Sex differences in the emotional behavior of three strains of laboratory rat*

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Sex differences in the emotional behavior of three strains of rats were investigated, using a variety of measures taken during the initial 2 min of open-field exposure and after a loud bell had sounded. Animals were tested either in a clean open field or in one containing the odor of a same-sex conspecific. No general overall sex differences for a variety of measures associated with emotional reactivity were found, so the results did not support the theory that male rats are more "emotional" than females. Sex differences for particular measures associated with emotional responsiveness were found, but these varied with the strain, measure, and odor condition: The latter was found to be particularly important.

Sex differences in the behavior of rodents in novel environments and to other test situations for "emotional" behavior have been frequently reported in the literature. Typically, females show more ambulation and less defecation in an open field (e.g., Broadhurst, 1957, 1958; Henderson, 1963; Gray, Levine, & Broadhurst, 1965), they emerge sooner from their home cage into a novel environment (e.g., Meyers, 1962; Woods, 1962; Pfaff & Zigmond, 1971), and they show faster acquisition of a shuttlebox avoidance learning task (studies reviewed by Gray, 1971; Beatty & Beatty, 1970) than do males. Gray (1971) interpreted these and other results as supporting the hypothesis that male rodents were more fearful than females.

Gray's theory was criticized by the present author (Archer, 1971) on several grounds. One of these was that sex differences in open-field measures were more variable than Gray suggested, strain differences being a particularly important determinant of this variation. Another criticism was that the commonly used open-field measures (ambulation and defecation) were equivocal, since their hypothetical inverse relationship is found in only some, but not all, studies involving the appropriate correlation data. It was further suggested (Archer, 1973) that variables other than emotional reactivity could influence these measures, e.g., ambulation could, under different circumstances, reflect causally different types of behavior (e.g., lack of "freezing," presence of escape behavior, lack of lying or sitting, or lack of sniffing at localized stimuli). A fuller discussion of these and related issues is included in Archer (1973).

Odors are particularly important in the social behavior of rodents (review by Cheal & Sprott, 1971). With a few recent exceptions (Whittier & McReynolds, 1965; Stevens & Koster, 1972; Davies & Bellamy, 1972), little attempt has been made in open-field studies to control

for the possibility that a previously tested animal might influence the behavior of a currently tested one.

The present paper describes an investigation of sex differences in the emotional behavior of rats in an open-field test, modified to overcome some of the limitations suggested above. Several methodological changes were introduced compared with previous work: First, three strains were used, so that general and strain-specific sex differences could be distinguished. Second, a range of measures of emotional behavior, rather than merely ambulation and defecation, were used: These included escape behavior, reluctance to leave the walls of the open field, and defecation and freezing under two different circumstances. They were chosen as providing a range of behavior associated with "emotional responsiveness;" thus, sex differences could be studied in more detail than on the basis of the more usual ambulation and defecation measures. Third, the present work provided separate data for rats tested in a clean (odor-free) open field and for rats tested in a field containing an odor from the same sex and strain. Thus, any effect of differential responsiveness to odors of the same-sex conspecific was not confounded with differential responsiveness to the open-field situation, as may have occurred in many previous studies.

METHOD

Adult male and female rats, 60 of each sex, aged 2-3 months, and housed five to a cage, were each tested once in an open field. The sample of rats comprised 40 Sprague-Dawley (albino), 40 Wistar (albino), and 40 Long-Evans (hooded) strains. Two open fields were used, both of the type described by Broadhurst (1957). The procedure involved testing two male rats from the same strain consecutively in one open field and two females, also from the same strain, consecutively in the other open field on each day. The floors of both open fields were then washed. Thus, the first rat tested on any particular day was exposed to a relatively clean apparatus (henceforth termed the NOD, or "no odor," condition), whereas the second rat was exposed to a field containing the recently deposited odor of a conspecific (only the feces were removed); this will be referred to as the OD, or "odor," condition.

