Pharmacology Biochemistry and Behavior, Vol. 64, No. 4, pp. 731–738, 1999

0091-3057/99/$–see front matter

ELSEVIER

PII S0091-3057(99)00139-2

Factor Analysis Shows That Female Rat Behaviour Is Characterized Primarily by Activity, Male Rats Are Driven by Sex and Anxiety

C. FERNANDES,* M. I. GONZÁLEZ,† C. A. WILSON† AND S. E. FILE[1]*

**Psychopharmacology Research Unit, Neuroscience Research Centre, GKT School of Biomedical Sciences, King's College London, Guy's Campus, London, UK; and*
†Department of Obstetrics and Gynaecology, St. George's Hospital Medical School, London, UK

FERNANDES, C., M. I. GONZÁLEZ, C. A. WILSON AND S. E. FILE. *Factor analysis shows that female rat behavior is characterized primarily by activity, male rats are driven by sex and anxiety.* PHARMACOL BIOCHEM BEHAV **64**(4) 731–738, 1999.—This experiment explored sex differences in behaviour using factor analysis to describe the relationship between different behavioral variables. A principal component solution with an orthogonal rotation of the factor matrix was used, ensuring that the extracted factors are independent of one another, and thus reflect separate processes. In the elevated plus-maze test of anxiety, in male rats factor 1 accounted for 75% of the variance and reflected anxiety, factor 2 represented activity, and accounted for 24% of the variance. This contrasted with the finding in female rats in which factor 1 was activity, accounting for 57% of the variance, with the anxiety factor accounting for only 34% of the variance. When behaviour in both the plus-maze and holeboard were analysed, a similar sex difference was found with anxiety emerging as factor 1 in males and holeboard activity as factor 1 in females. Locomotor activity in the inner portion of the holeboard loaded on the anxiety factor for males, but on activity for females. When behaviours in the plus-maze and sexual orientation tests were analysed, anxiety emerged as factor 1 in males, sexual preferences factor 2, and activity factor 3. In females, activity was factor 1, sexual preference factor 2, anxiety factor 3, and social interest factor 4. These results suggest caution should be exercised in interpreting the results from female rats in tests validated on males because the primary controlling factor may be different.
© 1999 Elsevier Science Inc.

Anxiety Motor activity Exploration Sexual preference Gender differences

SEXUAL differentiation is initially under genetic control with the sex-determining region of the Y chromosome (Sry) stimulating the development of the testes. However, further development of male characteristics in the fetus is under the hormonal control of testosterone secreted by the fetal testes (37). As well as the trophic effect on the male reproductive system, the testosterone, acting over a "critical period" (which in the rat extends over the last days in utero to about 10 days postpartum), masculinizes and defeminizes the brain, causing structural and neurochemical changes in the developing brain that do not occur in the female that is not exposed to androgen (25). Thus, in adulthood, there are sexual dimorphisms in the size of certain brain areas, in neuronal population and distribution, synaptic connections, and neurotransmitter concentrations and activity (5,9,34). There are sex differences in hypothalamic dopaminergic neuronal numbers and striatal uptake sites (26,29) and opioid (30) innervation in the adult brain. There are also transient sex differences in neurotransmitter activity over the neonatal period that disappear in adulthood, but seem to be important for the permanent structural dimorphisms (7,18,35,36). It is likely that these sexual dimorphisms are linked to the sex differences in various aspects of behaviour [see (19,22,36)].

It has been widely reported that sex differences exist in fear and anxiety-related behavior, as detected in many different behavioural test situations (3). Male rats are generally thought to exhibit more fear-related behaviors than females, as revealed by lower ambulation and rearing, and higher defecation in the open field (2,24), and reduced open-arm activity in the elevated plus-maze (20,21,23). Male rats have also been

[1] Requests for reprints should be addressed to Professor Sandra E. File, Psychopharmacology Research Unit, Hodgkin Building, Guy's Campus, London, SE1 9RT, UK.

shown to display more defensive behaviour in a social test situation as they approach and investigate conspecifics less than females (1).

The sex differences in behaviour measured in the open field and elevated plus-maze appear to be age-related, first emerging around day 60 of age (20,21,24). Furthermore, there is evidence that anxiety and activity, measured in the elevated plus-maze and open field, appeared to be determined mainly by brain sex following neonatal manipulations (10,23). However, exploration of the holeboard was determined by both the brain sex and hormones present at the time of testing (10).

