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Central and Peripheral Norepinephrine Metabolism in Rat Strains Selectively Bred for Differences in Response to Stress[1]

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SLATER, J., D. A. BLIZARD AND L. A. POHORECKY. *Central and peripheral norepinephrine metabolism in rat strains selectively bred for differences in response to stress.* PHARMAC. BIOCHEM. BEHAV. 6(5) 511–520, 1977. – Rats of the Maudsley nonreactive (MNRA) strain were found to contain higher levels of norepinephrine in heart, spleen, and hypothalamus than animals of the Maudsley reactive (MR) strain. Total adrenal catecholamines were also greater in nonreactive animals. There was a trend toward higher endogenous norepinephrine concentration in MR rats in brainstem and telencephalon, but this was not statistically significant. Turnover measurements calculated from the fall of norepinephrine at 1 and 4 hours after a single dose of *levo*-α-methylparatyrosine showed no significant strain differences in telencephalon or brainstem, but MNRA animals had a faster rate of norepinephrine decline in heart than had MR rats. Possibly indicative of a higher rate of norepinephrine metabolism, the percentage of ^{3}H-non-catechol metabolites relative to total counts was higher in brainstem of MNRA rats 90 min after intraventricular injection of ^{3}H-norepinephrine. However, the disagreement between this estimate of norepinephrine metabolism and that provided by the α-methylparatyrosine technique prevents a conclusive statement about norepinephrine metabolism in the two strains. The results are discussed in the light of the established differences in behavior between the strains as well as other work exploring relationships between catecholamine metabolism and emotionality.

Emotionality Maudsley reactive and non-reactive rats Norepinephrine α-Methylparatyrosine
Affective illness Autonomic nervous system

THE MAUDSLEY Reactive (MR) and Non-Reactive (MNR) rat strains have been selected and inbred by Broadhurst for high and low defecation scores in the open field [8]. His intention was to produce genetically determined differences in autonomic nervous system reactivity, held by Eysenck and others to be related to the behavioral dimension of emotionality [13].

Many experiments have shown that MR rats differ from MNR animals on a wide variety of tasks designed to measure emotional response [9,14]. In general, the consensus of this work is that the Non-Reactive (low-defecating, MNR) strain is less susceptible to frustration, learns better under stress, and exhibits less tendency to freeze in certain fear-provoking situations than the Reactive (high-defecating, MR) strain [18,28].

A number of physiologic and biochemical variables have been examined in an attempt to elucidate possible mechanisms critical to the expression of these differences [3, 16, 38]. From a neurochemical standpoint, Sudak and Maas showed that 5-HT concentrations in whole brain and limbic system were higher in MR males [34]. This paralleled similar differences in 5-HT levels between mouse strains shown to differ in open-field defecation [35]. The synthesis of GABA in the cerebral hemisphere has also been investigated in the MR and MNR strains, with either no differences found, or greater ^{14}C-GABA accumulation in MNRs, depending on the particular radioactive precursor used [26].

No comparison, however, of norepinephrine metabolism in the Maudsley strains has been reported. This is surprising in view of the evidence that has accumulated to suggest a relationship between central noradrenergic systems, located

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primarily in the brainstem, limbic system, and hypothalamus, and the behavioral parameters of emotionality, arousal, and fear [25,29]. The discovery of a difference in noradrenergic physiology in these strains might therefore be considered as evidence that the well-established behavioral differences between them are related to strain differences in this biochemical system.

There are several ways in which a difference in the central noradrenergic system might contribute to differences in emotional behavior. First, the threshold of activation of specific behavioral components might be altered by a difference in the biochemical system controlling them. Secondly, differences in noradrenergic function might be reflected in the intensity of expression of a given behavior. Third, a difference in the central organization of the noradrenergic system might modulate either directly or indirectly the input of sensory systems in arousing situations. Differences in central noradrenergic activity might, therefore, influence the reception of arousing stimuli, the likelihood of specific behavioral responses to it, and also the intensity of their motor expression.

Since differences in peripheral autonomic nervous system function have long been recognized as an important component of emotional behavior, we studied the peripheral noradrenergic system in these experiments. Peripheral differences in noradrenergic function might result from a direct effect of central noradrenergic function on the peripheral autonomic system. Secondly, differences in peripheral noradrenergic function might be brought about by the same genetic mechanisms (i.e., pleiotropic effects) that bring about changes in central noradrenergic mechanisms. Thirdly, they might be both genetically and functionally independent of changes in central noradrenergic function.

The present experiments represent a first step at discovering whether differences in central and peripheral norepinephrine metabolism exist in the Maudsley rats. It is not intended that these experiments should discriminate between the various alternatives outlined above. If differences in noradrenergic function are found between the strains, however, this would provide a stimulus to discovering what precise functional role such differences might play.

METHOD

Animals

Rats of the MR and MNRA strains [15] were maintained in our laboratory by random mating within each line for several generations before this experiment was conducted. Ancestors of this colony were kindly made available by Dr. Gordon M. Harrington, Department of Psychology, University of Northern Iowa, Cedar Falls, IA 50613. MNR animals were derived from the MNRA subline, which is similar in open-field behavior to the main MNR line [17]. All animals were weaned and separated by sex at about Day 23 and housed 3–5 per clear plastic cage on wood shavings. Purina lab chow and water were available ad lib and the diurnal cycle was 15 hr light and 9 hr dark.

Age range at sacrifice for the four subject groups was: Experiment 1, 210–281 days; Experiment 2, 200–239 days; Experiment 3, 200–345 days. With the exception of Experiment 4, where MR rats were older (MR: 220 days; MNRA: 150 days), strains were roughly age-matched for each study.

Biochemical Studies

In the first two experiments, comparisons of norepinephrine levels were made in three different brain regions and heart. In Experiment 1, spleens were also assayed for norepinephrine, while total adrenal catecholamines, largely epinephrine, were measured in Experiment 2. To study metabolism of norepinephrine, two additional investigations were conducted. The first measured the decline in brainstem, telencephalon and heart of norepinephrine concentrations after blockade of tyrosine hydroxylase with α-methylparatyrosine. In the second, a trace amount of ^{3}H-norepinephrine was injected intraventricularly and the amount of ^{3}H-norepinephrine and noncatechol metabolites measured 90 min later.

