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Brain 5-Hydroxytryptamine Correlates of Behavior: Studies Involving Spontaneously Hypertensive (SHR) and Normotensive Wistar Rats

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ROSECRANS, J. A. AND M. D. ADAMS. *Brain 5-hydroxytryptamine correlates of behavior: studies involving spontaneously hypertensive (SHR) and normotensive Wistar rats.* PHARMAC. BIOCHEM. BEHAV. 5(5) 559–564, 1976. – Brain area 5-hydroxytryptamine (5-HT) turnover and behavioral correlates were compared in spontaneously hypertensive rats (SHR) and normotensive Wistar rats. SHR appeared to have hyperfunctional 5-HT systems as evidenced by the finding that brain area 5-HT turnover times were 53% lower in SHR, with significant changes seen in the limbic forebrain. SHR, while less active upon initial testing of spontaneous motor activity than Wistar rats, did not habituate as readily to the testing procedures as evidenced by higher activity than Wistar rats in repeated testing situations. In the light of other data indicating that 5-HT systems are predominantly inhibitory to ongoing behavior, these data suggest that habituation, as determined in this study, is an active process that is retarded in the SHR because of hyperfunctional 5-HT systems. Spontaneous activity of SHR was inhibited less than that of Wistar rats by a conditioned stimulus which is consistent with the observations of others that 5-HT systems tend to suppress responsiveness to external stimuli. While spontaneous activity data suggested that SHR were more emotional than Wistar rats, the SHR also exhibited a higher avoidance rate in an unsignaled shuttle avoidance procedure which is usually indicative of a lower emotional state.

Brain 5–HT Spontaneous hypertension Motor activity Habituation

SEVERAL studies have been conducted in an attempt to better understand the role of brain 5-hydroxytryptamine (5-HT) projection systems in the control of certain animal behaviors. Much research so far conducted indicates that 5-HT pathways appear to be predominantly inhibitory resulting in a variety of changes in ongoing behaviors. Wise *et al.* [21], by the direct application of 5-HT to various brain areas, have also suggested that 5-HT systems subserve a punishment function by inhibiting normal catecholamine reward systems.

Research conducted in this laboratory has been directed toward an understanding of the relationship between 5-HT function and the ability of an animal to habituate to a novel environment. Data collected thus far indicate that rats who do not readily habituate to a novel environment (as indicated by a higher rate of activity after repeated exposures) exhibit a more functional 5-HT system upon chemical evaluation [13–16].

Preliminary experiments in this laboratory demonstrated a greater 5-HT turnover rate in certain brain areas of the spontaneously hypertensive rat (SHR) when compared to a normotensive Wistar control strain [17]. Thus, behavioral studies were conducted in these two strains of rats in order to obtain additional information concerning 5-HT systems and behavior.

METHOD

Male normotensive (Wister, MW-3) and genetically hypertensive rats (SHR) 12 weeks of age were obtained from Purina Laboratory Animals, Vincentown, N.J., U.S.A. Animals were housed two per cage and were allowed to adapt to their environment for a period of two weeks prior to experimentation. During this adaptation period, oxytetracycline hydrochloride was included in the drinking water (50 μg/ml). Following the two week equilibration period, the oxytetracycline was removed from the water, each animal was lightly etherized, and indirect systolic blood pressure measurements were made (tail cuff plethysmography). One week following the evaluation of blood pressures in normotensive and hypertensive rats, each animal was subjected to a variety of behavioral and chemical procedures.

Behavioral Procedures

Activity measurements. Rats of each strain were exposed

to two activity procedures in which daily activity counts were recorded during four repeated exposures. The first procedure utilized a circular path Woodward Activity Cage (Herndon, VA) and essentially measured horizontal activity. The second procedure utilized a 25 cm × 25 cm Lafayette activity (Lafayette, IN) platform which measured total activity. In this latter procedure rearing behavior was also simultaneously measured.

Activity was also recorded in an open field consisting of a circular arena 31 cm high and 62 cm in dia. The arena was painted white and the floor divided into four inch squares. A 60 W light bulb centrally suspended 45 cm above the arena provided illumination for the field. Hand counters were used to tally the number of squares (spontaneous activity) entered and standing up on both hind legs (rearing) during each trial.

