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Interstrain Comparison of Avoidance Behavior and Neurochemical Parameters of Brain Cholinergic Function

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BLAKER, W. D., D. L. CHENEY AND D. M. STOFF. *Interstrain comparison of avoidance behavior and neurochemical parameters of brain cholinergic function.* PHARMACOL BIOCHEM BEHAV **18**(2) 189–193, 1983.—Five rat strains (Long-Evans Hooded, Zivic Miller, Lewis, Buffalo and Fischer-344) were tested in a shuttlebox conditioned avoidance task and the differences in the performance levels among the strains were noted. In parallel experiments using naive rats, the acetylcholine concentrations in eight brain regions and the acetylcholine turnover rate in five brain regions were determined for these strains. Interstrain differences in these parameters were found but no correlation between avoidance performance and either of these measures was apparent in any brain region studied. In separate experiments, no differences were found in the hippocampal acetylcholine concentration or the turnover rate among good performing Hooded, poor performing Hooded or untested Hooded rats. Similarly, no differences in regional acetylcholine turnover rates were found between naive rats of the Iowa Reactive and Nonreactive strains. [^{3}H]-QNB (quinuclidinyl benzilate) binding was studied in three brain regions in the five strains, but no large interstrain differences in binding characteristics were found. In contrast to interpretations of other workers based on less direct assay methods involving fewer strains, we conclude that no strong correlation exists between avoidance performance ability and basal levels of brain cholinergic activity.

Acetylcholine levels Acetylcholine turnover Muscarinic receptor Conditioned avoidance response Strains

IT IS generally though that cholinergic pathways in the central nervous system of the rat play a role in avoidance response behavior (for reviews, see [2,9]). Various laboratories have attempted to demonstrate correlations between neurochemical indicators of basal cholinergic function and avoidance behavior performance among strains of rats and mice using choline acetyltransferase activity, acetylcholinesterase levels or acetylcholine concentrations as indicators of cholinergic activity [4,11]. However, none of these measures are sensitive or reliable measures of cholinergic activity. Choline acetyltransferase, acetylcholinesterase and acetylcholine concentrations do not show parallel variations among brain regions [10]. This is not surprising because: (1) choline acetyltransferase activity is not the rate-limiting step in acetylcholine synthesis [12]; (2) acetylcholinesterase activity is not located exclusively in cholinergic neurons [19] and only large changes in this enzyme affect behavior [7]; (3) acetylcholine content is maintained at steady state during activity. Therefore, it is impossible to assess from measurements of tissue concentrations of acetylcholine the dynamic state of cholinergic neurons [5]. Reliable indications of cholinergic function can be obtained by procedures whereby calculations of the efflux rates of acetylcholine are determined under steady-state conditions. Turnover rate measurements obtained by multiplying the fractional rate constant for acetylcholine efflux by the steady-state concentration of acetylcholine increase or decrease proportionally to the activity of the cholinergic neurons [13].

The present study was undertaken to investigate possible correlations between conditioned avoidance response performance and basal levels of brain cholinergic function using acetylcholine turnover rate determinations from various brain regions of several strains of rats. In addition, [^{3}H]-QNB (quinuclidinyl benzilate) binding was used to study variations in the number and affinity of muscarinic receptors.

METHOD

Fischer-344, Buffalo and Lewis rats were obtained from Microbiological Associates, Walkersville, Md. Long-Evans Hooded rats were obtained from Charles Rivers, Wilmington, MA, and Zivic-Miller rats (Charles River derived Sprague-Dawley strain) were obtained from Zivic-Miller Laboratories, Allison Park, PA. Iowa Reactive and Iowa Nonreactive rats were obtained from G. M. Harrington, University of Northern Iowa, Cedar Falls, Iowa. All rats

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were male and weighed 175–250 g. The rats were maintained in individual living cages on a 9:00 A.M.—9:00 P.M. light-dark cycle with ad lib food and water. All behavioral testing and sacrificing for biochemical analyses were performed between 10:00 A.M. and 2:00 P.M.