Clear sex differences in the motivation to respond to aversive stimuli have been reported (32). Female rats seem to display more anxiety and fearfulness than males in the Vogel conflict test (21), and more defensive behaviours in situations involving anticipatory threat [e.g., following exposure to cat odor; Blanchard et al. (4)]. It has thus been suggested that male rats tend to show a behavioural inhibition after previous aversive stimulation, whereas female rats more readily show active responding (1) and their behaviour appears to be less dependent on previous experiences (32,33).

The aim of this experiment was to exploit the methodological tool of factor analysis to further explore sex differences in the behaviour of male and female rats in three different tests. The tests used were the holeboard, which provides independent measures of motor activity and exploration (14,15), the elevated plus-maze test of anxiety (28), and the sexual orientation test for sexual preference (8,17). Factor analysis provides a useful tool for the interpretation of behavioural data as it moves beyond the description of behaviours to the definition of the different factors, and underlying motivations, to which they refer.

METHOD

Animals

Male (n = 57) and female (n = 59) Wistar rats (bred at St. George's Hospital Medical School), were housed in same-sex groups of five or six from weaning. All animals were housed in the same animal room, maintained at 22°C, in a reversed light regimen 12 h on:12 h off (lights off 0800 h). Food and water were freely available. The animals were tested at approximately 90 days of age. The experimental procedures carried out in this study were in compliance with the UK Animal Scientific Procedures Act 1986.

Apparatus

Holeboard. The holeboard was a black Perspex box 60 × 60 × 35 cm with four holes, each 3.8 cm in diameter, equally spaced in the floor. Each rat was placed in the centre of the holeboard for a 5-min trial and at the end of each trial any faecal boluses were removed and the box was wiped clean with a damp cloth. Behaviours recorded were the number of head-dips, the time spent head-dipping, locomotor activity in the internal and external areas of the box (number of squares crossed), and the number of rears. The holeboard apparatus provides independent measures of motor activity and exploration (13,14).

Elevated plus-maze. The elevated plus-maze was made of wood and had two open arms (50 × 10 cm) and two enclosed arms of the same size with walls 40 cm high, elevated 62 cm above the ground. Each rat was placed in the central square (10 × 10 cm), facing an enclosed arm, and allowed to freely explore the maze for 5 min. At the end of each trial, any faecal boluses were removed from the maze, which was wiped clean with a damp cloth. The numbers of entries onto, and the times spent on, open and closed arms were recorded. Four paws into, and two paws out of, an arm defining an arm entry, and exit, respectively. For further details, see Pellow et al. (28).

Sexual orientation test. This test was carried out in a wooden circular arena (90 cm diameter) with a 30 cm-high Perspex wall. Two small wire-mesh cages (15 cm^2) were fixed to the arena wall such that the front of the cage was flush with the wall and the two cages were opposite each other. Each box housed a stimulus animal: a sexually experienced male and a receptive female. The test animals were familiarized with the apparatus by placing them in the arena without the stimulus animals for a daily 10-min period 2 days prior to the test day. Each rat was placed in the centre of the arena, facing away from the stimulus animals, and allowed to freely explore the arena for 5 min. At the end of each trial, any faecal boluses were removed from the arena, which was wiped clean with a damp cloth. Frequency of investigations and the time spent investigating each stimulus animal were recorded. For further details, see Gonzalez et al. (17).

Procedure

Behavioural testing started when the experimental animals were about 90 days old. In the group of female rats, only those in proestrous, determined by daily vaginal smears, were selected for behavioural testing. On day 1, all animals were tested in the holeboard for 5 min. Immediately after the end of the holeboard test, animals were tested for 5 min in the elevated plus-maze. One week later, the sexual orientation test was carried out. Because of the experimental time constraints, only 44 male and 39 female rats were tested for sexual orientation. All testing took place under quiet conditions and dim red light, between 1100 and 1700 h (i.e., 3 h into the dark period). Testing was carried out in an order randomised for sex type.

