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# Chronic Phenytoin Induced Impairment of Learning and Memory With Associated Changes in Brain Acetylcholine Esterase Activity and Monoamine Levels

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SUDHA, S., M. K. LAKSHMANA AND N. PRADHAN. *Chronic phenytoin induced impairment of learning and memory with associated changes in brain acetylcholine esterase activity and monoamine levels.* PHARMACOL BIOCHEM BEHAV **52**(1) 119–124, 1995.—Groups of adult, male, Wistar rats were administered phenytoin (DPH) at 5, 12.5, 25, 50, or 75 mg/kg IP for 21 days. The learning and memory of these rats were assessed using the T-maze and passive avoidance tests. The plasma DPH levels, acetylcholine esterase (AChE) activity in different brain regions, and the levels of monoamines in the hippocampus were measured. The results indicate that DPH below the therapeutic plasma level did not significantly impair learning and memory. Correspondingly, no changes were noted in the brain 5-HT or AChE activity. However, DPH, at therapeutic plasma concentrations (i.e., 10.5 μg/ml and 14 μg/ml in the dosage range of 50 and 75 mg/kg, respectively), significantly impaired learning and memory in rats. The impaired learning and memory functions were associated with increased 5-HT levels and decreased AChE activity in the hippocampus. With a dose of 75 mg/kg DPH, there was a reduction in the AChE activity in the striatum, in addition to hippocampus. It is conjectured that the neurochemical changes brought about by DPH at therapeutic plasma levels may account for the impairment of learning, memory, and cognitive functions in epilepsy.

Phenytoin  Learning and memory  AChE activity  Monoamines  Plasma DPH levels

REPORTS in the literature suggest that a significant number of people with epilepsy experience memory disturbances (39). Several different biological and psychosocial factors may contribute to the origin of such memory disturbances. These factors may include antiepileptic drugs, underlying brain pathology, seizure type, frequency and severity of seizures, and psychosocial factors such as mood and expectations (40). The antiepileptic drugs have, with great probability, negative effects on memory (42). Phenytoin (Diphenyl hydantoin or DPH) is one of the cheapest and widely used anticonvulsant drug. Many studies have investigated the effects of acute administration of antiepileptic drugs on learning and memory (33,37). However, epilepsy is a chronic disorder, and antiepileptic drugs are administered on long-term basis to patients. For optimization of therapy, it is desirable to have complete seizure control without a therapy-induced memory disturbance in the patients. So, it is important to know to what extent the memory disturbances are caused by chronic DPH medication. However, such investigations are rather difficult to carry out clinically because many patients require treatment by more than one type of anticonvulsant. Experimental research with animals provides opportunities for such investigations that would not be possible in humans for obvious ethical reasons. This article presents further observations on the effect of chronic administration of DPH on learning and memory in normal rats.

DPH has been shown to decrease total brain concentrations of acetylcholine (1,13). The central cholinergic system is known to play an important role in learning and memory and is considered crucial for maze learning in rats (6,8,22). The

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activation of cholinergic system reportedly improves learning and memory (5), while blockade of cholinergic transmission impairs memory retention (18). Acute administration of DPH has been shown to impair learning and memory in mice (33). However, the contributions of the cholinergic system to impairments in learning and memory after chronic administration of different doses of DPH need to be investigated.

DPH reportedly increases 5-HT levels in the brain (9,10). The central serotonergic system is also involved in learning and memory. The intracranial or intrahippocampal administration of 5-HT impairs retention of avoidance tasks in rats (15). Moreover, 5-hydroxytryptophan and p-chloramphetamine also impair avoidance learning in rats (4,26). There are scant reports on the effects of DPH on central serotonergic system and its role in the memory.

In this study we have used the T-maze and passive avoidance test to evaluate the learning of rats. The objective of the present study was to ascertain if there were any changes in the learning of these tasks with chronic administration of DPH at different doses and if there were any associated changes in the cholinergic and monoaminergic systems. The levels of DPH in the plasma, the activity of acetylcholine esterase in different regions of the brain, and the levels of monoamines and their metabolites in the hippocampus were also assessed.