Conditioned suppression of spontaneous activity. A conditioned emotional response was established in both rat strains by 15 pairings of a 4 sec white noise (CS) with 2 sec of 0.6 ma scrambled shock. The intertrial interval was 11 sec and shock was presented during the last 2 sec of the CS. Three days after conditioning, rats were placed in a Lafayette activity cage and the CS reintroduced to the animal via a house speaker for six minutes. All rats were exposed to two sessions. Activity was recorded at 15 sec intervals.

Unsignaled avoidance responding. Rats of each strain were exposed to twelve 15 min sessions in shuttle boxes programmed to deliver an unsignaled avoidance schedule. Shock (1 mA; 0.2 msec) was presented every 5 sec until an animal made a response by crossing a hurdle to the other side of the apparatus. Shocks were presented every 20 sec if an animal did respond. An animal could delay or avoid a shock by crossing into the opposite chamber prior to the 20 sec shock period. Individual rates of spontaneous activity were determined during a 10 min period prior to being exposed to the avoidance procedure (Lafayette activity cage).

Chemical Techniques

Brain area serotonin (5-HT) turnover (synthesis) was estimated by measuring the increase in 5-HT levels at various time intervals following the inhibition of monoamine oxidase. These experiments were conducted in 15 week old W and SHR. In this experiment, three control and three SHR were sacrificed by decapitation at four time intervals (0, 10, 20, and 30 min) following the administration of the MAO inhibitor, pargyline (75 mg/kg; IP).

Following sacrifice, brains were removed and dissected into four parts which included the following areas: (1) cerebral cortex (CC); (2) limbic forebrain (LF; this area included portions rostral to the CC from the thalamus plus the caudate nucleus); (3) diencephalon (Dien.); and, (4) brainstem (BS). Each brain area was quickly frozen on dry ice blocks and was maintained at -20°C until the time of assay. 5-HT levels were measured by the technique of Rosecrans and Schechter [16]. Turnover times (time in minutes to synthesize a ng of 5-HT) were calculated from slopes and confidence limits determined from a regression analysis of the chemical changes measured (REGR routine, SAS package).

Statistical Analysis

Behavioral data was analyzed using a 2 × 2 analysis of variance. Appropriate t values were also determined and presented.

RESULTS

Brain Area 5-Hydroxytryptamine Turnover

Normotensive and hypertensive animals used for these studies displayed blood pressures (110 ± 5 mm Hg and 181 ± 9 mm Hg, respectively) of comparable magnitude to those used in the behavioral studies. While brain steady state 5-HT levels were 20–26% lower in SHR than normotensive Wistar rats, the turnover time (inverse of turnover rate) was less in the SHR in all brain areas (significantly less in the limbic forebrain; Table 1).

Spontaneous Activity in Normotensive Wistar Rats and SHR

Upon initial exposure to electronic spontaneous motor activity testing procedures, the SHR (blood pressure 169 ± 2 mm Hg) were less active than normotensive (blood pressure 108 ± 2 mm Hg) Wistar animals (Table 2). On

TABLE 1

5-HYDROXYTRYPTAMINE TURNOVER IN SPONTANEOUS HYPERTENSIVE (SH) AND CONTROL WISTAR (W) RATS

Brain Area	5-HT* Levels* nMoles/g ± SE W N = 6	5-HT* Levels* nMoles/g ± SE SH N = 6	Molar Turnover Time (min)† (95% Confidence Limits) W N = 12	Molar Turnover Time (min)† (95% Confidence Limits) SH N = 12
Cerebral Cortex	2.89 ± 0.25	2.22 ± 0.23	137 (88-286)	68 (47-128)
Limbic Forebrain	4.56 ± 0.55	3.40 ± 0.28	211 (73-245)	44 (36-57)
Diencephalon	6.10 ± 0.51	4.80 ± 0.26	113 (62-260)	54 (44-69)
Brainstem	4.69 ± 0.28	3.48 ± 0.35	71 (54-104)	48 (38-61)

*5-HT levels determined in six nondrug treated rats of each strain.