Statistics

The holeboard, elevated plus-maze, and sexual orientation data were analysed separately for males and females by factor analyses using a principal component solution with an orthogonal rotation (varimax) of the factor matrix, which ensures that the extracted factors are independent of one another and should, therefore, reflect separate processes. The number of factors extracted for each analysis was selected using a combination of two criteria—the 75% variance rule (the relevant matrix variance is accounted for when the sum of the proportionate contributions of the eigenvalues exceeds 0.75), and the root curve analysis (the point of inflection of a plot of the eigenvalues from largest to smallest). In the tables, the factors for eigenvalues $\geqslant 1$ are presented left to right in an order that corresponds to the decreasing size of the proportion of the original variance represented by each factor. The contribution of each behavioral variable to each factor is referred to as a factor loading. The higher the loading, the better the variable reflects a particular factor and, therefore, only factor loadings of >0.5 are reported in this study.

RESULTS

Factor Analysis on the Measures of Behavior in the Holeboard

In both sexes, two clear independent factors emerged from a factor analysis of the holeboard data—factor 1 reflecting exploration, and factor 2 reflecting motor activity (see Table 1).

TABLE 1

ORTHOGONAL FACTOR LOADINGS FOR MEASURES OF BEHAVIOUR IN MALE (n = 57) AND FEMALE (n = 59) RATS IN THE HOLEBOARD, ACCOUNTING FOR 79% AND 81% OF THE TOTAL VARIANCE, RESPECTIVELY

	Males		Females	
	Exploration (46%)	Motor Activity (33%)	Exploration (51%)	Motor Activity (30%)
Locomotor activity		0.86		0.80
No. of rears		0.78		0.91
No. of head dips	0.89		0.87	
Time spent head-dipping	0.90		0.94	

The percentage of the total variance accounted for by each factor is given in parentheses.

There were no sex differences in the factor analyses of holeboard data.

Factor Analysis on the Measures of Behaviour in the Elevated Plus-Maze

As can be seen from Table 2, two independent factors were extracted from the analysis of the elevated plus-maze measures in male and female rats.

In male rats, factor 1, on which open-arm activity loaded highly, was considered to be an index of anxiety. The number of entries into the closed arms and the total number of arm entries both contributed to factor 2. Factor 2 thus appears to be reflecting motor activity in the maze.

An important sex difference was observed in the factor analysis on behavioural measures in the elevated plus-maze. In female rats, the order of the factors (i.e., the proportion of the original variance represented by the factors) reflecting anxiety and motor activity were reversed so that measures of activity loaded on factor 1 and measures of anxiety loaded on factor 2.

Factor Analysis on the Behaviours Measured in the Holeboard and the Elevated Plus-Maze

Factor analysis was carried out on both the behaviours measured in the holeboard (including two extra measures of locomotor activity defined according to whether activity was in the internal or external parts of the holeboard) and in the elevated plus-maze.

In male rats, four independent factors were revealed, separately reflecting anxiety in the elevated plus-maze, activity in the holeboard, exploration in the holeboard, and activity in the elevated plus-maze (see Table 3). Factor 1 appeared to be reflecting anxiety, with the number of entries into, and time spent in, the open arms loading on this factor. Locomotor activity in the internal part of the holeboard also loaded on factor 1, suggesting that this may be a measure of anxiety in the holeboard in male rats.

Factor analysis on the behaviours measured in the holeboard, and the elevated plus-maze in female rats also revealed four independent factors, reflecting the same factors as found with male rats. However, the order of the factor reflecting anxiety in the elevated plus-maze and the factor reflecting activity in the holeboard was reversed in female rats compared with male rats (see Table 3). In addition, in female rats there was a shift in the pattern of factor loading of locomotor activity in the internal part of the holeboard, such that this measure loaded on the factor reflecting activity in the holeboard and not on the anxiety factor, as seen in male rats.

Factor Analysis on the Measures of Behaviour in the Sexual Orientation Test

Two clear independent factors emerged from the factor analysis on the behavioural measures in the sexual orientation test in male and female rats. Factor 1 appeared to be reflect-

TABLE 2

ORTHOGONAL FACTOR LOADINGS FOR MEASURES OF BEHAVIOUR IN MALE (n = 57) AND FEMALE (n = 59) RATS IN THE ELEVATED PLUS-MAZE, ACCOUNTING FOR 99 AND 91% OF THE TOTAL VARIANCE, RESPECTIVELY

	Males		Females	
	Anxiety (75%)	Motor Activity (24%)	Anxiety (57%)	Motor Activity (34%)
No. open-arm entries	0.94			0.82
Time spent in open arms	0.96			0.91
No. closed-arm entries		0.99	0.97	
Total No. arm entries		0.91	0.95	

The percentage of the total variance accounted for by each factor is given in parentheses.