Tissue Preparation

All animals were sacrificed by decapitation 7–10 hr after onset of the light cycle. Each animal was weighed immediately before sacrifice. Brain tissue was quickly dissected on crushed ice. The olfactory bulbs were removed and then, using iris scissors, the lateral and caudal/rostral outlines of the hypothalamus were cut, with the optic chiasm, caudal border of the mammillary bodies, and the mediolateral edges of the pyriform cortex serving as landmarks. The brain was then divided by a single vertical cut from the optic chiasm through the cortex perpendicular to the sagittal sinus. The hypothalamus was then dissected free by making a cut in the horizontal plane at the level of the anterior commissure. The remaining telencephalic portions were removed and combined. After the removal of the cerebellum, the remaining tissue was designated the brainstem sample and contained medulla, pons, midbrain, and thalamus. In the α-methylparatyrosine studies (see below), the striatum was removed from the rostral telencephalic portions for use in other studies.

To obtain peripheral tissues, hearts were quickly dissected free and trimmed of great vessels. Spleens and both adrenal glands were also trimmed of fat and removed. All tissues were rapidly wrapped in aluminum foil and immediately frozen on dry ice until assay no more than 1 week later.

Biochemical Assays

Endogenous norepinephrine. All tissues were homogenized with 0.4 N perchloric acid and centrifuged. After adjustment of the pH of each sample to 8.6 with the addition of 10 ml of 2 M Tris buffer [10], norepinephrine was extracted from the supernatant by adsorption on alumina [40]. After the alumina column containing the adsorbed catecholamines had been washed with 8 ml of 0.5 M Tris buffer (pH 8.6) and 10 ml of distilled water, the catecholamines were eluted with 3 ml of 0.2 N acetic acid. After oxidation by the method of von Euler and Lishajko [12], norepinephrine fluorescence was read at 395/505 nm on an Aminco-Bowman spectrophotofluorometer. Adrenal catecholamines were read at 395/520 nm, the fluorescent peak for epinephrine.

Several samples of pooled rat brain homogenate were run with each experimental group to assess recovery. A known amount of norepinephrine was added to half of the pooled rat-brain homogenate samples and recoveries were calculated for each assay. A consistent retrieval of approx-

imately 60% was observed and the tissue norepinephrine values were corrected accordingly.

Studies of Norepinephrine Metabolism

α-Methylparatyrosine. To block synthesis of norepinephrine, a dose of 200 mg/kg *levo*-α-methylparatyrosine (αMPT) (kindly donated by Merck, Sharp, and Dohme Laboratories) was given by intraperitoneal injection to 14 experimental animals of each strain comprising two-thirds of the third experimental group. Of these 28 animals, 7 of each strain were sacrificed at 1 hr, and the rest at 4 hr, after injection. The αMPT was dissolved in warm 1.0 N NaOH, brought to pH 2 with 0.1 N HCl, and cooled to room temperature before administration. This delivery regimen has been shown by other experimenters to produce satisfactory inhibition of tyrosine hydroxylation [19]. The use of the more soluble d,l-methyl ester was unfortunately precluded because its administration to Maudsley rats in a trial study resulted in the death of half of the subjects. Since the *levo* form is the more active stereo-isomer [32], we were able to administer a lower total dose with reduced toxicity and yet remain above the dose needed for complete inhibition.

^{3}H-Norepinephrine and ^{3}H-Metabolites. The 8 MNR and 7 MR rats in Experiment 4 were etherized, placed in a stereotaxic instrument, and injected in the right lateral ventricle with 6 μC ^{3}H-norepinephrine dissolved in 0.02 ml Ringer's solution. All animals were killed 90 min after injection for determination of radioactive norepinephrine and noncatechol metabolites in brainstem, telencephalon, and hypothalamus. The d,l-norepinephrine-7-^{3}H (3000 mC/ml) was purchased from Schwartz/Mann (Orangeburg, NJ).

^{3}H-Norepinephrine values (cpm/g) were determined by counting an aliquot of the alumina eluate after elution with 3 ml of 0.2 N acetic acid. The noncatechol metabolites, which are not retained on alumina, were counted in an aliquot of the initial alumina effluent [22]. Catechol metabolites, largely 3,4-dihydroxyphenylglycol, remained on the column and were not counted in this procedure.

Open-Field Testing

The open-field test apparatus was similar but not identical to that described by Broadhurst [8]. It consisted of a circular arena 32.75 in in diameter with an illumination level of approximately 30 FTC. Each animal's activity was automatically recorded whenever it traversed any one of 12 8 in square metal plates that comprised the arena floor. The defecation score was the number of boli excreted in the 2 min test. Animals were carried individually from their home cages in plastic containers lined with slightly damp toweling to remove dust from their footpads and insure a good contact with the floor of the field. After testing they were replaced in their original cages.

The animals in Experiment 2 (N = 14) were tested 3 hr before offset of the light cycle on 4 consecutive days. In Experiment 3 (N = 42), the animals received 9 open-field tests on consecutive days. Tests 1–4 and 9 were carried out in the dark portion of the cycle in connection with another study. The scores reported in Table 1 are for tests 5–8, which are conducted 3 hr before offset of the light cycle; i.e., the time at which they were later sacrificed. The animals in Experiment 4 (N = 16) had also been previously tested in the open field, and Table 1 reports the results of the open-field test carried out the day before sacrifice. The activity scores of animals in this group were recorded by hand-tracing the animal's path on a floor plan of the open field. The animals in Experiment 1 (N = 12) were not tested in the open field.

RESULTS

Norepinephrine Levels

Table 1 shows that in Experiment 1 MNRA rats had significantly higher norepinephrine levels than had MR animals in hypothalamus, heart, and spleen. The findings in heart and hypothalamus were largely repeated in Experiment 2. In that experiment, adrenals were analyzed instead of spleens, and MNRAs were found to have significantly higher adrenal catecholamines than had MRs (Table 1).