†Values were calculated from slopes and 95% confidence limits determined via a regression analysis of the rise in 5-HT after MAOI. 5-HT turnover times and confidence limits were determined by dividing the predicted nMole 5-HT steady state level by the nMole 5-HT turnover rate/min.

TABLE 2

RATE OF HABITUATION IN CONTROL (W) AND HYPERTENSIVE RATS (SHR)

Behavioral* Parameter	Strain	(N)	Initial Exposure	Last Exposure
Locomotor Activity Counts ± SE	W	(12)	169 ± 17	47 ± 9
	SHR	(12)	96 ± 12‡	61 ± 8†
Total Spontaneous Activity Counts ± SE	W	(18)	127 ± 12	58 ± 6
	SHR	(18)	110 ± 6	92 ± 12†
Rearings ± SE	W	(18)	18.1 ± 1.7	5.6 ± 0.9
	SHR	(18)	12.2 ± 1.5‡	9.4 ± 1.7†

*All rats were exposed to four 3-min. daily exposures in a specific activity cage. Locomotor activity refers to rats studied in a circular path activity cage. Total spontaneous activity refers to a different group of rats exposed to a Lafayette activity cage. Rearing behavior was determined simultaneously in the latter experiment.

†The data were subjected to a 2x2 analysis of variance which indicated a significant strain exposure interaction; minimal $p<0.05$.

‡Initial exposures were significantly different at $p<0.05$.

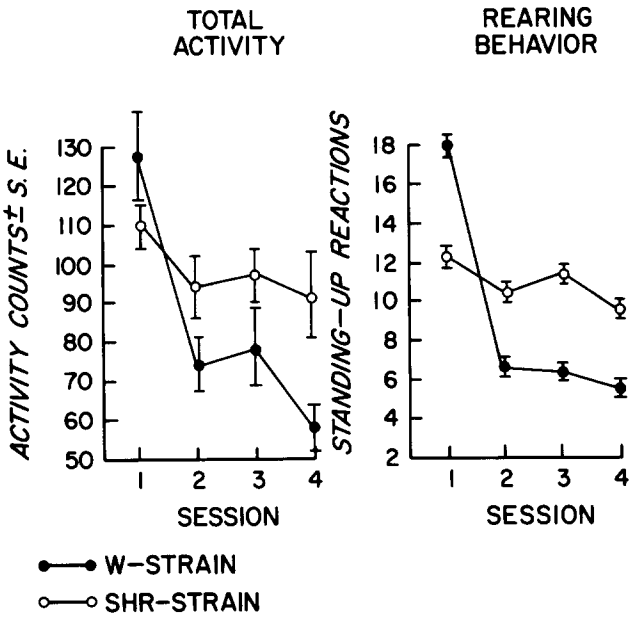

FIG. 1. Spontaneous locomotor activity and rearing behavior in the open field (see Methods). Asterisks indicate significant differences between strains. Vertical bars represent SE of means.

repeated testing, activity was observed to decline in both strains of rats. However, the magnitudes of decline in spontaneous motor activity were not similar for the two groups as evidenced by a significant strain-exposure interaction when the data in Table 2 were subjected to a 2 × 2 factorial analysis of variance [strains and exposure (initial or final)]. Thus, activity in the normotensive animals declined more (54–73%) between initial and final exposures than in the SHR (17–36%). Therefore, in contrast to the data recorded during the initial exposure, the SHR displayed