## METHOD

### *Animals*

Adult, male, Wistar rats weighing 200–250 g, obtained from the Central Animal Research Facility of the National Institute of Mental Health and Neurosciences, Bangalore, India, were used in this experiment. The rats were fed with 18 CW diet (a semisynthetic balanced diet of 18% casein, 78% wheat, and all the vitamins and minerals needed for the normal growth of the rat) and water ad lib. They were housed four per cage in Plexiglas cages and reared in a 12 L : 12 D cycle (lights on at 0700 h), temperature-controlled (23 ± 1°C) animal house.

### *Drugs*

DPH was a gift from Cadila Laboratories Ltd., India. DPH, suspended in 30% propylene glycol, was administered to groups of rats ($n$ = 6 in each group) intraperitoneally at volumes not greater than 1.0 ml/rat. DPH was given in doses of 5, 12.5, 25, 50, and 75 mg/kg/day for 21 days. Control rats were injected with equal volume of the vehicle. Kits for the assay of DPH were obtained from M/s Syva International, UK. All other chemicals were from Sigma Chemical Co., St. Louis, MO. Four hours after the drug administration, animals were tested in the T-maze or passive avoidance tasks.

### *Training Procedure in the T-Maze*

The T-maze had a start arm and a left and right arm (50 × 13 × 35 cm), all painted black. At the extremity of each arm, a 5 cm diameter, 0.5 cm deep food cup was located on the floor. The T-maze was located in a dimly illuminated room with a weak light (25 W). The animals were familiarized with the maze, food, and food containers on 2 consecutive days before the start of the experiments. On these days, two trials per rat was carried out. The animals were then food deprived for 24 h. On start of the experimental session, 15 trials per rat were carried out on the first day (i.e., 20 days after the beginning of chronic DPH treatment). The next day trials were carried out for each rat until it attained a criterion of nine correct choices out of 10 consecutive trials. Only one arm of the T-maze was baited with the food (pellets made out of the 18 CW diet). The correct choice was the left arm for half of the rats, while it was the right arm for the rest. The rat was put into the start arm and the door gently raised. When the rat reached one or the other arm, it was removed from the maze and put into a separate waiting box for 10 s and then returned to the maze as before. A correct trial ended with the rat eating the food. An incorrect trial (error) ended with the rat reaching the empty food cup. The time taken for each trial was also noted by a stop watch. On the first day, the number of errors, the percentage of errors, and average time taken for each trial were noted. On the second day, the number of trials to reach the criterion of nine correct responses, the number of errors, the percentage of errors, and the average time taken for each trial were noted.

### *Step-Down Passive Avoidance*

Naive rats were placed on a plastic platform (10 × 10 × 10 cm) placed in the center of a lighted box (30 × 30 × 30 cm) with an electrifiable grid floor. When the rats stepped off the platform they received a constant and continuous electric shock of 0.8 mA applied through the grid floor. The normal reaction of the rat was to jump back on to the platform. Twenty-four hours later each rat was placed back on the platform and tested again for step-down latency. The test ended when the rat stepped down or refrained from stepping down for at least 60 s. The percentage of rats that stepped down before 60 s was noted.

### *Blood Collection and Estimation of DPH Levels*

Within 1 h following the T-maze or passive avoidance tests, the rats were sacrificed by rapid decapitation and the blood was collected. The DPH levels in the plasma were estimated by EMIT assay method using kits from Syva (M/s Syva International, UK).

### *Brain Dissection*

After decapitation, the brains were rapidly removed. Subsequent dissection was performed on ice. Motor cortex, pyriform cortex, olfactory bulb, striatum, and hippocampus were dissected out by a slightly modified method of Glowinski and Iversen (20).

### *Estimation of Acetylcholine Esterase Activity*

Acetyl choline esterase (AChE) activity was measured on the basis of the yellow color produced due to reaction of thiocholine with dithiobis nitro benzoate ion (14).