To determine whether differences in norepinephrine concentrations in heart and spleen depended on strain differences in tissue weight, total norepinephrine values were calculated for each organ irrespective of weight. Although the degree of difference separating the strains was reduced by this comparison, it was found that MNRA rats still had significantly more total norepinephrine than had MRs (Table 2).

Although no significant strain differences in norepinephrine levels were found in the brainstem or telencephalon samples, there was a trend for MR rats to have higher levels than MNRA rats in Experiment 2. Zero-time

TABLE 1

NOREPINEPHRINE CONCENTRATIONS (NG/G)

	Brain stem		Telencephalon		Hypothalamus		Heart		Spleen	Adrenal*
Expt No.	1	2	1	2	1	2	1	2	1	2
MR	402 ± 32 N = 6	538 ± 13 N= 7	356 ± 22 N = 6	394 ± 21 N = 7	1660 ± 45 N = 6	1865 ± 110 N = 7	1291 ± 90 N = 6	929 ± 44 N = 7	1938 ± 200 N = 6	16.32 ± .64 N = 7
MNRA	459 ± 21 N = 6	485 ± 21 N = 7	362 ± 19 N = 6	355 ± 12 N = 7	2115 ± 99 N = 6	2138 ± 74 N = 7	1951 ± 108 N = 6	1199 ± 63 N = 7	3757 ± 236 N = 6	20.61 ± .99 N = 7
	t = 1.46 N.S.	t = 2.14 N.S.	t = 0.19 N.S.	t = 1.55 N.S.	t = 4.18 p<0.01	t = 1.98 p<0.10	t = 4.69 p<0.001	t = 3.50 p<0.01	t = 5.89 p<0.001	t = 3.27 p<0.01

*Adrenal catecholamine levels are reported as μg/pair of adrenals. Means ± SEM of norepinephrine concentrations.

TABLE 2

NOREPINEPHRINE (NG) PER ORGAN

	Heart		Spleen
Expt. No.	1	2	1
MR	1066 ± 63 N = 6	708 ± 31 N = 7	1262 ± 113 N = 6
MNRA	1464 ± 73 N = 6 t = 4.125 p <0.01	890 ± 64 N = 7 t = 2.56 p <0.05	1613 ± 100 N = 6 t = 2.32 p <0.05

Values are means ± SEM.

controls of the two strains from the αMPT study were compared to gain further insight into this possibility. Again, MR rats had higher norepinephrine levels than MNRA rats, but this was not statistically significant. Thus, there is a possibility that MR rats have higher norepinephrine levels in brainstem and telencephalon than MNRA rats, but it has not been possible to demonstrate it clearly in these experiments.

Norepinephrine Metabolism

Norepinephrine decline after αMPT. Three MNRA rats in the 4 hr group consistently showed only slightly lower norepinephrine levels in the telencephalon and brainstem than did control rats. The three rats mentioned above were therefore excluded from the statistical analysis. As precipitate from the injection was more often seen in the peritoneal cavity of MR than of MNRA rats 1 hr after injection, it is possible that MNRA rats clear the drug more quickly and that this accounts for release from inhibition in these three animals at 4 hr after drug administration. A small number of samples in other groups were spoiled and account for the reduced number at various time points.

Correlations and linear regression coefficients between log-transformed norepinephrine concentrations and time