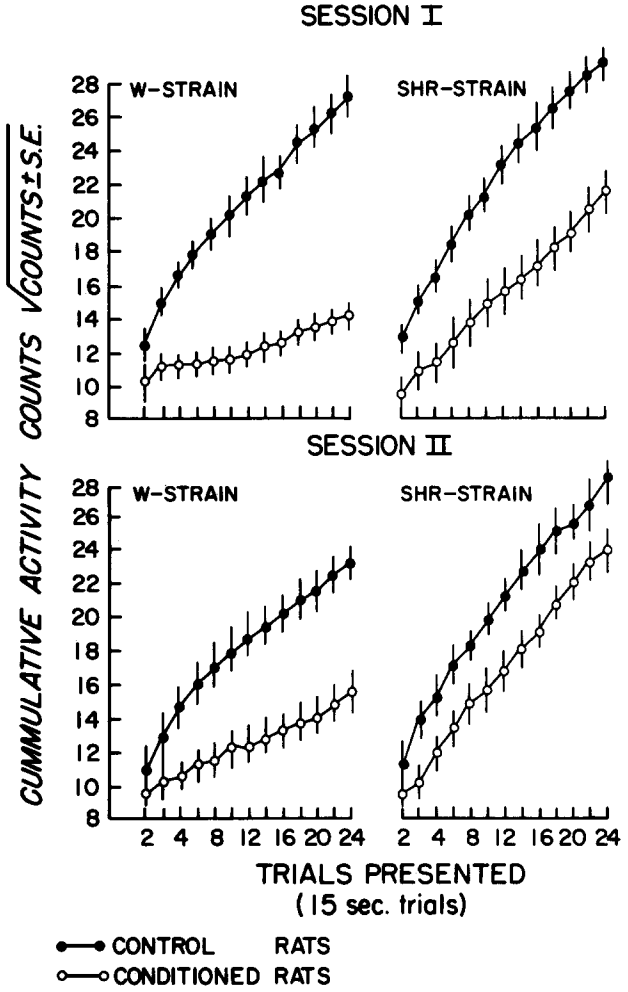

FIG. 2. Conditioned suppression of spontaneous activity in SHR and normotensive Wistar rats. Conditioned rats were exposed to 15 pairings of white noise and shock (0.6 ma) three days prior to activity exposure. Control rats were exposed only to the white noise. During sessions in the activity cage, only conditioning white noise was reintroduced. Vertical bars represent SE of means.

greater spontaneous activity during the final testing situation.

Similar data were obtained when activity was measured in the open field (Fig. 1). Although SHR animals demonstrated significantly reduced activity (compared to normotensive Wistar rats) upon initial testing, their activity declined very little upon subsequent exposures. In contrast, the activity of the normotensive Wistar strain declined dramatically upon subsequent exposures.

Conditioned Suppression of Activity in Normotensive Wistar Rats and SHR

In these experiments, mean blood pressures ± SE were 118 ± 5 mm Hg for Wistar and 187 ± 7 mm Hg for SHR. Animals of each strain were conditioned to associate a white noise (CS) with a scrambled electrical shock under motor activity testing conditions. After conditioning, these animals (conditioned) and naive (control) animals of each strain were exposed to two sessions in which spontaneous motor activity was measured in the presence of the white