TABLE 3

ORTHOGONAL FACTOR LOADINGS FOR MEASURES OF BEHAVIOUR IN MALE ($n = 57$) AND FEMALE ($n = 59$) RATS IN THE HOLEBOARD AND THE ELEVATED MAZE, ACCOUNTING FOR 84 AND 83% OF THE TOTAL VARIANCE, RESPECTIVELY

	Males				Females			
	Plus-Maze Anxiety (37%)	Holeboard Activity (21%)	Holeboard Exploration (15%)	Plus-Maze Activity (11%)	Holeboard Activity (38%)	Plus-Maze Anxiety (20%)	Holeboard Exploration (14%)	Plus-Maze Activity (11%)
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3	Factor 4
Locomotor activity (external part of the holeboard)		0.91			0.86			
Locomotor activity (internal part of the holeboard)	0.74				0.66			
Locomotor activity (total)		0.95			0.92			
No. of rears		0.50			0.73			
No. of head-dips			0.87				0.89	
Time spent head-dipping			0.90				0.89	
No. open-arm entries	0.81					0.81		
Time spent in open arms	0.88					0.88		
No. closed-arm entries				0.95				0.95
Total no. arm entries				0.91				0.93

The percentage of the total variance accounted for by each factor is given in parentheses.

ing sexual preference, with the number of investigations of, and time spent investigating, the opposite-sex stimulus animal, and the number of investigations of the same-sex stimulus animal loading on this factor (see Table 4). The time spent investigating the same-sex stimulus animal, possibly a measure of social interest, loaded on factor 2 in both sexes (see Table 4).

Although there were no sex differences in the order of the factors that emerged following factor analysis of sexual orientation data, a greater percent of the total variance was accounted for by factor 1 (sexual preference) in male rats (56%) compared with female rats (46%).

Factor Analysis on the Behaviours Measured in the Sexual Orientation Test and the Holeboard

Factor analysis on both the behaviours measured in the sexual orientation test and the holeboard revealed four independent factors in both sexes, separately reflecting sexual preference in the sexual orientation test, exploration in the holeboard, activity in the holeboard, and social interest in the sexual orientation test (see Table 5). The only difference in the factor analyses between male and female rats was regarding rearing activity. In male rats, the number of rears loaded on factor 3 and negatively loaded on 2, reflecting activity and exploration in the holeboard, respectively. However, in fe-

TABLE 4

ORTHOGONAL FACTOR LOADINGS FOR MEASURES OF BEHAVIOUR IN MALE ($n = 44$) AND FEMALE ($n = 39$) RATS IN THE SEXUAL ORIENTATION TEST, ACCOUNTING FOR 81 AND 77% OF THE TOTAL VARIANCE, RESPECTIVELY

	Males		Females	
	Sexual Preference (56%)	Social Interest (25%)	Sexual Preference (46%)	Social Interest (31%)
No. investigations female stimulus animal	0.93		0.59	0.71
Time investigating female stimulus animal	0.76			0.84
No. investigations male stimulus animal	0.83		0.94	
Time investigating male stimulus animal		0.99	0.72	

The percentage of the total variance accounted for by each factor is given in parentheses.

TABLE 5

ORTHOGONAL FACTOR LOADINGS FOR MEASURES OF BEHAVIOUR IN MALE ($n = 42$) AND FEMALE ($n = 33$) RATS IN THE SEXUAL ORIENTATION TEST AND THE HOLEBOARD, ACCOUNTING FOR 83 AND 84% OF THE TOTAL VARIANCE, RESPECTIVELY

	Males				Females			
	Sexual Preference (31%)	Holeboard Exploration (24%)	Holeboard Activity (16%)	Social Interest (12%)	Sexual Preference (29%)	Holeboard Exploration (22%)	Holeboard Activity (19%)	Social Interest (14%)
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3	Factor 4
No. investigations female stimulus animal	0.92				0.51		0.68	
Time investigating female stimulus animal	0.77							0.86
No. investigations male stimulus animal	0.85				0.89			
Time investigating male stimulus animal				0.98	0.81			
Locomotor activity			0.91				0.89	
No. of rears		−0.57	0.65				0.63	−0.55
No. of head dips		0.92				0.96		
Time spent head dipping		0.90				0.79		

The percentage of the total variance accounted for by each factor is given in parentheses.

male rats, rearing loaded on factor 3 and negatively loaded on factor 4, factors that reflect activity in the holeboard, and social interest in the sexual orientation test, respectively. Also, the number of investigations of the same-sex stimulus animal made by female rats loaded on both factors 1 and 3, so this measure may relate to both sexual preference and activity (see Table 5).