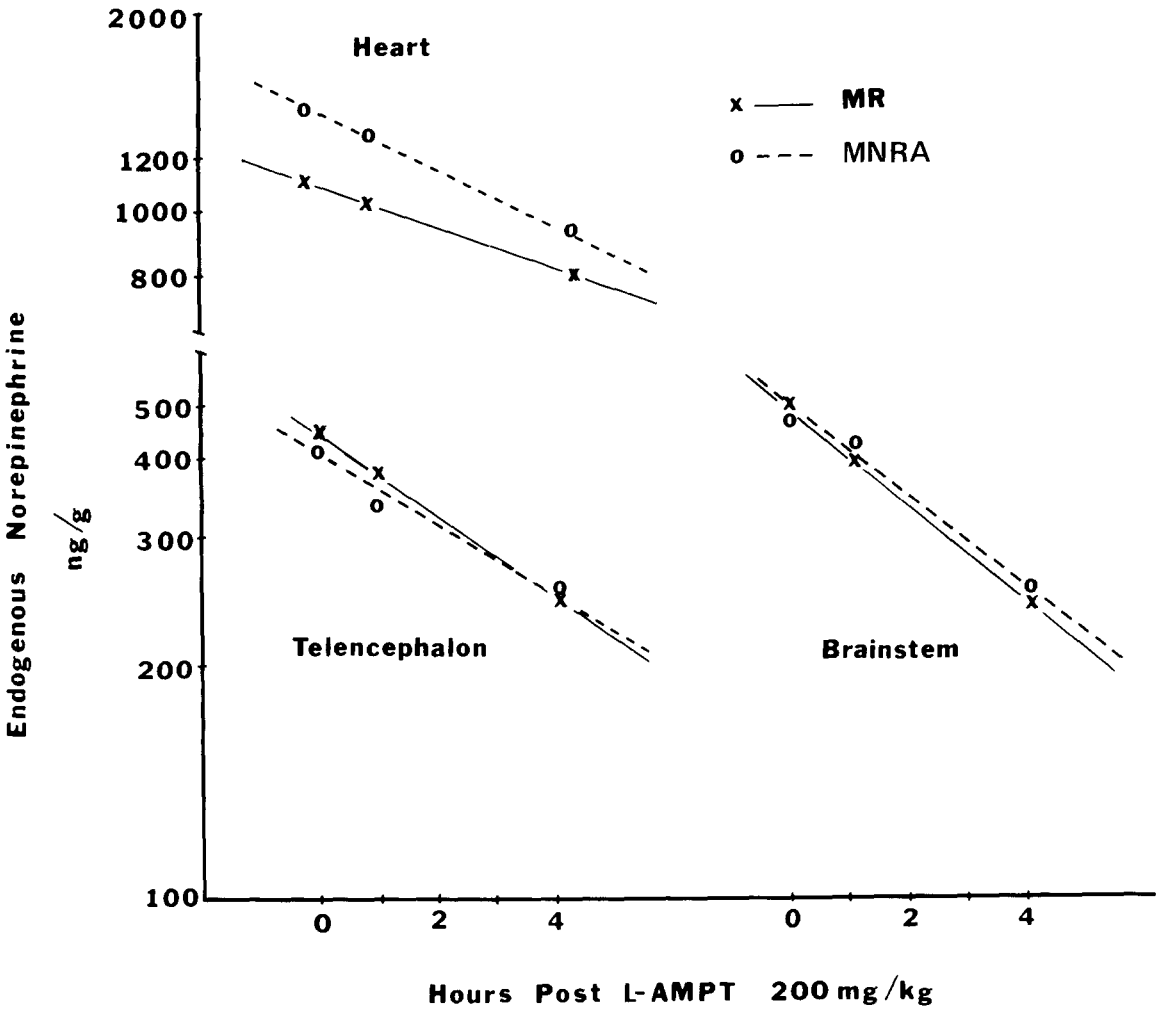

FIG. 1. Semi-log plot of the decline in norepinephrine concentration after injection of αMPT. Each value represents the mean of 3–7 rats. Regression lines were calculated from a least-squares analysis and the slope was used to indicate respective turnover values. The control animals (Time 0) were injected with vehicle alone. Strain differences in norepinephrine levels in heart are in agreement with those found in Experiments 1 and 2.

TABLE 3

REGRESSION COEFFICIENTS AND HALF-LIVES OF ENDOGENOUS NOREPINEPHRINE

Tissue	Regression Coefficient	r	p	T½
Brainstem				
MR	−.0789 ± .005	−.97		3.81
MNRA	−.0687 ± .006	−.96	N.S.	4.38
Cortex				
MR	−.0664 ± .005	−.97		4.53
MNRA	−.0571 ± .007	−.91	N.S.	5.27
Heart				
MR	−.0337 ± .007	−.80		8.93
MNRA	−.0579 ± .008	−.89	$p<0.05$	5.20

Linear regression coefficients (b) ± SEM and correlation coefficients (r) from Experiment 3. T½ was determined from the formula T½ = log 10^2/b.

TABLE 4

EXPERIMENT 4: ^{3}H CONTENT AS NOREPINEPHRINE AND NONCATECHOL METABOLITES IN VARIOUS BRAIN REGIONS

	Brainstem (cpm/g)		Telencephalon (cpm/g)		Hypothalamus (cpm/g)	
	Norepinephrine	Metabolites	Norepinephrine	Metabolites	Norepinephrine	Metabolites
MR	1689 ± 55	5155 ± 197	460 ± 24	1664 ± 69	1421 ± 78	5694 ± 152
	N = 6	N = 7	N = 7	N = 7	N = 7	N = 7
MNRA	1320 ± 53	4462 ± 160	380 ± 28	1449 ± 70	1183 ± 37	4641 ± 131
	N = 8	N = 8	N = 8	N = 8	N = 8	N = 8
	t = 4.77	t = 2.75	t = 2.07	t = 2.55	t = 2.87	t = 5.27
	$p<0.001$	$p<0.02$	N.S.	$p<0.05$	$p=<0.02$	$p<0.001$

All animals were injected with 6 μCi of ^{3}H-norepinephrine intraventricularly and sacrificed 90 min later. Values are means ± SEM and are not corrected for recovery.

were calculated by the method of least-squares [30]. The high negative correlation of norepinephrine concentration with time after αMPT administration in both strains indicates that inhibition of tyrosine hydroxylase was probably complete (Table 3). The T 1/2 values are in general agreement with those in previous studies [19].

Comparison of Norepinephrine Turnover in the Two Strains

Brainstem and telencephalon. There was no difference in norepinephrine turnover in these two brain regions (Fig. 1 and Table 3). However, in brainstem, although there were no significant differences in control (Time 0) norepinephrine levels, MR rats showed significantly greater percent decline from mean control values at 1 hr than did MNRA rats (MR: 78%, MNRAs: 91%, t = 4.15, $p<0.001$). A strain difference in onset of αMPT inhibition of tyrosine hydroxylase was considered as a possible explanation. However, the decline from control levels at 1 hr in telencephalon was significantly greater in MNRA rats (MNRA: 80%, MR: 86%, t = 2.32, $p<0.05$) and obviously did not support the latter interpretation as the difference was in the opposite direction.

Heart. In heart, MNRA rats showed a significantly faster turnover of norepinephrine than MR rats (Fig. 1 and Table 3).

^{3}H-Norepinephrine and Metabolites

The amount of ^{3}H-norepinephrine (cpm/g) remaining 90 min after intraventricular injection of ^{3}H-norepinephrine was significantly lower in MNRA rats in both brainstem and hypothalamus (Table 4). MNRA rats also had lower ^{3}H-norepinephrine in telencephalon, but this was not statistically significant. Similarly, MNRA rats had significantly lower ^{3}H-non-catechol metabolites than MR rats in all three brain regions (Table 4).

Not surprisingly in view of the previous findings, when total radioactivity in each brain region was calculated by summing cpm/g of ^{3}H-norepinephrine and cpm/g of ^{3}H-norepinephrine metabolites, it was found that MNRA rats had significantly fewer total counts than MR rats in all three brain regions (telencephalon: $t(13) = 2.77$, $p<0.02$; brainstem: $t(12) = 3.17$, $p<0.01$; hypothalamus: $t(13) = 7.39$, $p<0.001$).

