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Inverse Relationship Between Whole Brain Monoamine Levels and Audiogenic Seizure Susceptibility in Mice: Failure to Replicate

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LINTS, C. E., J. F. WILLOTT, P. Y. SZE AND L. H. NENJA. *Inverse relationship between whole brain monoamine levels and audiogenic seizure susceptibility in mice: Failure to replicate.* PHARMAC. BIOCHEM. BEHAV. 12(3) 385–388, 1980.—Whole brain levels of 5-HT and NE in audiogenic seizure susceptible DBA mice were compared with those of the nonsusceptible C57BL/6 strain at different ages and in two separate laboratories that used different assay procedures. Contrary to previous reports, there were no significant differences between the susceptible and nonsusceptible animals in whole brain levels of either amine at the age of peak seizure susceptibility. Differential stress associated with capturing the susceptible mice had no effect on brain levels of NE. The data question the suggestion that deficits in brain monoamine transmission capable of being indexed by lower whole brain levels of NE and/or 5-HT play a role in genetically determined susceptibility to audiogenic seizures.

NE 5-HT Brain levels Audiogenic seizures

SUSCEPTIBILITY of DBA/2J mice to audiogenic seizures (AGS) is related to age, beginning around 15 days post partum, reaching a peak between 19–23 days, and declining by 28–30 days [9,13]. Some age-related differences in brain monoamine levels between genetically susceptible and nonsusceptible strains of mice have also been reported to vary with development in a manner paralleling susceptibility to AGS. In one study whole brain levels of both norepinephrine (NE) and 5-hydroxytryptamine (5-HT) in 14 and 28 day-old DBA/2J mice were similar to those found in like-aged nonsusceptible C57BL/6J mice. At the age of peak seizure susceptibility (21 days), however, the whole brain levels of both these amines were significantly lower in the susceptible animals. Furthermore, this study reported an inverse correlation between seizure susceptibility and brain levels of NE and 5-HT across the DBA, C57BL, and the F1 hybrid of these two strains which shows seizure susceptibility intermediate between the parental strains [9]. Another study that assayed both forebrain and hindbrain monoamine levels reported that DBA mice had significantly lower levels of NE in both brain regions at 21 and 28 days of age when compared with the C57BL animals. The forebrain levels of 5-HT were also lower at 21 days of age in the seizure susceptible mice in this report, but the effect was not statistically significant [5]. Thus, although there is some lack of agreement between these two reports, the results taken together suggest that deficits in brain monoamine transmission capable of being indexed by lower whole brain levels of NE, and perhaps 5-HT, may play a role in genetically determined AGS susceptibility.

Experiment 1 of the present study was designed to replicate the above mentioned findings with respect to brain levels of NE and 5-HT in DBA/2J mice at their age of peak seizure susceptibility. Negative findings were obtained, so Experiment 2 tested the possibility that these discrepant results were related to differential handling of the seizure susceptible animals. Again, negative results were obtained and are supported in Experiment 3 by data from a different laboratory that used different assay procedures and different substrains of seizure susceptible and nonsusceptible mice.

EXPERIMENT 1: WHOLE BRAIN LEVELS OF 5-HT AND NE IN SUSCEPTIBLE AND NON-SUSCEPTIBLE MICE DURING THE PERIOD OF PEAK AGS SUSCEPTIBILITY

Brain levels of 5-HT and NE were assayed in DBA/2J

TABLE 1

WHOLE BRAIN LEVELS OF 5-HT AND NE IN 22–23 DAY-OLD MICE

Strain	Brain weight (mg)	5-HT (ng/g)	NE (ng/g)
C57BL/6J	412 ± 8 (9)	505 ± 33 (9)	445 ± 29 (6)
DBA/2J	323 ± 8 (9)*	494 ± 44 (9)	463 ± 26 (6)

*$t(16)=7.77$, $p<0.001$ (2-tailed).

Each value is the mean ± SEM with the number of animals indicated in parentheses.

mice during the period of peak seizure susceptibility and in like-aged, nonsusceptible C57BL/6J mice.

METHOD

Subjects

The subjects were DBA/2J and C57BL/6J mice of both sexes who were the offspring of stock obtained from the Jackson Laboratory, and were bred and reared in the Northern Illinois University Psychology Department mouse colony in controlled lighting (12 hr light/12 hr dark) and a moderate acoustic environment. The animals were weaned on the day of assay. Mice from this colony are constantly being tested for AGS susceptibility in conjunction with various experiments and demonstrate a near 100% incidence of seizure activity in 19–23 day-old DBA animals and a near 0% incidence in C57BL/6J animals. For example, of 88 DBA/2J mice tested for a recent study in this laboratory 82% displayed full tonic convulsions and no animals failed to show at least wild running.