Factor Analysis on the Behaviours Measured in the Sexual Orientation Test and the Elevated Plus-Maze

Three factors emerged from the factor analysis on the sexual orientation test and the elevated plus-maze in male rats (see Table 6). Factor 1 related to anxiety measured in the elevated plus-maze, factor 2 reflected sexual preference measured in the sexual orientation test, and factor 3 corresponded

TABLE 6

ORTHOGONAL FACTOR LOADINGS FOR MEASURES OF BEHAVIOUR IN MALE ($n = 42$) AND FEMALE ($n = 33$) RATS IN THE SEXUAL ORIENTATION TEST AND THE ELEVATED PLUS-MAZE, ACCOUNTING FOR 82 AND 84% OF THE TOTAL VARIANCE, RESPECTIVELY

	Males			Females			
	Plus-Maze Anxiety (38%)	Sexual Preference (29%)	Plus-Maze Activity (15%)	Plus-Maze Activity (29%)	Sexual Preference (26%)	Plus-Maze Anxiety (18%)	Social Interest (11%)
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 4
No. investigations female stimulus animal		0.94			0.63		
Time investigating female stimulus animal		0.75					0.98
No. investigations male stimulus animal		0.85			0.90		
Time investigating male stimulus animal			−0.77		0.81		
No. open-arm entries	0.95					0.75	
Time spent in open arms	0.94					0.84	
No. closed-arm entries			0.76	0.93			
Total no. arm entries	0.72		0.64	0.96			

The percentage of the total variance accounted for by each factor is given in parentheses.

TABLE 7

ORTHOGONAL FACTOR LOADINGS FOR TWO MEASURES OF BEHAVIOUR FROM EACH OF THE THREE TESTS CONDUCTED IN MALE ($n = 42$) AND FEMALE ($n = 33$) RATS, ACCOUNTING FOR 67 AND 72% OF THE TOTAL VARIANCE, RESPECTIVELY

	Males			Females		
	Sexual Preference/Social Interest (27%)	Plus-Maze Anxiety/Activity (21%)	Holeboard Exploration/Activity (19%)	Activity (32%)	Social Interest/ Exploration (22%)	Sexual Preference (18%)
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Time investigating female stimulus animal	0.75				0.83	
Time investigating male stimulus animal	0.73					0.93
Locomotor activity			0.63	0.87		
Time spent head-dipping			0.81		0.75	
Time spent in open arms		0.90			−0.50	
No. closed-arm entries	−0.65	0.52		0.83		

The percentage of the total variance accounted for by each factor is given in parentheses.

to activity in the elevated plus-maze. Interestingly, the total number of arm entries in the elevated plus-maze loaded on the anxiety factor (factor 1) as well as on the activity factor (factor 3), providing further evidence that this measure does not provide an independent measure of general activity in the elevated plus-maze (6,11,12,13).

In female rats, four independent factors were extracted from the factor analysis on the sexual orientation test and the elevated plus-maze (see Table 6). These factors appeared to be reflecting activity in the elevated plus-maze (factor 1), sexual preference in the sexual orientation test (factor 2), anxiety in the elevated plus-maze (factor 3), and social interest in the sexual orientation test (factor 4).

Factor Analysis on the Behaviours Measured in the Sexual Orientation Test, the Holeboard and the Elevated Plus-Maze

In both male and female rats, three independent factors emerged from a factor analysis of the data from the three behavioural tests (see Table 7). However, there were important differences in these factors between the two sexes. In male rats, factors (primarily defined by the behavioural measure that had the highest loading on the factor) of sexual preference (factor 1), anxiety (factor 2), and exploration (factor 3) emerged. In female rats, the three factors that emerged from the factor analysis were activity (factor 1), social interest/exploration (factor 2), and sexual preference (factor 3).