The testing procedure (described in Archer, 1973) involved a

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Table 1

Measure	Males (N = 60)	Females (N = 60)	p*
Latency to inner circle (seconds)	66	81.5	0.04
Open-field defecation (boli)	2.5	2.0	n.s.
Walking-sniffing (time samples)	4.5	5.0	0.1
Stationary-sniffing (time samples)	5.0	4.0	0.0016
Rearing (time samples)	1.5	2.0	n.s.
Grooming (time samples)	0	0	n.s.
Immobility (time samples)	0	0	n.s.
Bell: defecation (boli)	1	1	n.s.
Bell: flight distance (squares entered)	7	8	n.s.
Bell: latency to move head (seconds)	24	22	n.s.
Bell: latency to move paw (seconds)	69	40	0.1
Bell: latency to leave square (seconds)	120	120	n.s.

*Wilcoxon matched-pairs test, two-tailed probabilities (Siegel, 1956).

2-min exposure to the open field, followed by the ringing of a loud doorbell for 7 sec. after which there was a further 2-min observation period. The measures taken were, first, time samples every 10 sec. of behavior occurring during the first 2-min exposure, classified as walking and sniffing (WS), stationary and sniffing (SS), walking or running (W), rearing (R), immobility (I), and grooming (G). Second, various behaviors likely to be associated with emotional responsiveness were measured: defecation in the open field during the first 2-min exposure, defecation in the 2 min after the bell, latency to leave the squares adjacent to the walls and enter the center, the number of squares crossed when the bell was sounded ("flight distance"), latency to (a) move the head, (b) move the paws, and (c) leave the square after the bell had sounded (these measures indicate the time spent "freezing").

Treatment of the Results

Analysis was carried out as follows using two-sample nonparametric tests (Siegel, 1956): (1) a preliminary analysis of overall sex differences, all strains and odor conditions combined; (2) the three strains treated separately; (3) within each strain, OD and NOD conditions were compared within each sex, then the effect on sex differences of considering NOD and OD conditions separately was examined. For Analyses 1 and 2, Wilcoxon matched-pairs tests were carried out on animals from the same day and the same odor conditions. For Analysis 3 animals of the same sex tested on the same day were compared again using the Wilcoxon test.

RESULTS

(a) Overall Sex Differences

Table 1 shows the findings for overall sex differences for all strains combined. Only two measures are significantly different at the 0.05 level: males moving to the inner circles sooner than females and showing more "stationary and sniffing" (SS) during their time samples for the first 2 min.

(b) Sex Differences Within Strains

Table 2 shows the sex differences occurring for each strain. Several measures showed significant or near-significant sex differences for the Sprague-Dawley rats, the males entering the inner circles sooner,

defecating less in response to the bell and in the open field, and showing more SS during the first 2 min.

Wistar showed no significant sex differences for any measure, whereas Long-Evans rats showed significant differences only for "walking and sniffing" (WS), females showing more than males. (Males also showed more SS than females, $p = 0.051$).

(c) Differences between NOD and OD Conditions in Relation to Sex Differences

Sprague-Dawley

Table 3 shows the differences between rats tested in the NOD condition and in the OD condition in the open field for the two sexes separately. Measures not shown in the table were little different in the two conditions.

In males, the first four measures shown in Table 3, which are all likely indications of emotional responsiveness, were higher in the OD condition than in the NOD one, two of them significantly so. No such differences were apparent for the same measures in females.

The effect of these two different odor conditions on sex differences is shown in the two right-hand columns of Table 3. Rats tested first (NOD) showed more pronounced sex differences in the four measures than those tested second (OD); significance levels for sex differences between NOD rats were, therefore, higher than for the two odor conditions combined (Table 2), whereas those for the OD rats were much reduced and none were statistically significant.