For eight consecutive days, rats were given 100-trial shock avoidance sessions in a dual chambered shuttle box [20]. The shuttlebox was equipped with a centrally located tone transmitter and two lights, one in each compartment. The discriminative stimulus was a simultaneous presentation of the tone and lights. This was followed five seconds later by a negative reinforcer (1.6 mA scrambled electric footshock in the occupied chamber). Conditioned avoidance responses (i.e., crossings during discriminative stimulus but before shock onset) were automatically recorded. The time interval between trials was 30 seconds during which time intertrial crossings were recorded. For each rat, the avoidance performance level was taken as the percent of the combined trials of days 7 and 8 in which the rat displayed an avoidance response. Intertrial crossings were also taken as the average from these two days.

In other rats, the level and turnover rate of acetylcholine were determined following infusion of phosphoryl $(C^2H_3)_3$ choline chloride (KOR Isotopes, Cambridge, MA) in physiological saline into the lateral tail vein (15 μmole/kg/min) of unanesthetized rats for 9 minutes. The rats were killed by microwave irradiation focused on the head and the brain areas were dissected. Endogenous and deuterated acetylcholine and choline were analyzed using gas chromatography-mass fragmentography [21]. From the percent incorporation of the deuterated precursor into choline and acetylcholine, the fractional rate constant for acetylcholine efflux was determined [21]. The turnover rate of acetylcholine was obtained by multiplying the fractional rate constant by the steady-state concentration of acetylcholine.

To analyze [^{3}H]-QNB binding, rats were decapitated, the brains were dissected on ice and the tissues were stored at −70°C. Thawed brain segments were homogenized in 50 volumes of 50 mM sodium-potassium phosphate buffer pH 7.4 and centrifuged at 48,000 × g for 15 minutes. The pellet was rehomogenized and centrifuged as above and then suspended in 100 volumes of the buffer. The incubation mixture consisted of 100 μl of the particulate fraction (40–60 μg protein) and appropriate concentrations of L-[benzilic-4,4′-^{3}H] quinuclidinyl benzilate (40.2 Ci/mmol; New England Nuclear, Boston, MA) in a total volume of 5 ml buffer. Duplicate samples were incubated at 37°C for 60 minutes. Specific binding of [^{3}H]-QNB was defined as that displaced by 1 μM scopolamine. The incubation was terminated by filtration under vacuum through Whatman GF/B filters followed by three washes of the filter with cold buffer. The filters were extracted overnight in Aquasol (New England Nuclear, Boston, MA) and the radioactivity determined by liquid scintillation spectrophotometry.

RESULTS

The conditioned avoidance response performance of five strains is shown in Fig. 1. Although animal-to-animal variability was found (four of the strains consisted of rats from at least two performance categories), the strains did exhibit statistically significant differences by a one-way analysis of variance, $F(4,46)=5.71$, $p<0.05$, and were ranked according to their mean performance levels. Hooded rats showed the lowest mean performance level while the Fischer-344 strain showed the highest and by far the most uniform performance level. The avoidance performance levels vs. the frequency of spontaneous crossings during the intertrial intervals did not yield a statistically significant correlation coefficient.

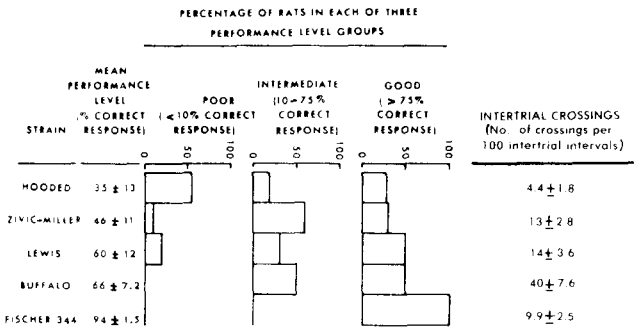

FIG. 1. Conditioned avoidance response performance of five rat strains. Ten rats of each strain were tested and performance levels were determined as described in the Methods. Mean performance levels and intertrial crossings are expressed as the mean±S.E.M.