Monoamine Assay

All animals were sacrificed by decapitation between 1–2 p.m. Their brains were quickly removed, weighed, frozen in liquid nitrogen and stored in a freezer (−20°C) until assay within one week. Each brain sample was assayed for levels of 5-HT and NE using a modification of the procedure reported by Maickel, Cox, Saillant, and Miller [6]. The tissue was homogenized in acid n-butanol and, after centrifugation, 2.5 ml of the supernatant was added to 7 ml iso-octane [1] and 0.2 ml 0.1 N HCl. The mixture was shaken mechanically for 5 min and then centrifuged. The organic layer was removed by aspiration and the aqueous layer was used for the determination of NE. A 0.1 ml aliquot was oxidized with iodine to develop the polyhydroxyindole derivatives, then heated at 100°C for 2 min, cooled, and fluorescence was measured at 385 mμ (excitation) and 485 mμ (emission). The remainder of the tissue homogenate was washed with an equal volume of borate buffer (pH 10.0–10.2) in order to remove the acidic indoles [3]. After centrifugation, the 5-HT in a second aliquot was extracted into 0.1 N HCl as above, treated with p-opthaldehyde (OPT) and, after heating for 10 min at 100°C, the fluorescence was read at 360 mμ (excitation) and 470 mμ (emission). The concentrations of each amine were determined as ng/g tissue (wet weight). The OPT and iodine were obtained from Regis Chemical Company (Morton Grove, IL), and the standards (5-HT creatinine sulfate and L-NE bitartrate) were obtained from Sigma Chemical Company (St. Louis, MO). Fluorescence was read in an Aminco-Bowman spectrophotofluorometer.

RESULTS

Contrary to previous reports [5,9], there were no significant differences in whole brain 5-HT and NE levels between the seizure susceptible and nonsusceptible strains of mice during the period of peak seizure susceptibility (Table 1). In agreement with Kellogg [5] and other investigators [4,12], but contrary to Schlesinger and Griek [9], the brains of the DBA mice consistently weighed significantly less than those of the C57BL strain, p's<0.001, Student's t-test, two-tailed.

EXPERIMENT 2: EFFECTS OF CAPTURE STRESS ON WHOLE BRAIN NE LEVELS IN 19 DAY-OLD DBA/2J MICE

Stress can result in reduced brain levels of NE in some circumstances [2]. Thus, stress associated with handling could be one factor accounting for the discrepant findings of the various laboratories with respect to the strain differences in brain levels of NE during the period of peak AGS susceptibility. That is, different investigators might have used different handling techniques or have been differentially skillful in capturing and decapitating the animals. In this experiment stressed and nonstressed DBA/2J mice were compared with respect to their whole brain NE levels.

METHOD

The subjects were 19 day-old DBA/2J mice housed and assayed for whole brain NE levels as in Experiment 1. Littermates were divided into two groups. Animals in the stressed group were purposefully pinched and chased about their cage for a period of 30 sec before decapitation and those in the control (nonstressed) group were captured routinely and rapidly, being decapitated within 15 sec of the onset of capture.

RESULTS

There were no significant differences in whole brain NE levels between the stressed and nonstressed (control) mice (Table 2) indicating that differential capture stress probably does not contribute to the reports of depleted brain NE levels in AGS susceptible DBA/2J mice.

EXPERIMENT 3: WHOLE BRAIN LEVELS OF 5-HT AND NE IN 16, 20, AND 28 DAY-OLD DBA/1BG AND C57BL/6BG MICE

Since different types of environmental factors have been demonstrated to affect both brain levels [2] and metabolism [8,11] of the biogenic amines in experimental animals, we felt that it would be important to confirm the negative results of

TABLE 2

EFFECTS OF CAPTURE STRESS ON WHOLE BRAIN NE LEVELS IN 19 DAY-OLD DBA/2J MICE

Treatment group	N	Brain weight (mg)	NE (ng/g)
Control	7	342 ± 4	520 ± 19
Stressed	7	341 ± 2	516 ± 24

Each value is the mean ± SEM.

the previous experiments in another laboratory where housing conditions and handling techniques would be different. Furthermore, different assay procedures were used in this experiment which compared whole brain levels of 5-HT and NE in 16, 20, and 28 day-old DBA/1 Bg and C57BL/6Bg mice at the Department of Biobehavioral Sciences, University of Connecticut, Storrs, CT.