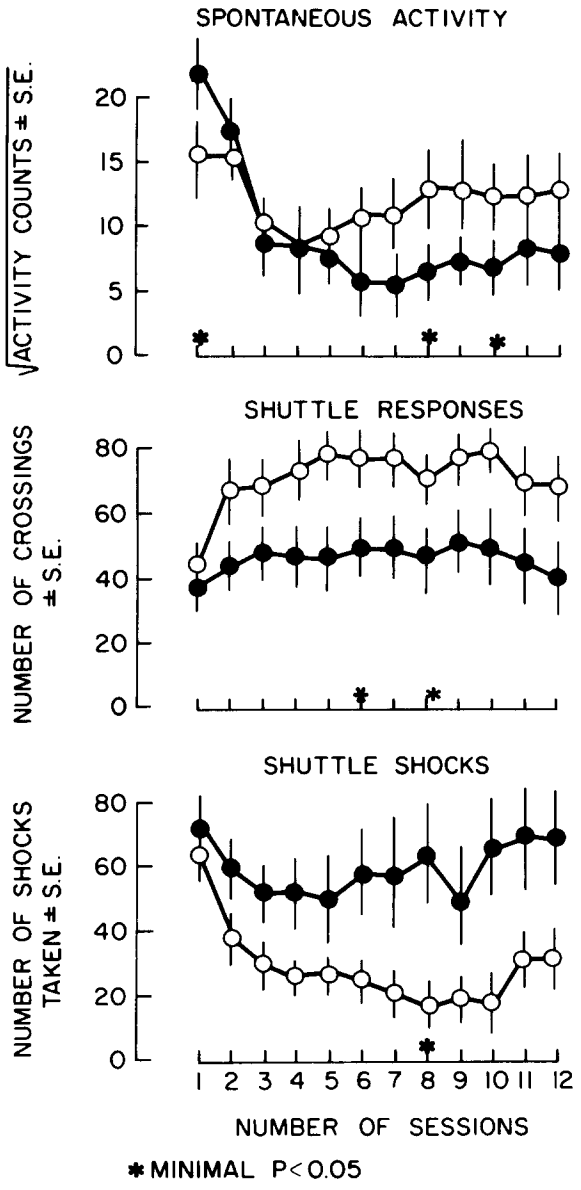

FIG. 3. Activity and shuttle responses of SHR and Wistar rats exposed to an unsignaled avoidance procedure. Rats were exposed to an activity cage for 10 min just prior to shuttle exposure. Each avoidance session was 15 min in duration. SHR are represented by the open circles whereas the closed circles represent Wistar controls. Asterisks indicate significant differences between strains. Vertical bars represent SE of means.

noise. Activity of conditioned Wistar animals was found to be greatly reduced when compared to naive animals of the same strain although some extinction of this conditioned behavior became apparent in the second session (Fig. 2). Some suppression of activity was observed in the conditioned SHR when compared to control SHR during Session I. However, the magnitude of this conditioned suppression was obviously less than in the normotensive Wistar rats. In the second session, no conditioned suppression of activity was seen in the SHR.

Unsignaled Avoidance Responding in Normotensive Wistar Rats and SHR

When tested in an unsignaled avoidance procedure, SHR were found to be more responsive than normotensive Wistar animals (Fig. 3). These animals shuttled twice as much and received far fewer shocks than the Wistar rats. As observed previously, measurement of spontaneous motor activity indicated that SHR animals were initially less active but that their activity declined significantly less than that of the normotensive Wistar animals upon continued testing.

DISCUSSION

Most studies regarding the role of 5-HT projection systems in behavior have indicated that such pathways are inhibitory in nature. Thus, lesions of the midbrain raphe nucleus, an important site for tryptaminergic neurons, have been shown to produce increases in motor activity [9], increased sensitivity to foot shock [10] and facilitated acquisition of conditioned avoidance behavior [11] in rats. Furthermore, rats depleted of 5-HT by parachlorophenylalanine (PCPA) demonstrate increased sensitivity to electric shock [20]. On the other hand, Sheard and Aghajanian [19] reported that electrical stimulation of the raphe nucleus produced an enhancement of the startle response to acoustic stimuli and Fechter [6] demonstrated that administration of the 5-HT precursor, 5-hydroxytryptophan had a facilitatory effect on the startle response.

In the present study, SHR demonstrated enhanced 5-HT turnover rates (decreased turnover times) in all brain areas examined when compared to a normotensive Wistar strain. While it is conceivable that differences in 5-HT turnover could merely reflect a compensation for lack of 5-HT activity at postsynaptic sites, it is generally agreed that such measurements are a reflection of the neural activity of 5-HT pathways. Therefore, behavioral studies were conducted to determine if this biochemical alteration could be correlated with changes in behavior.

Previous behavioral studies with SHR have been rather limited. Eichelman *et al.* [5] studied aggression and found no difference in aggressive behavior between SHR and normotensive control rats. In another study Pappas *et al.* [12] found SHR to be more active (open field and activity wheels) and less emotional (less defecation in the open field) than a normotensive Wistar strain. Saari and Pappas [18] also observed that foot-shock induced suppression of drinking behavior was less in SHR than in normotensive Wistar animals and thus concluded that SHR are less fearful.