DISCUSSION

Head-dipping is considered to be a measure of directed exploration in the holeboard because it reflects novel aspects of the environment, manipulated by placing objects under the holes, and results in information storage, indicated by habituation on repeated exposures (15). There is also pharmacological and factor analytical evidence for a separation of head-dipping and locomotor activity (12,13,15). Far greater variability between the scores of different sample groups from the same population was found for locomotor activity than for head-dipping (14). The present study also found that locomotor activity and head-dipping loaded on independent factors in both male and female rats, and there were no sex differences in the factor loadings on these measures. This suggests that in both sexes, the holeboard can be used to provide independent measures of exploration and locomotor activity. The same two independent factors of anxiety and activity emerged for both sexes in the plus-maze, which is in agreement with previous studies (6,11–13). However, an important sex difference emerged in the relative importance of these factors. Whereas in male rats the strongest factor was an anxiety factor, activity was the strongest factor in female rats. This would suggest that the behavior of female rats in the elevated plus-maze is characterized more by activity than anxiety. This suggests that although it would be possible to use the plus-maze to measure anxiety in female rats, it would be less sensitive to anxiolytic and anxiogenic drugs or treatments than in male rats. Furthermore, the plus-maze would be more sensitive to drug-induced changes in general activity in female, than in male, rats.

Analysing the measures from both the holeboard and the elevated plus-maze revealed independent factors, separately reflecting anxiety in the elevated plus-maze, activity in the holeboard, exploration in the holeboard, and activity in the elevated plus-maze in both sexes. Flaherty et al. (16) have found that the measures of anxiety in the plus-maze load on a different factor from the measures in the home-cage emergence test, but that the total arm entries in the plus-maze loaded negatively on the emergence factor. Once again, there was a key difference between the order of these factors between male and female rats, with activity again emerging as the strongest factor in female behaviour. This sex difference in the relative importance of the two factors controlling behaviour in the plus-maze reinforces the caution urged by Johnston and File (21) in not drawing general conclusions about whether male or female rats exhibit greater anxiety in the plus-maze.

The emergence of two factors related to activity in the two different tests provides further evidence that the nature of the activity measured in the holeboard is different from that measured in the elevated plus-maze, and that these behaviours are not interchangeable in either male and female rats. Interestingly, the activity of male rats in the internal part of the holeboard (internal ambulations) had a high factor loading on the anxiety factor, suggesting that internal ambulations in the holeboard may be a measure of anxiety in this test. This is of significance to the interpretation of the open field as a test of anxiety (31) as the holeboard resembles an open-field arena. This suggests that central activity in the holeboard could provide a useful measure of anxiety in male rats, but would not provide a valid measure in female rats. The problems inherent in using measures of exploratory behavior to measure anxiety are discussed in detail by Pellow (27). In some tests (i.e., the black–white crossing and home-cage emergence test), it is very difficult to determine whether a drug-induced change reflects a change in exploration or anxiety. The separation of measures in the plus-maze allows firmer conclusions to be drawn. It is clear from these results that more extensive validations are needed for several of the tests, and that they cannot be assumed to be valid for the use in female rats if the validation studies only used males.

In male rats the number of investigations of the same sex rat loaded on the sexual preference factor rather than on the social interest factor (see Tables 4 and 5). This raises the question as to whether the number of investigations is a good measure of sexual preference. Although this measure was recorded in the study by Gonzalez et al. (17), the only data presented were for the ratio of times spent investigating male and female stimulus animals. For the data analysed in the present study, the male rats made a mean ($\pm$SEM) of 12.1 $\pm$ 0.9 investigations of the female and 9.9 $\pm$ 0.7 investigations of the male. In contrast, the times spent investigating the stimulus animals showed a strong sexual preference with 160.4 $\pm$ 14.1 s spent investigating a female, compared with 79.1 $\pm$ 6.4 s spent investigating the male. It is possible that investigations of the same sex stimulus animal reflects primarily information gathering as to its sexual identity. For the female rats, the number of investigations loaded on both factors (Table 4), suggesting that it was performing both an information-gathering role as well as reflecting social interest.

