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Relationships Between Hyponeophagia, Diazepam Sensitivity and Benzodiazepine Receptor Binding in Eighteen Rat Genotypes

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SHEPHARD, R. A , H. F. JACKSON, P L BROADHURST AND J F W. DEAKIN. *Relationships between hyponeophagia, diazepam sensitivity and benzodiazepine receptor binding in eighteen rat genotypes* PHARMACOL BIOCHEM BEHAV **20**(6) 845–847, 1984 —Rats of 18 genotypes derived from the selected Roman strains were tested for hyponeophagia in a control condition and following diazepam (1 mg/kg) Subsequently, benzodiazepine receptor binding was measured in the cortical/striatal region. Hyponeophagia in the control condition correlated strongly with diazepam sensitivity, but benzodiazepine receptor titres did not correlate significantly with either control behavior or drug responsivity. These findings are discussed in the contexts of the arousal hypothesis of hyponeophagia and of postulated relationships between benzodiazepine receptors and emotionality

Hyponeophagia Diazepam Benzodiazepine receptors Genotype Rats

THAT benzodiazepine receptor binding in several brain regions correlates negatively with emotional defecation in the open field has been suggested on the basis of studies with the Maudsley strains of rats [8] and inbred strains of mice [7]. Moreover, in the Roman strains of rats, which differ in open-field defecation [2], in inhibition of feeding due to novelty or hyponeophagia and in diazepam sensitivity [9,12] this relationship between emotionality and benzodiazepine receptors was supported by greater binding in the less emotional Roman High Avoidance strain than in Roman Low Avoidance rats in all brain regions tested [3]. This result was also found in other work, but benzodiazepine binding was lower still in the behaviorally-intermediate Roman Control Avoidance strain [13]. Since the largest of these correlational studies employed only four genotypes [7], none of them permit any statistical test of this postulated relationship

The arousal hypothesis of hyponeophagia [10,11] predicts that subjects with high arousal levels will show strong hyponeophagia and also greatest sensitivity to treatments (including drugs) affecting arousal, since they will be at an extreme point on the inverted-U curve where the slope is steep. These predictions have been supported by data from a number of pharmacological treatments [9, 10, 11] and also for the Roman strains [9,12]. However, the results cited also lack a statistical test of the putative correlation between control behavior and drug sensitivity

The present communication reports results from 18 rat genotypes which permit both of these hypotheses to be tested in a statistically satisfactory way.

METHOD

A total of 288 rats comprising equal numbers of males and females were used for the hyponeophagia tests. They were composed of equal numbers of the parental Roman strains (3 genotypes), reciprocally-bred F_1's (6 genotypes) and non-reciprocally-bred F_2's (3 genotypes) and backcrosses (6 genotypes). Half of these subjects, randomly chosen but also comprising equal numbers of each genotype and sex were used for the benzodiazepine receptor assays.

Hyponeophagia tests used experimentally naive subjects at 79.7±(SD) 3.4 days of age and measured the food-directed

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TABLE 1

CORRELATIONS OF HYPONEOPHAGIA AND DIAZEPAM SENSITIVITY WITH BENZODIAZEPINE RECEPTOR BINDING (SPEARMAN S RHO N=18)

		Condition		
		Control	Diazepam	Difference
Measure	Approach latency	−0 067	−0 148	−0 009
	Eating latency	+0 020	−0 053	+0 038
	Amount eaten	−0 041	−0 125	−0 057

Note all correlations are non-significant

TABLE 2

CORRELATIONS BETWEEN BEHAVIOUR IN DRUG CONDITIONS (SPEARMAN'S RHO, N=18)

		Comparison		
		Control/ Diazepam	Control/ Difference	Diazepam/ Difference
Measure	Approach latency	+0 716‡	+0 968‡	+0 539‡
	Eating latency	+0 826‡	+0 955‡	+0 683‡
	Amount eaten	+0 871‡	−0 618‡	−0 268