Several explanations could account for these differences in total counts. First, the initial uptake of ^{3}H-norepinephrine might differ in the two strains. Secondly, removal of ^{3}H-norepinephrine metabolites could proceed at a different rate in the two groups. Third, ^{3}H-norepinephrine-related counts may have been lost along other metabolic routes than those we assayed. Finally, injection procedures could have been differentially successful in the two strains resulting in poorer distribution of ^{3}H-norepinephrine in one strain than in the other. This final possibility seems least likely to have been the reason for the differences between MNRA and MR rats in total counts. Cannulas were stereotaxically placed after considerable pilot work had been previously carried out, and were

TABLE 5

STRAIN DIFFERENCES IN OPEN-FIELD BEHAVIOR

	Open-Field Defecation			Open-Field Activity		
Expt No.	2	3	4	2	3*	4†
MR	3.5 ± 0.35	3.21 ± 0.28	4.6 ± 0.96	30.6 ± 2.3		10.7 ± 1.9
	N = 7	N = 21	N = 8	N = 7		N = 8
MNRA	1.78 ± 0.50	2.17 ± 0.30	1.1 ± 0.44	42.0 ± 2.8		19.0 ± 2.5
	N = 7	N = 21	N = 8	N = 7		N = 8
	t = 2.79	t = 2.50	t = 3.30	t = 3.12		t = 2.61
	$p<0.02$	$p<0.02$	$p<0.01$	$p<0.01$		$p<0.05$

Scores in each column are mean defecation and activity ± SEM per 2-min daily test for male rats of the MR and MNRA strains.

*Scores lost because of mechanical failure.

†Scores lower because these rats were tested manually.

TABLE 6

STRAIN DIFFERENCES IN BODY WEIGHT (GRAMS)

Experiment No.	1	2	3	4
MR	293 ± 11	276 ± 14	303 ± 7.7	315 ± 8.6
	N = 6	N = 7	N=21	N = 8
MNRA	227 ± 8	228 ± 12	246 ± 5.5	258 ± 13
	N = 6	N = 7	N = 21	N = 8
	t = 4.79	t = 2.56	t = 5.90	t = 3.65
	$p<0.001$	$p<0.05$	$p=<0.001$	$p<0.01$

Mean body weights ± SEM of MR and MNRA rats in the four experiments.

manipulated in each case until cerebrospinal fluid was seen to well up in the cannula.

^{3}H-Norepinephrine Metabolites as a Percentage of Total Counts

An attempt was made to circumvent the differences in total counts between the two strains along the lines suggested by Stone [33]. ^{3}H-Norepinephrine metabolites were expressed as a percentage of total counts in each brain region for each animal and the two strains compared as before.

In this analysis only one statistically significant strain difference appeared. In brainstem, MNRA rats had a significantly larger percentage of ^{3}H-norepinephrine metabolites relative to total counts than MR rats ($t(12) = 2.591$, $p<0.05$). MNRA rats showed the same trend (greater percentage of ^{3}H-norepinephrine metabolites) in telencephalon, but this was not statistically significant. There was no difference between the strains on this measure in hypothalamus.

Open-Field Behavior

MR rats defecated significantly more in the open field than did MNRA rats in all tests (Table 5). In Experiments 2 and 4, MNRA animals exhibited significantly higher mean activity scores during the 2 min test than did MR animals (Table 5). Activity scores of Experiment 3 were lost because of equipment malfunction.

Body Weight

MR rats weighed significantly more than MNRAs in all experimental groups (Table 6).

Tissue Weights

MR rats had significantly heavier adrenals and spleens

TABLE 7

WEIGHTS OF PERIPHERAL TISSUES (GRAMS)

	Heart			Adrenal				Spleen
Expt No.	1	2	3*	1	2	3†	4	1
MR	0.8293	0.7673	0.8838	0.0368	0.0389	0.0380	0.0426	0.6587
	± .02	± .04	± .02	± .0015	± .0022	± .004	± .0001	± .024
	N = 6	N = 7	N = 18	N = 6	N = 7	N = 7	N = 7	N = 6
MNRA	0.7542	0.7398	0.8116	0.0297	0.0292	0.0296	0.0313	0.4314
	± .03	± .03	± .02	± .0003	± .0008	± .004	± .0001	± .016
	N = 6	N = 7	N = 14	N = 6	N = 7	N = 7	N = 8	N =6
	t = 2.12	t = .615	t = 2.33	t = 4.59	t = 4.01	t = 3.72	t = 5.57	t = 7.62
	N.S.	N.S.	$p<.05$	$p<.001$	$p<.01$	$p<.01$	$p<.001$	$p<.001$

*The N is reduced in this experimental group, compared with that reported in the text, because tissue weight comparisons were made for a subgroup of animals that could be exactly age-matched.

†Adrenals were removed only from control animals in Experiment 3.

Values are means ± SEM.

TABLE 8

BRAIN WEIGHTS (GRAMS)

Expt No.	1	2	3	4
MR	1.5933	1.4553	1.5200	1.4593
	± .043	± .019	± .01	± .029
	N = 6	N = 7	N= 18	N = 7
MNRA	1.6053	1.4789	1.523	1.5004
	± .035	± .023	± .02	± .073
	N =6	N = 7	N = 14	N = 8
	t = 0.217	t = 0.44	t = 1.15	t = 1.39
	N.S.	N.S.	N.S.	N.S.

Brain weight comparisons were made from the sum of the frozen tissue weights of hypothalamus, brainstem, and telencephalon. Values are means ± SEM.

(Table 7), but did not consistently differ in heart or brain weights (Tables 7 and 8).

Organ Weight:Body Weight Ratio

Analysis of the size of the various organs relative to the difference in body weight between the two strains revealed the following significant differences. MNRA rats had significantly larger hearts per gram of body weight than MRs (Experiment 1: $t(10) = 4.44$, $p<0.01$; Experiment 2: $t(12) = 5.28$, $p<0.001$; Experiment 3: $t(34) = 2.07$, $p<0.05$). MNRA rats also had larger brains per gram of body weight (Experiment 1: $t(10) = 5.33$, $p<0.001$; Experiment 2: $t(12) = 3.5$, $p<0.01$; Experiment 3: $t(34) = 4.7$, $p<0.001$; Experiment 4: $t(13) = 4.24$, $p<0.001$).