Of the two factors revealed in the factor analysis of the sexual orientation test, that reflecting sexual preference accounted for more of the total variance than the other factor (relating to social interest) in male compared with female rats. This would suggest that the behaviour of male rats in this test is more strongly characterized by measures reflecting sexual preference than social interest, compared with female rats. Subsequent factor analysis on the behavioural measures in the sexual orientation test and the holeboard did not reveal any major sex differences, but there were important sex differences when the behaviours in the sexual orientation test were analyzed with those measured in the elevated plus-maze. The behaviour of male rats in these two tests appears to be defined primarily by anxiety and sexual preference, while female behaviour is characterized by activity and sexual preference. Factor analysis on the behavioural measures from all three tests further describes this sexually dimorphic pattern, with the behaviour of male rats appearing to be driven by sex and anxiety and female behaviour being characterized primarily by activity and exploration.

In conclusion, the results of these factor analyses provide further evidence that behavioural tests validated on male rats may not necessarily by applicable to female rats and, therefore, may provide less reliable measures. This probably reflects sex differences in behavioural strategies and response priorities, and extensive neurobiological studies are needed before these can be fully understood.

REFERENCES

1. Albonetti, M. E.; Farabollini, F.: Effects of single restraint on the defensive behaviour of male and female rats. Physiol. Behav. 57:431–437; 1995.
2. Archer, J.: Rodent sex differences in emotional and related behaviour. Behav. Biol. 14:451–479; 1975.
3. Beatty, W. W.: Gonadal hormones and sex differences in nonreproductive behaviors in rodents: Organizational and activational influences. Horm. Behav. 12:112–163; 1979.
4. Blanchard, R. J.; Blanchard, D. C.; Weiss, S. M.; Meyer, S.: The effects of ethanol and diazepam on reactions to predatory odors. Pharmacol. Biochem. Behav. 35:775–780; 1990.
5. Breedlove, S. M.: Sexual differentiation of the human nervous system. Annu. Rev. Psychol. 45:389–418; 1994.
6. Cruz, A. P. M.; Frei, F.; Graeff, F. G.: Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol. Biochem. Behav. 49:171–176; 1994.
7. Davies, A. M.; Gratton, D. R.; Selmanoff, M.; McCarthy, M. M.: Sex differences in glutamic acid decarboxylase mRNA in neonatal rat brain: Implications for sexual differentiation. Horm. Behav. 30:538–552; 1996.
8. De Jonge, F. H.; Burger, J.; van Haaren, F.; Overduk, H.; van de Poll, N. E.: Sexual experience and preference for males or females in the female rat. Behav. Neurol. Biol. 47:369–383; 1987.
9. de Vries, G. J.: Sex differences in neurotransmitter systems. J. Neuroendocrinol. 2:1–14; 1990.
10. Farabollini, F.; File, S. E.; Johnston, A. L.; Wilson, C. A.: An analysis of sex differences in the open field and tests of exploration and anxiety. Br. J. Pharmacol. 90:263P; 1987.
11. Fernandes, C.; File, S. E.: The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol. Biochem. Behav. 54:31–40; 1996.
12. File, S. E.: The biological basis of anxiety. In: Meltzer, H. Y.; Nerozzi, D., eds. Current practices and future developments in the pharmacotherapy of mental disorders. Amsterdam: Excerpta Medica; 1991:159–166.
13. File, S. E.: Behavioural detection of anxiolytic action. In: Elliott, J. M.; Heal, D. J.; Marsden, C. A., eds. Experimental approaches to anxiety and depression. London: John Wiley & Sons Ltd; 1992:25–44.
14. File, S. E.; Wardill, A. G.: The reliability of the holeboard apparatus. Psychopharmacologia 44:47–51; 1975.
15. File, S. E.; Wardill, A. G.: Validity of head-dipping as a measure of exploration in a modified holeboard. Psychopharmacologia 44:53–59; 1975.
16. Flaherty, C.; Greenwood, A.; Martin, J.; Leszczuk, M.: Relationship of negative contrast to animal models of fear and anxiety. Anim. Learn. Behav. 26:397–407; 1998.
17. González, M. I.; Albonetti, E.; Siddiqui, A.; Farabollini, F.; Wilson, C. A.: Neonatal organizational effects of the 5-HT_2 and 5-HT_{1A} subsystems on adult behavior in the rat. Pharmacol. Biochem. Behav. 54:195–203; 1996.
18. Gratton, D. R.; Selmanoff, M.: Sex differences in the activity of gamma-aminobutyric acidergic neurons in the rat hypothalmus. Brain Res. 715:244–249; 1997.
19. Hull, E. M.; Lorrain, D. S.; Du, J.; Matuszewich, L.; Bitrin, J. D.; Nishita, J. D.; Scaletta, L. L.: Organizational and activational effects of dopamine on male sexual behaviour. In: Ellis, L.; Ebertz, L., eds. Males, females and behaviour: Towards biological understanding. Westport, CT: Praeger Publ; 1998:79–86.
20. Imhof, J. T.; Coelho, Z. M. I.; Schmitt, M. L.; Morato, G. S.; Carobrez, A. P.: Influence of gender and age on performance of rats in the elevated plus-maze apparatus. Behav. Brain Res. 56:177–180; 1993.