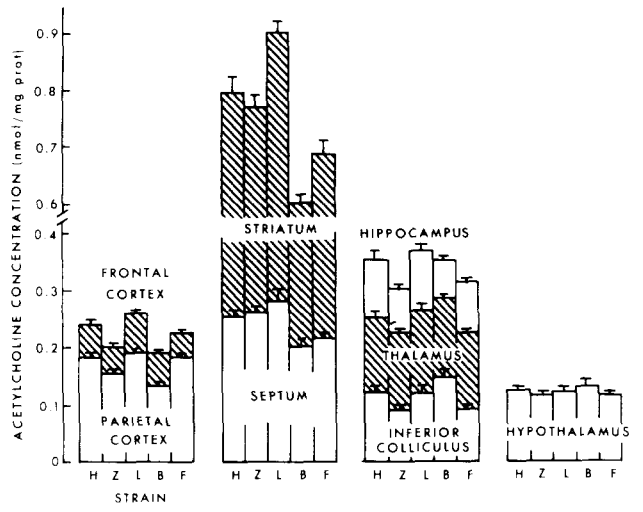

FIG. 2. Regional acetylcholine concentration of five rat strains. Strains are ordered in increasing conditioned avoidance response performance level: H=Long-Evans Hooded, Z=Zivic-Miller, L=Lewis, B=Buffalo, F=Fischer-344. Results are expressed as the mean±S.E.M. of 10–21 animals per group.

The acetylcholine concentration in eight brain regions of naive rats from these five strains is shown in Fig. 2 with the strains being ordered according to their behavioral performance. The acetylcholine content of frontal and parietal cortex shows a similar pattern among the strains. The other regions differ in their interstrain pattern from that of the cortex, but they too can be grouped. Thus, the septum and straitum have a similar interstrain pattern as do the hippocampus, thalamus, and inferior colliculus. In contrast, the hypothalamus shows no strain differences in the acetylcholine content. Despite this variability in the interstrain pattern of acetylcholine content, there is no structure in which the mean acetylcholine concentration vs. the mean conditioned

TABLE 1

SPECIFIC BINDING OF [^{3}H]-QNB TO PARTICULATE FRACTION

Tissue	[QNB] (pM)	Strain Hooded	Zivic-Miller (pmol/g protein)	Lewis	Buffalo	Fischer-344
Frontal Cortex	600	1310 ± 28	1240 ± 26	1280 ± 45	1300 ± 26	1470 ± 95
	20	640 ± 45	590 ± 17	640 ± 19	630 ± 19	730 ± 81
Striatum	600	1520 ± 160	1490 ± 60	1500 ± 88	1550 ± 88	1390 ± 90
	20	730 ± 83	730 ± 55	780 ± 120	840 ± 110	700 ± 25
Hippocampus	600	1220 ± 50	1200 ± 86	1180 ± 20	1290 ± 30	1180 ± 86
	20	630 ± 53	590 ± 5	610 ± 13	700 ± 18	620 ± 30

Values are the mean±S.E.M. of six animals per group.

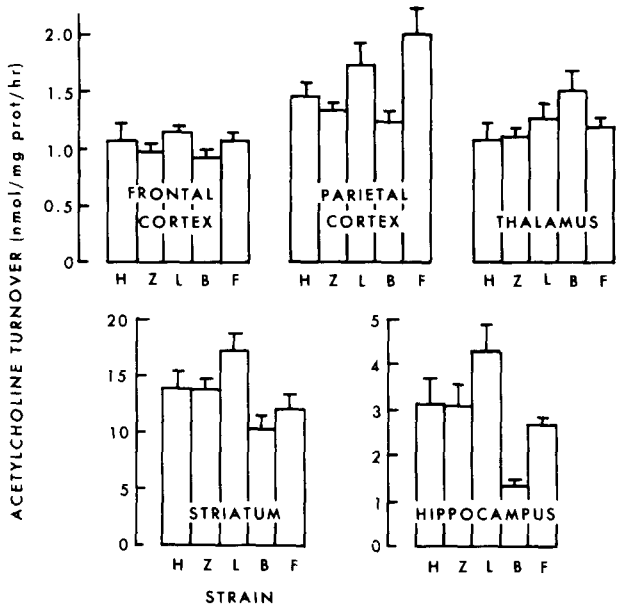

FIG. 3. Regional acetylcholine turnover rate of five rat strains. Abbreviations are described in Fig. 2. Results are expressed as the mean±S.E.M. of 10–18 animals per group.

avoidance response performance yields a statistically significant correlation coefficient. In addition, the Hooded and Fischer-344 strains display the poorest and best performances, respectively, but do not constitute the extremes in acetylcholine concentrations in any brain region studied.