When the body weight correction was applied to the spleen data, the tendency remained for MR spleens to be heavier, but this did not quite achieve statistical significance ($t(10) = 2.14$, $p<0.10$). When adrenal weights were corrected for strain differences in body weight, no significant differences between MR and MNRA groups were found. As was the case for spleens, however, MR adrenals still tended to be heavier and probably would have reached statistical significance with larger sample sizes.

Comparison with Previous Results in MR and MNR Lines

The strain differences in open-field defecation and activity, and in body weight, are all consistent with earlier reports comparing the strains on these measures [8]. The existence of similar differences in our lines suggests strongly that, despite the relaxation of selection pressure and inbreeding in our colony, these rats were representative, in both a behavioral and a physiological sense, of previous samples of Maudsley rats.

The significance of the strain differences in brain/body weight and heart/body weight ratios is not readily apparent, but these ratios provide additional background information about the strains.

DISCUSSION

These experimental results demonstrate a variety of differences in the structure and function of norepinephrine-containing neurons in both peripheral and central nervous system tissues of the Maudsley strains.

Norepinephrine Levels

MNRA animals were characterized by higher norepinephrine concentrations (ng/g) in heart, spleen, and hypothalamus, as well as by more total catecholamines in the adrenal glands. This finding in hypothalamus and three peripheral tissues is suggestive of either structural, functional, and/or metabolic differences in the norepinephrine system of MR and MNRA strains. It is interesting to note that the hypothalamus, a brain region long thought to have a strong connection with the peripheral autonomic nervous system [27], showed a significant difference in norepinephrine level consistent with the direction seen in three peripheral tissues. This may simply be fortuitous, but it also raises the possibility that a difference in the character of the sympathetic branch of the autonomic nervous system as a whole may be present in these strains.

The higher norepinephrine levels obviously cannot be taken per se as indicative of higher metabolic activity. Indeed, Iversen and Glowinski have presented evidence that brain regions with high norepinephrine levels tend to have relatively lower turnover rates [19]. Among other possibilities suggested by the differences in norepinephrine levels are: (a) a greater innervation by norepinephrine-containing neurons in the various tissues, and (b) greater norepinephrine concentration per nerve ending, either because of differences in receptor sensitivity, reflected through feedback mechanisms, or primary variations in tyrosine hydroxylase activity.

Several findings indicate that physiologic parameters, such as established differences in endocrine function between the strains, are not solely responsible for the differences in norepinephrine levels found in this study. For example, although the Maudsley strains have been shown to differ in thyroid activity, it is unlikely that the thyroid-norepinephrine relationships described by Prange and others [23,24] can by themselves sufficiently account for the data. If this were so, one might expect strain-specific catecholamine characteristics to appear in all brain regions and peripheral tissues studied. Since telencephalon and brainstem tend to exhibit the opposite differences to those found in the other four tissues, additional factors other than a single endocrine influence probably are operative.

Metabolic Studies

Taken together, the studies of norepinephrine metabolism suggest that MNRA rats have faster peripheral turnover of norepinephrine and the possibility of faster turnover in certain brain regions. However, for reasons detailed below, a definitive characterization of norepinephrine metabolism in the two strains must await the completion of additional studies.

αMPT. The higher norepinephrine turnover in hearts of MNRA rats and lack of difference in brainstem and telencephalon parallels the higher norepinephrine levels in heart in MNRA rats and opposite group trends in brainstem and telencephalon in norepinephrine levels. A study of norepinephrine turnover in hypothalamus as well as additional peripheral tissues would indicate whether this correlation of differences in norepinephrine concentration with turnover were general in these strains.

A cautionary note concerning the data derived by the αMPT technique is required because the validity of turnover measurements derived from this method are based on the assumption of an equivalence in drug action between

experimental groups [39]. The wide range of endocrinological and physiological differences between these strains make differential drug handling a distinct possibility and, as pointed out by Wurtman *et al.* [41], may lead to erroneous interpretations of norepinephrine metabolism. It would be valuable therefore to assess turnover in the animals by several different techniques. Determination of steady-state activity of tyrosine hydroxylase would be an additional approach.

^{3}H-Norepinephrine

The presence of less ^{3}H-norepinephrine (cpm/g) in brainstem and hypothalamus and a similar but nonsignificant trend in telencephalon of MNRA rats 90 min after injection of ^{3}H-norepinephrine might suggest that MNRA animals release newly taken up norepinephrine faster than do MRs in these areas. This interpretation is supported in brainstem (although not for telencephalic or hypothalamic samples) in that MNRA rats also had a greater percentage of ^{3}H-norepinephrine metabolites relative to total counts than MRs in this brain area. However, the accumulation of more ^{3}H-noncatechol metabolites in all brain regions of MR animals is not consistent with this interpretation. Furthermore, the lack of a difference between the strains in the turnover of norepinephrine in brainstem as measured by the αMPT technique raises additional questions about the hypothesis.

In the light of possible strain differences in ^{3}H-norepinephrine utilization mentioned earlier (see RESULTS), it can only be said that ^{3}H-norepinephrine is either taken up or metabolized differently in the central nervous system of each strain and emphasizes the need for more detailed studies.

Possible Role of Norepinephrine System in Strain Differences in Behavior

The Maudsley strains were originally selected for high and low defecation in the open-field test [8]. This index was considered to reflect relative degrees of autonomic arousal. Subsequent studies have generally supported the notion that there is indeed a basic difference in autonomic function between the strains [3,4]. Thus, it is interesting to consider whether the present neurochemical findings provide a basis for the explanation of the differences in autonomic responsivity between the lines.