The present study demonstrated that SHR exhibited less conditioned suppression of spontaneous motor activity than normotensive Wistar animals (Fig. 2) which is consistent with the data of Saari and Pappas [18]. Furthermore, the enhanced responsiveness of SHR in the unsignaled conditioned avoidance procedure (Fig. 3) is typical of the behavior of low emotional rats [13]. On the other hand, the present study also showed that SHR exhibited decreased spontaneous activity (in contrast to data of Pappas *et al.*) when compared to a normotensive Wistar control strain upon initial exposure to spontaneous motor activity recording cages or an open field.

Previous studies in this laboratory suggest that habituation to a novel environment, as measured by the rate of decline in spontaneous motor activity during consecutive testing sessions, is inversely correlated with brain 5-HT

turnover [16]. The present study confirms this observation, in that SHR animals while demonstrating greater brain 5-HT turnover (decreased turnover times) than normotensive Wistar animals, did not as readily habituate to spontaneous activity recording conditions. This finding, in conjunction with the many reports that 5-HT serves to inhibit behavior might be interpreted to mean that this form of habituation is an active process which is modulated by the inhibitory influence of 5-HT.

The effects, if any, of 5-HT on habituation are probably quite complex and might depend greatly on the type of test system employed. Thus, data in the current study from animals subjected to an auditory stimulus designed to inhibit activity in subsequent tests, indicated that the SHR, while demonstrating less conditioned suppression of activity in an initial testing procedure, appeared to adapt more readily upon a second test exposure (Fig. 2).

Other investigators have examined the role of 5-HT systems in habituation to the startle response. Aghajanian and Sheard [1] reported that electrical stimulation of the midbrain raphe area caused dishabituation of a habituated startle response. On the other hand, Conner *et al.* [3] found that depletion of brain 5-HT with PCPA slowed down but did not prevent the development of habituation to the startle response. More recently, Davis and Sheard [4] found that, while raphe lesions enhanced the startle response (sensitization to the response), such lesions did not alter the rate of startle response habituation. These latter investigators concluded that repetitive stimulus exposures produces both habituation and sensitization and that different neural systems might regulate these two behavioral phenomena.

This study was not designed to study the role of altered brain 5-HT turnover in the etiology or maintenance of the hypertensive state of the SHR. The role(s) of central 5-HT pathways in blood pressure regulation remain to be clearly established. At present there is data to implicate brain 5-HT systems in both the elevation and depression of blood pressure. Recent studies in the SHR indicate that orally administered PCPA decreases blood pressure [8] and that intraventricular administration of 5,6-dihydroxytryptamine (a chemical which selectively destroys 5-HT-containing nerve terminals) to young SHR retards the development of hypertension [2]. Thus it would seem reasonable to infer that the differences in brain 5-HT turnover between SHR and normotensive Wistar animals could be important in the development and/or maintenance of the hypertensive condition. However, there are two factors which must temper such speculation. The first is the selection of a standard normotensive Wistar control strain with which to compare SHR. The Kyoto Wistar is the strain from which the SHR were initially derived and is probably the best strain for cardiovascular comparisons with the SHR [22]. Such a strain was not available from the supplier at the time of this study. Secondly, it has also been shown that intraventricular administration of 6-hydroxydopamine (a compound which destroys central noradrenergic nerves) also retards the development of hypertension in SHR [7]. Therefore, it is quite possible that the more rapid brain 5-HT turnover demonstrated in the SHR is not at all related to the hypertensive condition or may be just one of many biochemical defects leading to the development of elevated blood pressure.

In summary, the present study has demonstrated an enhanced 5-HT turnover rate in several brain areas of the SHR when compared to a normotensive Wistar strain. Correlated with this biochemical alteration were behavioral changes which indicated that SHR animals may less readily habituate to a novel environment, suggesting that 5-HT systems may serve to inhibit habituation as measured in this study. While initial spontaneous activity was less in SHR than in Wistar rats, the inhibition of activity elicited by a conditioned stimulus was also less which is consistent with the observations of others that 5-HT systems suppress responses to external stimuli. Initial spontaneous activity data suggested that SHR were more emotional than Wistar rats. However, the observation that SHR exhibited a higher avoidance rate in an unsignaled conditioned avoidance procedure is consistent with the behavior of low emotional animals.

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