Wistar

Table 4 shows the differences between NOD and OD conditions for Wistar rats. In contrast to the Sprague-Dawleys, there was no consistent increase in measures of emotional responsiveness from the first to the second tested males, but there was a significant increase in defecation following the bell. This difference

Table 2
Sex Differences in Open-Field Behavior of Three Strains of Rats (Medians)

Measure	Sprague-Dawley			Wistar			Long-Evans		
	Male (N = 20)	Female (N = 20)	p*	Male (N = 20)	Female (N = 20)	p*	Male (N = 20)	Female (N = 20)	p*
Latency to inner circle (seconds)	33.5	61.5	0.02	120	120	n.s.	46	71.5	n.s.
Open-field defecation (boli)	4	5	0.056	1	1	n.s.	1.5	0.5	n.s.
Walking-sniffing (time samples)	3	5.5	0.1	3	4	n.s.	5	6	0.1-0.02
Stationary-sniffing (time samples)	4	2	0.02-0.01	6	5	n.s.	4.5	3.5	0.1-0.05
Rearing (time samples)	2	3	n.s.	1	1	n.s.	1	2	n.s.
Grooming (time samples)	1	1	n.s.	0	0	n.s.	0	0	n.s.
Immobility (time samples)	0	0	n.s.	0	1	n.s.	0	0	n.s.
Bell: defecation (boli)	0	1	0.05	1	1	n.s.	1	1	n.s.
Bell: flight distance (squares entered)	5	6	n.s.	6.5	7.5	n.s.	8	9.5	n.s.
Bell: latency to move head (seconds)	13.5	13	n.s.	33	36	n.s.	23	20.5	n.s.
Bell: latency to move leg (seconds)	23	22.5	n.s.	63	53.5	n.s.	98.5	40	n.s.
Bell: latency to leave square (seconds)	101	80.5	n.s.	120	120	n.s.	120	120	n.s.

*Wilcoxon matched-pairs test, two-tailed probabilities.

Table 3
Behavior of Sprague-Dawley Rats Tested in "No-Odor" (NOD) and "Odor" (OD) Conditions in the Open Field (Medians)

Measure	Males				Females				p† Value for Sex Difference	
	NOD	OD	Median Increase	p*	NOD	OD	Median Increase	p*	NOD	OD
Open-field defecation	3.5	4.0	+2	n.s.	4.5	5	-0.5	n.s.	0.05	n.s.
Median latency to inner circle (seconds)	27.5	48.5	+18	n.s.	61.5	62	+1	n.s.	< 0.01	n.s.
Bell: median flight distance (squares entered) N = 10	5	7	+3	0.05	6	5	-1	n.s.		
Bell: median defecation	0	1	+1	0.05	1	1	0	n.s.	< 0.01	n.s.

*Wilcoxon matched-pairs test, two-tailed probabilities

†Mann-Whitney U test, two-tailed probabilities

Table 4
Behavior of Wistar Rats Tested in "No-Odor" (NOD) and "Odor" (OD) Conditions in the Open Field (Medians)

Measure	Males				Females				p Value for Sex Difference	
	NOD	OD	Median Increase	p*	NOD	OD	Median Increase	p*	NOD	OD
Open-field defecation	1.5	0.5	0	n.s.	1.5	0	0	n.s.	n.s.	n.s.
Median latency to inner circle (seconds)	120	120	0	n.s.	120	120	0	n.s.	n.s.	n.s.
Bell: median flight distance (squares entered)	4.5	7	+4	n.s.	6.5	8.5	+1	n.s.	n.s.	n.s.
Bell: median defecation	1	2.5	+2	< 0.02	2	0	0	n.s.	n.s.	0.1-0.05
Stationary-sniff (time samples)	6.5	5.5	-2	0.1-0.05	5	4.5	-2	n.s.	n.s.	n.s.
Walk-sniff (time samples)	3	5	+2	0.01**	4	4	+1.5	n.s.**	n.s.	n.s.
Immobility (time samples)	1	0	-1	n.s.†	1.5	0.5	-2.5	0.08†	n.s.	n.s.

*Wilcoxon matched-pairs test, two-tailed probabilities

**p value for males and females combined 0.02-0.01

†p value for males and females combined 0.05-0.02

had only a marginal effect on the separate significance levels for sex differences in NOD and OD rats (Table 4, two right-hand columns).