Our principal findings indicate that MNRA rats have both higher norepinephrine/catecholamine levels in the hypothalamus and periphery (spleen, heart, and adrenals) and higher norepinephrine turnover in the heart. Since it is well known that the intestine is inhibited by sympathetic activity, it is conceivable that an alteration in the sympathetic system reflected in the biochemical differences cited above may be responsible for the strain differences in autonomic arousability. Thus, the low rates of defecation in the open field exhibited by MNRA rats relative to MR could be achieved by the existence of higher norepinephrine levels in the bloodstream of these rats. Alternatively, if activity of noradrenergic neurons controlling the gut is heightened in MNR rats, this could directly inhibit peristaltic activity. If further investigations confirmed the proposed mechanisms, they would provide support for a hypothesis originally advanced by Watson [38]. He suggested that MNR rats, although putatively selected for low susceptibility to stress, in fact exhibited greater sympathetic arousal under stress. This hypothesis is paradoxical, given the usual interpretation of the behavioral differences between the strains, as greater sympathetic arousal is usually held to be characteristic of a larger stress response.

The present experiments do not elucidate precisely how the central and peripheral noradrenergic systems might mediate differences in more complex behaviors (see Introduction for a discussion of different potential mechanisms). Further experiments will be needed to distinguish between the broad classes of possibilities previously outlined. However, the existence of strain differences in the norepinephrine system together with their confirmation and extension [20] suggest that further experimentation along these lines would be worthwhile.

A cautionary note should be added concerning the relevance of the present biochemical results, gathered largely under basal or nonstress conditions, to the etiology of behavioral differences between the strains in stressful situations. Perhaps strain differences in transmitter levels and/or metabolism might be altered if they were studied under stressful conditions. There are some indications in the data that this might be so. Although no overall differences in turnover were found in brainstem and telencephalon in the αMPT studies, it should be noted that the animals were in a relatively nonstressed behavioral state, having simply been returned to their home cages while αMPT was exerting its effect. However, the fact that MR rats had a greater percent fall of norepinephrine in brainstem than MNRA rats 1 hr after αMPT, a time when one might expect differences in response to the aversive manipulations of injection, may indicate that strain differences in central norepinephrine metabolism exist only under conditions of prolonged stress. Many studies indicate a higher turnover of norepinephrine in brainstem and hypothalamus after induction of stress [33,36] and provide precedents for this idea.

MR and MNR Strains as Models for Neurotransmitter/Behavior Research

Neurotransmitter levels and associated regulatory enzymes have recently been extensively studied in inbred mice with the long-term view that the discovery of relevant differences may provide a suitable model for the study of genetically based mental disorders [2]. Such studies provide valuable information about the possible existence of genetic control of neurotransmitter systems in the first instance, but are much more limited when the purpose is to provide evidence about the putative association between neurochemical and behavioral events. If this is the major purpose of the experiment, then it is a more powerful strategy to concentrate by genetic selection positive and negative alleles influencing a particular behavioral trait within separate lines and then study associated characters within these lines. The present findings indicating differences in structure and function of the norepinephrine-containing neurons in both peripheral and central tissues of the Maudsley rat strains are somewhat more interesting from this point of view. These strains have been selected from a common base population on a behavioral/physiological criterion involving differences in susceptibility to stress. Thus, the discovery of a difference in the norepinephrine system in these strains raises the possibility that this system may be involved in the mediation of their behavioral differences.

Other recent experiments [5] confirm the existence of a difference in the central serotonergic system of these strains that was previously claimed by Sudak and Maas [34]. In addition, a difference between the lines has recently been demonstrated in the central dopaminergic system [7]. These results suggest that the behavioral differences between the strains may rest on differences in more than one neurochemical system.

The nature of the genetic system controlling the differences we have found is not elucidated by these experiments. Since the genetic control of most behavioral traits is considered to be polygenic, it is possible that the neurochemical differences we have demonstrated are also polygenically based. However, the possibility has recently been raised that a major gene may account for a substantial proportion of the variation between the strains in open-field behavior [6]. Thus, a similar possibility may hold true for the biochemical alterations we have documented. In this case, since single genes can be manipulated with far more ease than polygenic systems, the prospect of investigating the covariation of the noradrenergic system and behavior would be correspondingly more promising.

As pointed out by Lader, the investigation of central mechanisms underlying emotional behavior and its pathological counterpart, anxiety, is hampered by the lack of good animal models [21]. Since a substantial amount of pharmacological data in humans implicate adrenergic systems with the behavioral manifestations of arousal, emotionality, and fear, the selection of animal stocks with differences in these behaviors facilitates more detailed and extensive biochemical investigations of their mechanisms [11,37]. The Maudsley strains, despite disagreement about interpretation of their behavioral differences [1] and widely disparate physiological make-up, can serve some useful functions in this area.