### *Estimation of Monoamines*

The 5-hydroxy tryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA), dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), and homovanillic acid (HVA) levels in the hippocampus were assessed by HPLC with electrochemical detection as described previously (36).

### *Statistical Analysis*

The data was analyzed using a statistical package (SYSTAT: SYSTAT Inc., Evanston, IL) on an IBM PC-AT. The mean and standard error of mean was calculated for all the parameters. Comparisons between the various groups were made using the one-way analysis of variance. Further com-

TABLE 1

PERFORMANCE OF THE RATS GIVEN DIFFERENT DOSES OF DPH AND THE CONTROLS IN THE T-MAZE ON THE FIRST DAY (MEAN + SEM)

| Parameter | Control | 5 mg | 12.5 mg | 25 mg | 50 mg | 75 mg |
|---|---|---|---|---|---|---|
| First day errors | 5.167 ± 0.477 | 6.167 ± 0.946 | 7.167 ± 1.493 | 7.333 ± 0.715 | 4.667 ± 0.422 | 6.500 ± 0.619 |
| Percentage of errors | 34.450 ± 3.181 | 41.118 ± 6.301 | 47.767 ± 9.959 | 48.883 ± 4.711 | 31.100 ± 2.807 | 43.330 ± 4.119 |
| Time taken for each trial | 43.833 ± 5.620 | 37.633 ± 3.302 | 52.717 ± 9.419 | 48.533 ± 6.908 | 78.900 ± 8.337 | 93.920* ± 6.791 |

$n = 6$.
*Signicantly different from controls ($p < 0.05$).

parisons were performed using Tukey's Honestly Significant Difference test using the 5% level of significance. Pearson's correlation coefficients were also calculated between DPH concentration, and errors in T-maze learning and the number of trials taken to reach criterion. The relationship between the learning results and the 5-HT and DA levels were examined.

## RESULTS

Prior to the start of the T-maze experiment, each rat was separately kept in a waiting box and observed for 5 min. No behavioral abnormalities were noted in experimental rats exposed to DPH up to 25 mg/kg. The 50 and 75 mg/kg dose group appeared to be relatively inactive and drowsy. The body and brain weights of the DPH rats did not differ significantly from that of the controls.

### *DPH Levels*

The average DPH levels in the plasma were 0.5, 1.0, 3.5, 10.5, and 14 μg/ml at 5, 12.5, 25, 50, and 75 mg/kg DPH, respectively. Thus, the 50 and 75 mg/kg groups had therapeutic levels of DPH in the plasma (i.e., between 10 and 20 μg/ml).

### *T-Maze and Passive Avoidance Tests*

The performance of the rats on the first and second days in the T-maze and passive avoidance tests is summarized in Tables 1 and 2. One-way ANOVA demonstrated a significant difference between the groups in the number of trials taken to reach criterion, $F(5, 30) = 7.335$, $p < 0.05$. The administration of DPH significantly impaired learning in the higher doses, i.e., the 50 and 75 mg/kg doses ($p < 0.05$). These rats also made significantly more errors in the T-maze on the second day, $F(5, 30) = 8.930$, $p < 0.05$. One-way ANOVA also demonstrated a significant difference between the groups in the time taken for each trial on the first day, $F(5, 30) = 10.053$, $p < 0.05$, and the second day, $F(5, 30) = 5.482$, $p < 0.01$. The time taken for each trial was also significantly higher for the 75 mg/kg group on both the first and second days. A significant positive correlation of concentration of DPH levels in the plasma was found with errors made on the second day ($r = 0.752$, $p < 0.01$) and number of trials to criterion ($r = 0.715$, $p < 0.05$). The rats treated with 50 and 75 mg/kg DPH also performed poorly in the passive avoidance test when compared to the controls.