The turnover rate of acetylcholine in five of the brain structures studied is shown in Fig. 3. As with the acetylcholine concentrations, a variety of rank orders of turnover rates is seen, and is reminiscent of the acetylcholine concentrations with two exceptions. In the parietal cortex, Fischer-344 has the highest turnover rate and in the hippocampus the Buffalo strain has an exceptionally low turnover rate. With the above exception, the Hooded and Fischer-344 strains generally show intermediate turnover rates and again, no significant correlation between behavior and turnover rate is found in any region.

The specific binding [^{3}H]-QNB reveals no significant differences in either the frontal cortex, striatum or hippocampus of naive rats from the five strains when subjected to a one-way analysis of variance (Table 1). That no differences are found using either a 600 or 20 pM [^{3}H]-QNB concentration (the determined saturating and K_d concentrations for the tissues used) would indicate that no significant differences exist in either the Bmax or K_D of the [^{3}H]-QNB binding sites.

In a separate experiment, Hooded rats have been tested as previously described in the conditioned avoidance response paradigm and selected rats have been grouped into poor and intermediate-to-good performance categories. One week later, the rats were analyzed for acetylcholine levels and turnover rates. No significant differences are found among the acetylcholine concentrations or turnover rates in the hippocampus of the good performance, poor performance and untested (naive) control groups (Table 2).

The acetylcholine concentration and turnover rate in brain regions of naive Iowa Reactive and Iowa Nonreactive rats are shown in Table 3. Iowa Nonreactive rats are known to perform considerably better in conditioned avoidance response paradigms than Iowa Reactive rats [8]. The only statistically significant differences between the two strains are seen in the frontal and parietal cortices where the Iowa Reactive strain has a higher acetylcholine concentration than the Iowa Nonreactive strain.

DISCUSSION

Numerous pharmacological studies have indicated that central cholinergic mechanisms play a role in conditioned avoidance responding in the rat, but no clear consensus as to the nature of that role has emerged [2,9]. As another approach to the problem, various studies employing indirect biochemical measures of acetycholine metabolism in two or three strains have suggested that basal levels of cholinergic parameters may correlate with avoidance performance ability. For example, the choline acetyltransferase activity in the temporal cortex is higher in two inbred strains of mice showing good avoidance performance than in a third strain showing poor performance whereas other cortical regions generally show no differences [11]. The F_1 offspring of matings between these good and poor performance strains also show this enzyme activity-performance relationship. It should be

TABLE 2

ACETYLCHOLINE CONCENTRATION AND TURNOVER RATE IN THE HIPPOCAMPUS OF HOODED RATS

Group	Conditioned Avoidance Response Performance (% Correct Responses)	Acetylcholine Concentration (nmol/mg protein)	Acetylcholine Turnover (nmol/mg prot/hr)
Untested Controls	—	0.30 ± 0.022	1.4 ± 0.16
Intermediate and Good Performers	47 ± 11	0.32 ± 0.016	1.3 ± 0.068
Poor Performers	1.0 ± 0.61	0.31 ± 0.0036	1.1 ± 0.11

Values are the mean±S.E.M. of 5–7 animals per group.