Note significant at ‡=5% level, †=1% level, ‡=0 1% level

behavior of 23 hr deprived, meal-fed rats in a novel environment following either diazepam (1 mg/kg), a dose which produced maximal effects on hyponeophagia [9,11] suspended in 1% Tween, 0.9% NaCl or a control injection (2 ml/kg). Injections were IP, 30 min prior to testing. An approach latency, eating latency and amount eaten were recorded in a 10 min period, the method being described fully elsewhere [11]. Results for approach latency, eating latency and amount eaten are reported for the present 18 genotypes in the control condition and following diazepam (1 mg/kg) administration. The difference between control and diazepam means gave a measure of the responsivity of each genotype to the drug.Approximately four months after hyponeophagia testing subjects were stunned, decapitated and their brains were removed, frozen in solid CO_2 and transferred from Birmingham to London. Tissue from the cortical/striatal region, which shows highest benzodiazepine receptor binding, of individual subjects were homogenized in 40 vol of TRI-HCl buffer (pH—7 4) in a tight-fitting Potter-Elvehjem homogenizer The homogenates were centrifuged at 22,000 rpm for 20 min, the liquid poured off and the centrifugation repeated. The resulting P_2 pellet was resuspended in 40 vol TRIS HCl (pH 7.4) and specific (^{3}H) diazepam binding was assayed by the filtration technique [1] A fixed, final concentration of 1.6 nM diazepam was used in the determinations, which were performed in duplicate. Binding was expressed in fM/mg protein, protein assays were as described elsewhere [5]

RESULTS

ANOVA of the behavioral data showed significant ($p<0.001$) effects of genotype, diazepam and interaction between these for all three measures [9] but no significant effects of sex ANOVA of the benzodiazepine receptor data showed significant effects of genotype ($p<0.05$), but no significant effect of sex, which is consistent with previous work [13] given the tissue used Consequently, sex was disregarded in calculating the correlations between benzodiazepine receptors and behavior given in Table 1, and the correlations between behavior in different drug conditions given in Table 2 Average specific benzodiazepine receptor binding was 178 17 fM/mg protein.

The negative correlations relating to the amount eaten measured in Table 2 are due to rats from relatively non-emotional strains eating such large amounts (up to 2 g in 10 min) in the control condition that further increase was unlikely, a ceiling effect [9] which will not be a considered further.

DISCUSSION

The present genotypic variation in benzodiazepine receptor binding appears to be uncorrelated with emotionality (first column of Table 1). These results agree with some data [13], conflict with others [7,8], but constitute the only statistical test of this hypothesis. A possible explanation of these discrepancies is that whereas in the present work and [13] hyponeophagia was used as the major index of emotionality, other reports use open field defecation scores [7,8]. However, these two measures of emotionality are long known to intercorrelate strongly [4] and, moreover, benzodiazepine receptor binding in the present 18 genotypes does not correlate significantly with either open field ambulation or defecation scores [5]. The use of tissue for several other biochemical assays [5] prevented us from performing Scatchard analyses, however, previous studies have shown strain differences in specific benzodiazepine binding to be due to receptor number, rather than affinity, differences. Likewise previous studies, including those of the Roman strains [3,13] have not shown strain differences in benzodiazepine receptors to depend upon brain region. Therefore we suggest that reports of negative relationships across genotype between benzodiazepine receptors and emotionality [7,8] may be artifacts of the low numbers of genotypes and of subjects within genotype used

Also noteworthy is the failure of benzodiazepine receptor binding to correlate with either hyponeophagia following diazepam or diazepam responsivity (respectively columns 2 and 3 of Table 1). Since diazepam (1 mg/kg) largely obscured strain differences in hyponeophagia [9] the first finding is not surprising but the failure of specific benzodiazepine receptor binding to correlate with a direct measure of diazepam sensitivity clearly raises the question of what biochemical paramaters diazepam responsivity does depend upon. The parental Roman strains differ with respect to GABA metabolism [6] but present knowledge of the neurochemical events which follow stimulation of benzodiazepine receptors, and of genotypic differences in these, do not justify further speculation.