### *Acetylcholine Esterase Activity*

The activity of AChE in different brain regions are detailed in Table 3. The activity of AChE in the motor cortex, pyriform cortex, and olfactory bulb did not differ significantly from the controls in any of the doses of DPH administered. In the striatum, one-way ANOVA demonstrated a significant difference between the groups, $F(5, 30) = 2.857$, $p < 0.05$. The 75 mg/kg group has significantly lower AChE activity when compared to the controls ($p < 0.05$). In the hippocampus, one-way ANOVA showed a significant difference between the groups, $F(5, 30) = 4.563$, $p < 0.01$. The 50 and 75 mg/kg groups had significantly lower AChE activity than the controls ($p < 0.05$). A significant negative correlation of the activity of AChE in the hippocampus was found with errors made by the animals on the second day ($r = -0.467$, $p < 0.01$) and the number of trials to criterion ($r = -0.355$, $p < 0.05$).

### *Monoamine Levels*

The levels of monoamines and their metabolites in the hippocampus are given in Table 4.

TABLE 2

PERFORMANCE OF THE RATS GIVEN DIFFERENT DOSES OF DPH AND THE CONTROLS IN THE T-MAZE ON THE SECOND DAY AND THE PASSIVE AVOIDANCE TESTS (MEAN + SEM)

| Parameter | Control | 5 mg | 12.5 mg | 25 mg | 50 mg | 75 mg |
|---|---|---|---|---|---|---|
| Second day errors | 5.333 ± 0.494 | 4.833 ± 0.792 | 5.333 ± 0.919 | 5.333 ± 1.116 | 9.833* ± 0.703 | 10.333* ± 0.917 |
| Percentage of errors | 28.362 ± 1.946 | 27.062 ± 4.284 | 25.153 ± 3.263 | 25.105 ± 3.843 | 36.975 ± 2.378 | 36.938 ± 3.367 |
| Time taken for each trial | 53.333 ± 4.620 | 50.567 ± 5.521 | 68.667 ± 15.051 | 91.983 ± 12.210 | 95.200 ± 10.183 | 112.000* ± 11.163 |
| Trials to criterion | 18.667 ± 0.615 | 18.000 ± 1.211 | 21.000 ± 2.000 | 19.833 ± 2.272 | 27.000 ± 2.066 | 28.000* ± 0.258 |
| Passive avoidance | | | | | | |
| % of rats stepped down | 66.700 | 66.700 | 66.700 | 66.700 | 100.000 | 100.000 |

$n = 6$.
*Signicantly different from controls ($p < 0.05$).

TABLE 3

ACTIVITY OF ACETYLCHLORINE ESTERASE IN DIFFERENT REGIONS OF BRAIN CONTROL RATS AND RATS GIVEN DIFFERENT DOSES OF DPH (MEAN ± SEM, $n$ = 6) EXPRESSED AS MICROMOLES HYDROLYZED/g WET WEIGHT/MINUTE

| Region | Control | 5 mg | 12.5 mg | 25 mg | 50 mg | 75 mg |
|---|---|---|---|---|---|---|
| Motor cortex | 4.531 ± 0.167 | 4.676 ± 0.240 | 4.451 ± 0.185 | 4.958 ± 0.309 | 4.087 ± 0.215 | 4.848 ± 0.217 |
| Pyriform cortex | 38.246 ± 3.891 | 35.976 ± 4.279 | 38.685 ± 2.773 | 39.395 ± 3.863 | 39.950 ± 5.420 | 43.459 ± 2.427 |
| Olfactory bulb | 4.879 ± 0.193 | 4.492 ± 0.271 | 4.474 ± 0.243 | 4.448 ± 0.599 | 4.541 ± 0.484 | 4.557 ± 0.385 |
| Striatum | 34.047 ± 2.272 | 29.960 ± 3.895 | 27.223 ± 1.702 | 31.514 ± 2.378 | 24.734 ± 1.791 | 23.226* ± 1.158 |
| Hippocampus | 5.943 ± 0.250 | 5.852 ± 0.495 | 4.902 ± 0.323 | 4.683 ± 0.702 | 3.638* ± 0.326 | 3.709* ± 0.548 |

*Signicantly different from controls ($p < 0.05$).