More striking was the general pattern for both sexes that WS increased and immobility (I) decreased from the NOD to the OD condition. Since these differences were largely common to both sexes, they did not affect the

magnitude of the sex difference calculated separately for OD and NOD rats (Table 4, two right-hand columns).

Long-Evans

Table 5 shows the differences between Long-Evans rats tested under NOD and OD conditions for the two

Table 5
Behavior of Long-Evans Rats Tested in Clean or Odor-Containing Open Fields (Medians)

Measure	Males				Females				p Value for Sex Difference	
	NOD	OD	Median Increase	p*	NOD	OD	Median Increase	p*	NOD	OD
Open-field defecation	3	1	-1	0.02	1	0.5	0	n.s.	0.05	n.s.
Median latency to inner circle (seconds)	47	46	-1.5	n.s.	76.5	60.5	-19.5	n.s.	n.s.	n.s.
Bell: median flight distance (squares crossed)	9.5	7	-1.5	n.s.	7	13	+4.5	< 0.01	n.s.	.1
Bell: median defecation	1	1.5	+0.5	n.s.	1	1.5	+0.5	n.s.	n.s.	n.s.
Stationary-sniff (time sample)	4.5	5	+1	n.s.	3.5	3.5	0	n.s.	n.s.	.05-.02
Walk-sniff (time sample)	5	5	0	n.s.	5	7	+1.5	0.02	n.s.	.02-.002
Rearing (time sample)	1	1.5	0	n.s.	1	2	+1	n.s.	n.s.	n.s.

*Wilcoxon matched-pairs test, two-tailed probabilities

sexes. There was no indication of increased emotional responsiveness from the first to the second tested male rats, as there was for the Sprague-Dawleys. In fact, open-field defecation showed a significant *decline* from the first to the second tested rat.

Females showed significantly increased "flight distance" and significantly reduced WS from the NOD to the OD condition (also a significant increase in R and a decrease in the latency to enter the inner circle).

DISCUSSION

General Sex Differences in Emotional Behavior

On the basis of measures such as open-field ambulation and defecation taken over repeated test days, Gray (1971) suggested that male rats are more "emotional" than females. The present open-field measures involved more direct and varied indications of initial emotional responsiveness to novel and startling stimuli; however, they revealed no clear-cut sex differences in emotional behavior throughout the three strains. They, therefore, support the case presented elsewhere (Archer, 1971) that the sex differences discussed by Gray may be more readily explained as several different effects, not necessarily related to initial emotional responsiveness to novel stimuli.

The only two significant sex differences found for all the three strains combined (Table 1) were in SS (stationary sniffing) and in latency to enter the inner circle. Males showed more SS, which is consistent with previous findings that they are generally less active than females (reviewed by Gray, 1971; Archer, 1971). On other measures, however, the males showed no indication of greater emotional responsiveness, which is Gray's interpretation of the activity difference. The present findings that males entered the inner circles *sooner* than females, despite their lower level of ambulation (measured by WS), is a strong argument

against Gray's interpretation of the sex differences in activity.

When data for the three strains were examined separately, there was no evidence that either Wistar or Long-Evans rats showed sex differences in general emotional reactivity. However, Sprague-Dawley males showed significantly lower values than Sprague-Dawley females for three measures associated with emotional reactivity (latency to enter the inner circle, open-field defecation, defecation following the bell), but there were no significant differences for two other such measures (flight distance and freezing times following the bell). In this strain, therefore, three measures indicated lesser emotional responsiveness by males than by females, the opposite of the general case argued by Gray (1971). It is also interesting to note that open-field defecation (i.e., during the first 2 min) was higher for *females* than males: The reverse has been more generally reported in the literature in studies involving several consecutive trials (reviewed by Gray, 1971). It seems likely that the duration of testing could account for this discrepancy.

Effects of Odors from Conspecific

Previous studies of sex differences have generally involved little or no control of odor cues, and it is, therefore, difficult to assess the separate sex differences for NOD and OD rats in relation to previous findings.

