig. 4B). There was no effect of sex or strain on total fluid consumption during the test (average total fluid intake: SD males 10±2 ml; WKY males 11±2ml; SD females 11±3 ml ; WKY females 7±1 ml).

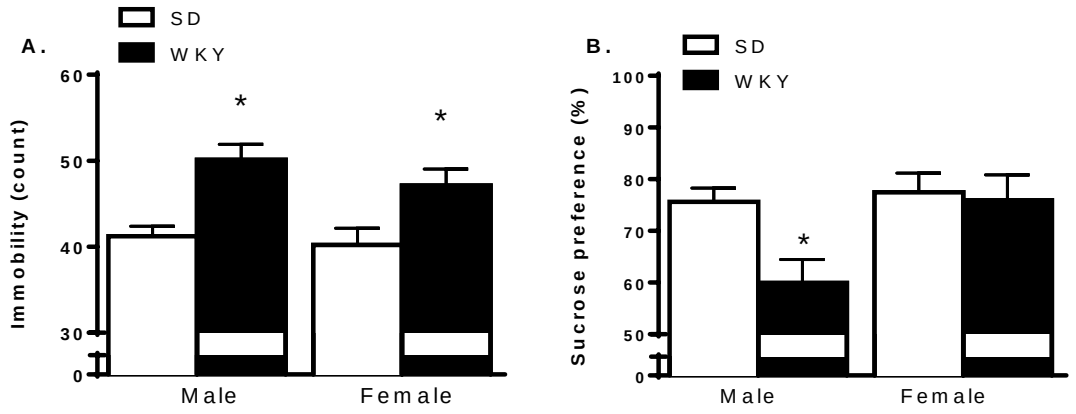

Figure 4. Effect of sex and strain on (A) immobility in the FST and (B) sucrose preference. Data expressed as Mean ± SEM (n=9 group). *P<0.01 vs. SD controls.

1.3.6 Summary of physiological and behavioural changes in WKY male and female rats

	WKY Male	***WKY Female***
Weight gain	⇓ weight gain	⇔
Home-cage	⇔	⇔
Open Field	⇓ LMA,⇓ time in centre, ⇑ defecation in OF	⇓ LMA,⇓ time in centre, ⇑ defecation in OF
Marble burying	⇔	⇑ marble burying (ns)
Novelty-induced hypophagia	⇑ NIH	⇔
FST	⇑ time spent immobile	⇑ time spent immobile
Sucrose preference test	⇓ sucrose preference	⇔

Table 2. Summary of physiological and behavioural changes in WKY rats when compared to SD counterparts. LMA (locomotor activity), NIH (Novelty-induced hypophagia), OF (open field test).

1.4 Discussion

We have shown that both male and female WKY rats demonstrate robust anxiety- and depressive-like behaviours in a battery of behavioural paradigms in comparison to SD counterparts. The precise expression of these behaviours is mostly maintained between the sexes but with some noted differences. WKY males and females exhibit consistent behaviour in classical tests of anxiety (open field test) and depressive-like behaviour (FST). However, only male WKY rats exhibit reduced weight gain, and enhanced novelty-induced hypophagia and anhedonia when compared to SD counterparts.

Rodents have an innate aversion to exposed, brightly-lit areas due to vulnerability to predators but are curious, exploratory animals. Exploration-based anxiety paradigms exploit the conflict between avoidance and the motivation to explore, thus are ethologically-relevant [52]. In accordance with previous reports [13, 14, 20, 51, 53], WKY rats in the present study demonstrated pronounced anxiety-like behaviour in the open field, compared with SD counterparts. Hypolocomotion was similarly observed in both sexes of WKY rats in the open field (versus SD controls), however this was not seen in the home-cage, underlining the critical differences in the behaviour expressed during these tests. The home-cage is non-stressful and perhaps a more reliable marker of general activity levels, compared to the novel open field, which is rather a measure of emotional reactivity. This suggests that WKY rats do not have an overt deficit in locomotor ability, but that this hypolocomotion is unmasked in a novel, aversive context, indicating a behaviourally inhibited phenotype, a risk factor for the development of anxiety disorders in humans [54]. Previous studies have shown that WKY rats demonstrate normal locomotor activity in a wheel-turn avoidance task [55], a running wheel [56], and the rotarod test [57]. Although WKY females were more active in the open field than WKY males, as previously reported [19, 58], they still remained significantly less active compared to SD females. Previous studies have shown that female rats tend to be more active compared to their male counterparts [59]. Taken together, the hypolocomotion observed in both male and female WKY rats in the open field may not confound other behavioural tests with a locomotor component in the absence of an aversive context, and may represent a behaviourally-inhibited phenotype.