*5-HT.* One-way ANOVA showed a significant difference between the groups, $F(5, 30) = 7.244$, $p < 0.01$. The 50 and 75 mg/kg groups had significantly higher 5-HT levels than the controls ($p < 0.05$). A significant positive correlation of the 5-HT levels was found with errors on the second day ($r = 0.661$, $p < 0.01$) and the number of trials to criterion ($r = 0.591$, $p < 0.01$).

*5-HIAA.* There was no significant difference in the 5-HIAA levels between the DPH and control rats.

*DA.* The 12.5, 25, 50, and 75 mg/kg groups had significantly higher DA levels when compared to controls ($p < 0.05$). A significant positive correlation of the DA levels was found with errors on the second day ($r = 0.330$, $p < 0.05$).

*DOPAC.* There was no significant difference between the groups in the DOPAC levels.

*HVA.* One-way ANOVA demonstrated a significant difference between the groups, $F(5, 30) = 10.568$, $p < 0.001$. All the rats treated with DPH had significantly higher HVA levels when compared with the controls ($p < 0.05$).

## DISCUSSION

Our results indicate that DPH impaired learning and memory in rats at the therapeutic levels. These results are consistent with earlier reports where acute administration of DPH has been shown to impair memory in mice (33). The plasma level of DPH that we observed in our rats were 10.5 μg/ml for the 50 mg/kg group and 14 μg/ml for the 75 mg/kg group. These fall within the therapeutic range of 10–20 μg/ml. The other doses of DPH administered had plasma DPH values below the therapeutic range and did not significantly impair memory. In our study, the rats with therapeutic range of DPH in plasma performed poorly when compared to the controls in the T-maze and passive avoidance tests. Although impairment of mental function at toxic levels of DPH is perhaps understandable, slightly more disconcerting is the possibility that impairment of learning and memory may occur with more modest or therapeutic levels of DPH. Similar findings are also reported in humans with mean blood values within the optimum range (41). In humans, DPH treatment for 1 month significantly impaired memory performance (31,32). In new referrals with epilepsy, patients receiving DPH performed consistently poorer on memory tasks than those untreated (3). Intellectual dulling and impaired memory has been observed in patients receiving DPH, and such effects are often not detectable until serum levels are well into the therapeutic range and are much more likely at high serum levels (12). However, it is not clear whether performance of rats in T-maze and passive avoidance tests can be correlated with cognitive performance in humans.

The animals receiving the higher doses of DPH, i.e., 50 and 75 mg/kg performed poorly in the T-maze and passive avoidance tasks. These rats appeared to be relatively inactive and drowsy and, in fact, required longer periods of time to complete each trial in the T-maze task. The drowsiness observed in these animals could have contributed to the learning deficits.

The above evidence suggests that DPH can impair learning and memory in normal rats. But the mechanism of action is still not clear. The central ACh system plays an important role in learning and memory (6). We have measured the AChE activity in different brain regions as a marker enzyme for cholinergic function. Comparative studies with AChE and choline acetyl transferase (ChAT) as a marker enzyme for cholinergic perikarya and their processes (28) show that ChAT immunoreactivity and AChE staining pattern in the hippocampus are virtually identical (19,30). Quantitative analyses of the two cholinergic marker enzymes, ChAT and AChE within the hippocampus also indicate that the relative amounts of both enzymes are very similar. Hence, AChE seems to be a reasonably good indicator of cholinergic functions.