Male Sprague-Dawleys tested in the OD condition showed higher values for measures of emotional responsiveness than those tested in the NOD condition. One obvious explanation is that the first rat produced a "fear odor" (since it has been startled and induced to flee and freeze by the bell) and that this increased the general emotional reactivity of the second tested rat, as indicated by its higher scores for flight distance, latency to enter the inner circle, and defecation scores. Valenta and Rigby (1968) have shown that Sprague-Dawley rats (sex unspecified) can distinguish between the odors of a shocked and a nonshocked conspecific, but they did not

investigate whether the odor from the shocked rat acted as an "alarm" or "fear" signal. The present findings suggest that there may be a fear odor in this strain of rat, but further evidence will be required before we can make a more definite and general statement.

These results are also interesting for the way in which they affect sex differences. Sprague-Dawley rats tested in the NOD condition showed clear sex differences in emotional responsiveness (males defecating less in the open field and to the bell and entering the inner circles sooner), but no such differences were found for the OD condition. This result could have important implications for interpreting previous studies of sex differences in this strain, in that an odor from a previously tested male might increase the emotional reactivity of males but females would not be affected by female odor. The precise effect would, of course, depend on the order of testing and how odors from one sex affect the emotional reactivity of the opposite sex.

In contrast to the Sprague-Dawleys, the results for Wistars showed no evidence of a possible "fear odor." Generally, in this strain the differences between NOD and OD conditions had little effect on sex differences. Of more interest was an effect that occurred in both sexes: The second tested rat showed more walking and sniffing (WS) and less immobility (I) and stationary sniffing (SS). Van Oortmerssen (1971), studying mice, also found more extensive locomotion in a novel cage when odor cues from another conspecific were present.

In the Long-Evans strain, females showed more WS and longer flight distances in the OD than the NOD conditions; since the difference in flight distance was not part of a general difference in emotional reactivity (Table 5), these results suggest that the residual odor produced a general increase in motor activity. Since the effect was confined to females, sex differences for the OD and NOD conditions were affected: In the former case, SS and WS were significantly different (less SS and more WS in females), whereas they were not different in the NOD condition.

The results for the Long-Evans rats showed no evidence for a "fear odor." On the contrary, open-field defecation showed a significant decline from the NOD to the OD condition in males but not in females (Table 5). This might possibly indicate that defecation functions as part of a marking system in this sex and strain. Other species of mammals are known to mark their feces with a secretion from anal glands and, in some cases, a territorial marking function has been demonstrated in the male (e.g., in rabbits: Mykytowycz, 1966; Hesterman & Mykytowycz, 1968). This type of function has also been suggested for defecation in male mice (Collins, 1966; Bruell, 1969; Brain & Nowell, 1969), but there is no direct evidence at present. A similar form of marking response could account for the present results, in that the first male rat shows general scent marking (feces, urine, and other odor sources) in the clean field and the second one marks to a lesser extent (including

defecation) owing to the (inhibiting) effect of the odor from another male. Females would be assumed not to show this response, since they defecated at a lower level in both the NOD and OD conditions. This suggestion must be regarded with caution until further evidence is available, because, in the present study, the feces from the NOD rat were removed and other possible aspects of marking (e.g., urination) were not recorded. However, if confirmed, the finding might influence interpretation of previous results for sex differences in this strain, in that the present data showed that males defecated significantly more than females when tested in a "clean" field (Table 5) but that there were no differences in the OD condition.

In conclusion, the present study found no general overall sex differences in a variety of measures of emotional behavior but did find some sex differences in particular measures associated with emotional responsiveness; these varied with the strain, the measure used, and whether or not an odor from a rat of the same sex and strain was present in the testing apparatus. The findings do not agree with the theoretical position that male rats are more emotional than females (Gray, 1971). Rather, the results were more complex, and a number of factors (discussed above) appeared to be involved. Some aspects of the present findings indicated that odors might be particularly important in this type of research.

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