METHOD

Subjects

The two inbred strains, C57BL/6Bg and DBA/1Bg, were obtained from Dr. Benson E. Ginsburg's colony at the University of Connecticut. Both strains were originally acquired from the Jackson Memorial Laboratory and have been separately inbred in Dr. Ginsburg's colony for at least 50 generations. The mice were maintained under a 12 hr light/12 hr dark cycle and at a temperature of 22 ± 1°C and a relative humidity of 52 ± 2%. Mice from this colony are continuously being tested for AGS susceptibility and demonstrate a 40–50% incidence of seizure activity on Day 20 and a 80–90% incidence on Day 28 in DBA/1, and a near 0% incidence in C57BL/6 at all ages. Pregnant females were housed singly and litters, with their birth monitored to an accuracy of ±0.5 days, were not disturbed until the time of experimental use. A total of 193 mice (C57BL/6, n=92; DBA/1, n=101) of both sexes were used in the experiment.

Monoamine Assay

All animals were sacrificed by decapitation at 9–10 a.m. and the brains were quickly removed and weighed. Each brain was assayed for levels of 5-HT and NE by the method of Mead and Finger [7] as modified by Wise [14]. The dissected brain was immediately homogenized in 4 vol of 0.01 N HCl. The homogenate was transferred to 5 ml of n-butanol containing 0.5 g of NaCl. After shaking thoroughly for 15 min, the layers were separated by centrifugation. Five ml of the butanol layer was then added to 5 ml of n-heptane and 0.6 ml of 0.01 N HCl. The mixture was shaken vigorously for 5 min and then centrifuged. The organic layer was removed by aspiration and the aqueous layer was used for the determination of the monoamines. For NE, 150 μl of the aqueous extract was added to 100 μl of 0.2 M sodium phosphate buffer (pH 6.3) and 25 μl of 0.25% potassium ferricyanide. After exactly 3 min, 100 μl of a freshly prepared alkaline ascorbate solution (2% ascorbic acid-ethylene diamine-4 N NaOH, 10:2:90 by volume) was added and the mixture allowed to stand for 40–60 min. Fluorescence was then measured at 405 mμ (excitation) and 508 mμ (emission). For 5-HT, 250 μl of the aqueous extract was added to 100 μl of concentrated HCl. Fluorescence was immediately measured at 295 mμ (excitation) and 550 mμ (emission). L-NE bitartrate and 5-HT creatinine sulfate (Sigma Chemical Co., St. Louis, MO) were used as standards. Fluorescence was read in a Farrand spectrophotofluorometer.

RESULTS

No significant differences in whole brain 5-HT and NE levels were found between DBA/1Bg and C57BL/6Bg mice at any of the three ages examined (Table 3).

TABLE 3

WHOLE BRAIN LEVELS OF 5-HT AND NE IN TWO STRAINS OF MICE

Age (day)	Strain	5-HT* (ng/g tissue)	NE* (ng/g tissue)
16	C57BL/6	548 ± 68 (11)†	228 ± 12 (18)
	DBA/1	511 ± 32 (15)	249 ± 18 (20)
20	C57BL/6	521 ± 27 (10)	331 ± 20 (15)
	DBA/1	541 ± 33 (14)	340 ± 17 (16)
28	C57BL/6	500 ± 27 (20)	486 ± 32 (18)
	DBA/1	511 ± 18 (20)	452 ± 39 (16)

*There was no difference between males and females. Therefore, the data were pooled.

†Each value is the mean ± SEM with the number of animals indicated in parentheses.

DISCUSSION

The results of the present experiments seriously question the hypothesis that strain and age-dependent differences in whole brain levels of 5-HT and NE are causally related to parallel differences in genetically determined AGS activity. Biochemical assays conducted independently by two laboratories failed to replicate earlier demonstrations of such a relationship [5,9]. The brain levels of these monoamines in the seizure susceptible DBA mice were not reliably lower than those of like-aged, nonsusceptible, C57BL/6 mice during the period of peak susceptibility.

The reasons for these contradictory findings are not clear. However, the present series of experiments seems to rule out several possibilities. Stress associated with general housing conditions, handling procedures, or methods of capture did not differentially influence assay values. Furthermore, since different assay procedures failed to produce positive results, the assays would seem to be reliable. Finally, since the animals in Experiments 1 and 2 were sacrificed between 1–2 p.m. (when they are usually tested for AGS activity) and those in Experiment 3 were sacrificed between 9–10 a.m., the circadian rhythm of the amines does not appear to be a factor. It must therefore be concluded that the previously reported age- and strain-related difference in whole brain NE and 5-HT levels are not robust and may be affected by subtle unknown factors. The audiogenic seizure, on the other hand, is a very robust phenomenon, and seizures were readily elicited in all laboratories. Apparently, a change in these amine levels is not a necessary condition for AGS suceptibility.

Highly inbred strains of mice like the DBA and C57BL differ with respect to many genes and many behavioral, anatomical, and neurochemical phenotypes, and determination of their causal interrelationships must be approached with caution (see [10]). Although there are a considerable number of studies suggesting that deficits in monoamine transmission may play a role in genetically determined AGS susceptibility [9], it appears that any such deficits cannot be reliably indexed by whole brain levels of NE or 5-HT.

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