TABLE 3

ACETYLCHOLINE CONCENTRATION AND TURNOVER RATE IN VARIOUS BRAIN REGIONS OF IOWA REACTIVE AND IOWA NONREACTIVE RATS

Tissue	Acetylcholine Concentration (nmole/mg prot)		Acetylcholine Turnover (nmole/mg prot/hr)	
	Iowa Reactive	Iowa Nonreactive	Iowa Reactive	Iowa Nonreactive
Frontal Cortex	0.28 ± 0.0032	0.22 ± 0.010*	1.2 ± 0.053	1.4 ± 0.31
Parietal Cortex	0.21 ± 0.0042	0.17 ± 0.0072*	1.5 ± 0.12	1.8 ± 0.41
Striatum	0.75 ± 0.016	0.81 ± 0.057	8.6 ± 2.0	9.7 ± 2.5
Septum	0.22 ± 0.021	0.24 ± 0.031	—	—
Hippocampus	0.33 ± 0.0093	0.31 ± 0.0090	2.6 ± 0.5	3.8 ± 1.1
Thalamus	0.24 ± 0.0079	0.24 ± 0.0072	1.5 ± 0.27	1.3 ± 0.17
Inferior Colliculus	0.10 ± 0.0051	0.084 ± 0.011	—	—
Hypothalamus	0.14 ± 0.012	0.14 ± 0.0078	—	—

Values are the mean±S.E.M. of 5–6 animals per group.
*$p<0.005$ by two-tailed Student t-test.

noted, however, that two of the above inbred mouse strains and a third inbred strain have been analyzed for regional acetylcholine turnover rates [6] and this measure does not appear to correlate with their conditioned avoidance response performance [1].

The activity of choline acetyltransferase in the cerebral cortex is the same in Roman High Avoidance and Roman Low Avoidance rat strains (rat strains produced by selective breeding for fast or slow conditioning in a two-way conditioned avoidance paradigm) [16] although individual cortical regions have not been analyzed. No significant differences exist between the acetylcholinesterase activity of Roman strains in the cerebrum [16], whole brain, or subcortical regions [4]. However, Roman High Avoiders have lower acetylcholine levels than Low Avoiders in whole brain and four brain regions [4]. Table 3 shows that the Iowa Reactive strain has lower levels of acetylcholine in the frontal and parietal cortex than the Iowa Nonreactive strain. These results cannot be generalized since the differences in acetylcholine levels we have found in cortex are not accompanied by significant differences in acetylcholine turnover rates. Moreover our results also show a lack of correlation when analyzing brain acetylcholine levels in Long-Evans Hooded, Zivic-Miller, Lewis, Buffalo and Fischer rat strains.

In the Hooded strain, differences in conditioned avoidance response performance is not accompanied by differences in hippocampal acetylcholine concentration or turnover rate. That we also see no correlation between basal levels of regional acetylcholine turnover rates in naive rats and avoidance performance ability in seven different strains may indicate that the role which brain cholinergic systems play in conditioned avoidance response performance is reflected by transient changes in activity occurring during the animals' performance in the paradigm rather than by "basal" activity levels. For example, the Zivic-Miller strain performs more poorly than the Fischer-344 strain in the conditioned avoidance response paradigm, mainly due to stress-induced motor suppression in the Zivic-Miller strain [15]. Significantly, the apparent turnover rate of acetylcholine in the dorsal hippocampus increases during acute footshock in the Zivic-Miller but not in the Fischer-344 strain [18]. This suggests that a hippocampal cholinergic system may mediate a suppressive behavioral response to stress accounting for the poor conditioned avoidance response performance of the Zivic-Miller strain. It should be noted that possible immobilization stress occurring during infusion for the measurement of the turnover rate of acetylcholine in the present study is not accompanied by changes in this measure in the stress-susceptible Zivic-Miller strain [3]. Hence, such stress probably does not interfere with the present interstrain

comparison of basal acetylcholine metabolism. Furthermore, in agreement with results from Roman High and Low Avoidance strains [14], we have found no correlations between regional QNB binding and avoidance performance ability. However, transient increases in QNB binding have been found in forebrain of chicks after passive avoidance learning [17].

Of the parameters discussed, only turnover studies which calculate the efflux rates of acetylcholine under steady-state conditions would give reliable indications of cholinergic function. In the studies reported here there are clear interstrain differences in regional acetylcholine turnover rates of naive rats and in conditioned avoidance response performance. Nevertheless, we must conclude that there is no convincing correlation between performance ability and basal levels of regional acetylcholine concentration, acetylcholine turnover rate and QNB binding using seven different strains of rats.

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