REFERENCES

1. Archer, J. The Maudsley reactive and non-reactive strains of rats: The need for an objective evaluation. *Behav. Genet.* **5**: 411-415, 1975.
2. Barchas, J. D., R. D. Ciaranello, J. A. Dominic, T. Deguchi, E. K. Orenberg, J. Renson and S. Kessler. Genetic differences in mechanisms involving neurotransmitters. *J. Psychiat. Res.* **11**: 347–360, 1974.
3. Blizard, D. A. Autonomic activity in the rat: Effects of genetic selection for emotionality. *J. comp. physiol. Psychol.* **76**: 282–289, 1971.
4. Blizard, D. A. Colonic reactivity in curarized rats of the MR and MNR strains: Differential response to novel stimuli and classical conditioning procedures. *Behav. Genet.* **5**: 90, 1975. (abstract)
5. Blizard, D. A. and B. Liang. Differences in the central 5-HT system in the Maudsley Reactive and Non-Reactive rats: A re-evaluation. In preparation, 1977.
6. Blizard, D. A. and B. Liang. The Maudsley experiment: Evidence of major gene involvement. In preparation, 1977.
7. Blizard, D. A., J. Slater, B. Liang and L. Shenkman. Serum prolactin and hypothalamic dopamine in rat strains selectively bred for differences in susceptibility to stress. *Neuroendocrinology*, in press, 1977.
8. Broadhurst, P. L. Experiments in psychogenetics. In: *Experiments in Personality: Psychogenetics and Psychopharmacology*. Vol. I, edited by H. J. Eysenck. London: Routledge and Kegan Paul, 1960, pp. 3-102.
9. Broadhurst, P. L. The Maudsley reactive and non-reactive strains of rats: A survey. *Behav. Genet.* **5**: 299–319, 1975.
10. Brodie, B. B., E. Costa, A. Dlabac, N. H. Neff and H. H. Smookler. Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. *J. Pharmac. exp. Ther.* **154**: 493–498, 1966.
11. Corrodi, H., K. Fuxe, P. Lidrink and L. Olsen. Minor tranquilizers, stress and central catecholamine neurons. *Brain Res.* **29**: 1–16, 1971.
12. Euler, U. S. von and F. Lishajko. Improved technique for the fluorometric estimation of catecholamines. *Acta physiol. scand.* **51**: 348–356, 1961.
13. Eysenck, H. J. *The Biological Basis of Personality*. Springfield: Charles C. Thomas, 1967.
14. Eysenck, H. J. and P. L. Broadhurst. Experiments with animals: Introduction. In: *Experiments in Motivation*, edited by H. J. Eysenck. Oxford: Pergamon, 1964, pp. 285–291.
15. Festing, M. F. W. and J. Staats. Standardized nomenclature for inbred strains of rats: Fourth listing. *Transplantation* **16**: 221–245, 1973.
16. Feuer, G. and P. L. Broadhurst. Thyroid function in rats selectively bred for emotional elimination. *J. Endocr.* **24**: 127–136, 1962.
17. Harrington, G. M. Strain differences in open-field behavior of the rat. *Psychon. Sci.* **27**: 51–53, 1972.
18. Imada, H. Emotional reactivity and conditionability in four strains of rats. *J. comp. physiol. Psychol.* **79**: 474–480, 1972.
19. Iversen, L. L. and J. Glowinski. Regional studies of catecholamines in the rat brain. II. Rate of turnover of catecholamines in various brain regions. *J. Neurochem.* **13**: 671–682, 1966.
20. Liang, B. and Blizard, D. A. Central and peripheral norepinephrine levels in rats selectively bred for differences in response to stress: Confirmation and extension. Submitted for publication, 1977.
21. Lader, M. The peripheral and central role of the catecholamines in the mechanisms of anxiety. *Int. Pharmacopsychiat.* **9**: 125–137, 1977.
22. Masuoka, D. R., W. Prell, H. F. Shott, A. F. Alcoraz and E. L. James. Separation of catecholamines and their metabolites. *Analyt. Biochem.* **5**: 426–432, 1963.
23. Noth, R. H. and S. W. Spaulding. Decreased serum dopamine-β-hydroxylase in hyperthyroidism. *J. clin. Endocr. Metab.* **39**: 614–617, 1974.
24. Prange, A. J., J. L. Mack and M. A. Lipton. Catecholamines: Diminished rate of synthesis in rat brain and heart after thyroxine pretreatment. *Life Sci.* **9**: 901–907, 1970.
25. Reis, D. J. Consideration of some problems encountered in relating specific neurotransmitters to specific behaviors or disease. *J. Psychiat. Res.* **11**: 145–148, 1974.
26. Rick, J J., A. K. Huggins and G. A. Kerkut. The comparative production of γ-amino butyric acid in the Maudsley reactive and non-reactive strains of rat. *Comp. Biochem. Physiol.* **20**: 1009–1012, 1967.
27. Sarnat, H. B. and M. G. Netsky. *Evolution of the Nervous System*. New York: Oxford University Press, 1974, pp. 198–219.
28. Savage, R. D. and H. J. Eysenck. The definition and measurement of emotionality. In: *Experiments in Motivation*, edited by H. J. Eysenck. Oxford: Pergamon, 1964, pp. 292–314.
29. Schildkraut, J. J. and S. S. Kety. Biogenic amines and emotion. *Science* **156**: 21–30, 1967.
30. Snedecor, G. W. *Statistical Methods*. Ames: Iowa State College Press, 1956.

31. Sorenson, G. A. and G. D. Ellison. Nonlinear changes in activity and emotional reactivity scores following central noradrenergic lesions in rats. *Psychopharmacologia* **32**: 313–325, 1973.
32. Spector, S., A. Sjoerdsma and S. Udenfriend. Blockade of endogenous norepinephrine synthesis by αMPT, an inhibitor of tyrosine hydroxylase. *J. Pharmac. exp. Ther.* **147**: 86–95, 1965.
33. Stone, E. A. Adrenergic activity in rat hypothalamus following extreme muscular exertion. *Am. J. Physiol.* **224**: 165–169, 1973.
34. Sudak, H. S. and J. W. Maas. Behavioral-neurochemical correlation in reactive and non-reactive strains of rats. *Science* **146**: 418–420, 1964.
35. Sudak, H. S. and J. W. Maas. Central nervous system serotonin and norepinephrine localization in emotional and non-emotional strains in mice. *Nature (Lond.)* **203**: 1254–1256, 1964.
36. Thierry, A. M., F. Javoy, J. Glowinski and S. S. Kety. Effects of stress on the metabolism of norepinephrine, dopamine, and serotonin in the central nervous system. I. Modification of NE turnover. *J. Pharmac. exp. Ther.* **163**: 163–171, 1968.
37. Tyrer, P. J. and M. H. Lader. Clinical response to propranolol and diazepam in somatic and psychic anxiety. *Br. med. J.* **2**: 14–16, 1974.
38. Watson, R. H. J. Constitutional differences between two strains of rats with different behavioral characteristics. *Adv. Psychosom. Med.* **1**: 160–165, 1960.
39. Weiner, N. A critical assessment of methods for the determination of monoamine synthesis rates *in vivo*. In: *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes*, edited by E. Usdin. New York: Raven Press, 1974, pp. 143–160.
40. Whitby, L. G., J. Axelrod and E. Weil-Malherbe. The fate of H^3-norepinephrine in animals. *J. Pharmac. exp. Ther.* **132**: 193–201, 1961.
41. Wurtman, R. J., F. Anton-Tay and S. Anton. On the use of synthesis inhibitors to estimate brain norepinephrine synthesis in gonadectomized rats. *Life Sci.* **8**: 1015–1022, 1969.