To our knowledge, this is the first study examining WKY rats in the marble-burying test, with WKY females burying almost twice as many of the novel objects compared to SD females, though this failed to reach statistical significance. Male WKY rats were previously shown to bury an electrified probe earlier and for a longer duration than other strains [24, 53] and exhibit shock-exacerbated neophobia in the emergence test [60]. The marble-burying test is a much less stressful test, and therefore a stronger stressor may be required to unmask this behaviour in males. Burying of a novel/noxious object is an active behaviour that has been suggested to represent anxiogenesis or impulsivity and is useful paradigm to include in test batteries. Additionally, novelty-induced hypophagia is an ethologically-relevant paradigm which is largely free of the confound of alterations in locomotor activity. The novelty-induced hypophagia test has also made a valuable contribution to the field of depression research, given its sensitivity to chronic but not acute antidepressant administration [48], mimicking the clinical picture. We observed that male, but not female, WKY rats exhibited augmented novelty-induced hypophagia when compared to their SD counterparts. Male WKY rats have previously been shown to exhibit an increased latency to approach food and reduced amount consumed in the novelty suppressed feeding test when compared to male SD rats [51], with similar trends seen by others [53]. WKY females did not exhibit the sex-dependent increase in novelty-induced hypophagia seen in SD females. WKY females also consumed more treats compared to WKY males, despite both groups showing a relatively long latency, suggesting that WKY females may exhibit less novelty-induced hypophagia compared to controls, which may be due to increased preference or appetite [for review see 48], although these groups had identical consumption levels in the home cage. Of note, sex differences in WKY rats ability to develop avoidance and conditioned behaviours has been reported previously [61, 62], with deficits observed in males, but not females. Other studies examining sex differences in WKY rats have shown that both male and female WKY rats have an increased incidence of developing stress-related ulcers [63]. Interestingly, the behavioural phenotype (i.e. anxiety- and depressive-like behaviour) of a substrain of WKY males was observed from early adolescence, in contrast to their female counterparts where increased depressive- and anxiety-like behaviour appeared only in adulthood [64]. The effects of stress appear to be more striking in female than in male WKY rats, and female WKY rats are more vulnerable to chronic stress, as they

do not adapt to repeated stress [19]. Further probing the impact of chronic stress on female WKY rats may reveal additional behavioural deficits.

As previously reported, male and female WKY rats exhibited greater immobility in the FST when compared to SD counterparts, indicating increased behavioural despair [14, 16, 19, 20]. Males and females of this strain display this phenotype to an equal degree, providing a justification for using both sexes when screening for novel antidepressant compounds using this test. In contrast, the sucrose preference test revealed an anhedonic response in male, but not female, WKY rats. Reduced preference for a sweet solution in male WKY rats has been demonstrated in some [65, 66], but not all studies [51, 67]. Inconsistencies are likely due to differences in the protocols [e.g. comparator strain (Wistar vs. SD); sucrose percentage (1% vs. 20%), number of training days, or vehicle administration]. Using a self-administration paradigm, it has been shown that male WKY rats have decreased consumption of sucrose pellets, when compared to Wistar rats [68]. Differences in taste discrimination between sexes [69] may account for the failure of female WKY rats to exhibit anhedonia. It has been shown that males and females differ in their short-term and long-term preferences of sugars [70]. Future studies should use varying concentrations of sucrose and using a longer exposure to the test to determine if anhedonia can be revealed in female WKY rats.

Clinical findings have highlighted differences in the presentation of depressive symptoms between men and women. The landmark Sequenced Treatment Alternatives to Relieve study (STAR*D) by the National Institute of Mental Health mapped the symptoms of depression between the sexes. Out of the 30 symptoms characterised in depression, there were significant gender differences in 11 typical depressive symptoms [33]. Women tended to have increased appetite, increased weight gain and more somatic/gastrointestinal complaints. Men tended to have weight loss, psychomotor changes and suicidal ideation. Though these differences may relate in part to social pressures, biological factors, such as gonadal hormones, dysregulation of the hypothalamic–pituitary–adrenal axis, or genetics, undoubtedly contribute to sexual dimorphisms in symptomatology.

1.6 Conclusion

The data presented herein support the use of WKY rats of both sexes as a genetic model of depression with comorbid anxiety and extend the literature showing that WKY rats encapsulate a number of components relevant to depression including physiological changes, psychomotor retardation, comorbid anxiety and behavioural despair. In addition to these well-characterised phenotypes, we show that WKY rats exhibit sexually dimorphic behaviours in motivation, neophobia and anhedonia.

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