In relation to the latency measures, columns 1 and 3 of Table 2 respectively show that the 18 genotypes largely re-

tain their ordering of control hyponeophagia when treated with diazepam (1 mg/kg), and that those genotypes showing largest latencies under diazepam (and hence generally also in the control condition) also show greatest drug sensitivity. Column 2 of Table 2 clearly shows diazepam responsivity to be strongly positively correlated with control behavior. This greater diazepam sensitivity in subjects of genotypes with strongest hyponeophagia can be explained by the inverted-U shaped relationship between performance in this paradigm and constructs such as arousal [9, 10, 11, 12]. When the neurochemical paramaters which presumably mediate this relationship are identified, substantial progress towards understanding of the biological basis of emotionality and anxiety, as well as the mechanism of action of benzodiazepines, will have been made. On present evidence, specific benzodiazepine binding does not appear to provide this link, but studies of genotype differences in sub-populations of benzodiazepine receptors may be useful, provided that sufficient genotypes are used to permit statistical tests of the postulated relationships.

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REFERENCES

1 Braestrup, D and R F Squires Brain specific benzodiazepine receptors in rats characterised by high-affinity ^{3}H-diazepam binding. *Proc Natl Acad Sci USA* **74:** 3805–3809, 1977.
2. Drewek, K J and P L Broadhurst. Alcohol selection by strains of rats selectively bred for behavior *J Stud Alcohol* **40:** 723–728, 1979.
3. Gentsch, C , M Lichtsteiner and H Feer ^{3}H-diazepam binding sites in Roman high- and low-avoidance rats *Experentia* **37:** 1315–1316, 1981
4 Hall, C. Emotional behavior in the rat 1. Defecation and urination as a measure of individual differences in emotionality. *J Comp Psychol* **18:** 385–403, 1934
5. Jackson, H F *The Effects of Parachlorophenylalanine on Open-Field and Avoidance Behavior Multivariate, Biometrical-Genetic and Neurochemical Analysis in Rats* Unpublished PhD Thesis, University of Birmingham, 1981.
6 Rick, J T , G. Tunicliff, G A Kerkut, D W. Fulker, J. Wilcock and P. L Broadhurst GABA production in brain cortex related to activity and avoidance behavior in eight strains of rat. *Brain Res* **32:** 234–238, 1971
7 Robertson, H A. Benzodiazepine receptors in "emtional" and "non-emotional" mice Comparison of four strains *Eur J Pharmacol* **56:** 163–166, 1979.
8. Robertson, H. A , I. L. Martin and J. M. Candy. Differences in benzodiazepine receptor binding in Maudsley reactive and Maudsley non-reactive rats *Eur J Pharmacol* **50:** 455–457, 1978
9 Shephard, R. A. *Psychopharmacogenetics of Neophagia and Conflict Behaviour in Rats*. Unpublished PhD thesis, University of Birmingham, 1980.
10 Shephard, R A and P L Broadhurst Effects of diazepam and of serotonin agonists on hyponeophagia in rats *Neuropharmacology* **21:** 337–340, 1982.
11 Shephard, R. A and P L Broadhurst. Hyponeophagia and arousal in rats Effects of diazepam, 5-methoxy N, N dimethyltryptamine, d-amphetamine and food deprivation. *Psychopharmacology (Berlin)* **78:** 368–372, 1982.
12. Shephard, R. A. and P L Broadhurst. Hyponeophagia in the Roman rat strains· Effects of 5-methoxy N, N dimethyltryptamine, diazepam, methysergide and the steroisomers of propranolol *Eur J Pharmacol* **95:** 177–184, 1983.
13 Shephard, R. A., E. B. Nielsen and P. L Broadhurst. Sex and strain differences in benzodiazepine receptor binding in Roman Rat strains *Eur J Pharmacol* **77:** 327–330, 1982