21. Johnston, A. L.; File, S. E.: Sex differences in animal tests of anxiety. Physiol. Behav. 49:245–250; 1991.
22. Libertun, C.: The male and female nervous system or the nervous system as a secondary sexual characteristic: Introduction. Cell Mol. Neurobiol. 17:563–564; 1997.
23. Lucion, A. B.; Charchat, H.; Pereira, G. A. M.; Rasia-Filho, A. A.: Influence of early postnatal gonadal hormones on anxiety in adult male rats. Physiol. Behav. 60:1419–1423; 1996.
24. Masur, J.; Schutz, M. T.; Boerngen, R.: Gender differences in open-field behavior as a function of age. Dev. Psychobiol. 13:107–110; 1980.
25. McClusky, N. J.; Naftolin, F.: Sexual differentiation of the central nervous system. Science 211:1294–1303; 1981.
26. Morisetti, M.; di Paolo, T.: Sex and estrous cycle variations of rat striatal dopamine uptake sites. Neuroendocrinology 58:16–22; 1993.
27. Pellow, S.: Anxiolytic and anxiogenic drug effects in a novel test of anxiety: Are exploratory models of anxiety in rodents valid? Methods Find. Exp. Clin. Pharmacol. 8:557–565; 1986.
28. Pellow, S.; Chopin, P.; File, S. E.; Briley, M.: Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
29. Simerley, R. B.; Swanson, L. W.; Gorski, R. A.: Demonstration of a sexual dimorphism in the distribution of serotonin-immunoreactive fibers in the MPN of the rat. Comp. Neurol. 225:151–166; 1984.
30. Simerley, R. B.; McCall, L. D.; Watson, S. J.: Distribution of opioid peptides in the pre-optic region: Immunohistochemical evidence for a steroid sensitive enkephalin sexual dimorphism. J. Comp. Neurol. 276:442–449; 1988.
31. Simon, P.; Soubrie, P.: Behavioral studies to differentiate anxiolytic and sedative activity of the tranquillizing drugs. In: Boissier, J. R., ed. Modern problems of pharmacopsychiatry, vol 14. Differential psychopharmacology of anxiolytics and sedatives. Basel: S. Karger; 1979: 99–143.
32. Steenbergen, H. L.; Heinsbroek, R. P. W.; van Hest, A.; van de Poll, N. E.: Sex-dependent effects of inescapable shock administration on shuttlebox-escape performance and elevated plus-maze behavior. Physiol. Behav. 48:571–576; 1990.
33. Steenbergen, H. L.; Farabollini, F.; Heinsbroek, R. P. W.; van de Poll, N. E.: Sex-dependent effects of aversive-stimulation on holeboard and elevated plus-maze behavior. Behav. Brain Res. 43:159–165; 1991.
34. Tobet, S. A.; Hanna, I. K.: Ontogeny of sex differences in the mammalian hypothalamus and pre-optic centre. Cell Mol. Neurobiol. 17:565–601; 1997.
35. Vathy, I.; Rimanoczy, A.; Eaton, R. C.; Katay, L.: Sex dimorphic alterations in postnatal brain catecholamines after gestational morphine. Brain Res. Bull. 36:185–193; 1995.
36. Wilson, C. A.; Gonzalez, M. I.; Albonetti, M. E.; Farabollini, F.: The involvement of neonatal 5-HT receptor-mediated effects on sexual dimorphism of adult behaviour in the rat. In: Ellis, L.; Ebertz, L., eds. Males, females and behaviour: Towards biological understanding. Westport, CT: Praeger Publ; 1998:109–128.
37. Wilson, J. D.; George, F. W.; Griffin, J. R.: The hormonal control of sexual development. Science 211:1278–1284; 1981.