TABLE 4

LEVELS OF 5-HT, 5-HIAA, DA, DOPAC, AND HVA (MEAN ± SEM, $n$ = 6) IN THE HIPPOCAMPUS OF CONTROL RATS AND RATS GIVEN DIFFERENT DOSES OF DPH IN ng/mg

| Parameter | Control | 5 mg | 12.5 mg | 25 mg | 50 mg | 75 mg |
|---|---|---|---|---|---|---|
| 5-HT | 0.222 ± 0.014 | 0.186 ± 0.020 | 0.216 ± 0.021 | 0.206 ± 0.029 | 0.304* ± 0.023 | 0.324* ± 0.017 |
| 5-HIAA | 0.150 ± 0.021 | 0.103 ± 0.017 | 0.181 ± 0.025 | 0.177 ± 0.023 | 0.223 ± 0.044 | 0.259 ± 0.031 |
| DA | 0.016 ± 0.010 | 0.220 ± 0.014 | 0.234* ± 0.025 | 0.236* ± 0.027 | 0.258* ± 0.037 | 0.245* ± 0.039 |
| DOPAC | 0.401 ± 0.034 | 0.360 ± 0.034 | 0.465 ± 0.022 | 0.431 ± 0.035 | 0.456 ± 0.033 | 0.391 ± 0.034 |
| HVA | 0.124 ± 0.016 | 0.249* ± 0.033 | 0.330* ± 0.028 | 0.339* ± 0.028 | 0.361* ± 0.026 | 0.363* ± 0.038 |

*Signicantly different from controls ($p < 0.05$).

Our results also indicate that there is a reduction in AChE activity in the hippocampus and striatum of the rats treated with therapeutic doses of DPH. These rats also showed impaired performance in the T-maze and passive avoidance tests. DPH administration has been shown to decrease the levels of acetylcholine in the brain (1,13). DPH has been shown to decrease synthesis of acetylcholine by changing the activity of choline acetyl transferase (43). DPH depressed synaptic transmission and affected the release of ACh at neuromuscular junctions (45). In epileptic guinea pigs, DPH suppressed the convulsive symptoms and reduced the abnormally high cortical ACh outflow (7). In spite of the reduction in acetylcholine esterase activity seen in our study, the levels of acetylcholine in the brain might have been decreased because DPH has been shown to decrease the activity of choline acetyl transferase also (43). Thus, DPH may decrease the cholinergic transmission. Experimental blockade of cholinergic transmission has been shown to impair learning and memory (2,23). Reduced activity of AChE might indicate reduced cholinergic transmission and might be responsible for the observed impairments in learning and memory. An alternative hypothesis might be that the reduced activity of AChE actually increased acetylcholine levels. This increased concentration of ACh might have induced the deficits in learning and memory, because it is well known that excessive cholinergic stimulation can induce a delirium characterized by poor learning and memory. However, no definite conclusions can be drawn because acetylcholine levels were not measured in this study.

Similarly, the central serotonergic system is known to be involved in learning and memory. Activation of central serotonergic systems impairs learning and memory (15,26). Electrical stimulation of the dorsal raphe nucleus disrupted memory by a process involving 5-HT (16). Our finding indicate that 5-HT levels are increased in the hippocampus in the rats treated with therapeutic doses of DPH, i.e., 50 and 75 mg/kg groups. These rats also showed impaired learning and memory. The increase in 5-HT levels caused by DPH might have played an important role in the impairment of learning and memory.

The 5-HT related memory impairment caused by DPH may be mediated by a dysfunction of ACh transmission. It has long been postulated that hippocampus has an important role in memory, and the involvement of cholinergic mechanisms has been suggested (25). It has been suggested that hippocampal theta activity, which involves the septo-hippocampal cholinergic neurons, is related to the consolidation of memory (17). p-Chloramphetamine-induced amnesia can be attenuated by AChE inhibitors (29). It has been reported that serotonergic neurons from the median raphe nucleus appear to tonically inhibit cholinergic neurons in the cortex and hippocampus, whereas those from the dorsal raphe nucleus appear to tonically inhibit cholinergic neurons in the cortex, hippocampus, and striatum (24). Therefore, the DPH-induced impairment in learning and memory may be partially mediated by a decrease in ACh release. Probably DPH increased the levels of 5-HT thereby inhibiting ACh release.

In our study, DA levels were increased in the hippocampus of the 12.5, 25, 50 and 75 mg/kg groups. HVA levels were also increased in all the experimental groups. But only the 50 and 75 mg/kg groups showed impairment in learning and memory. Dopaminergic mechanisms have been shown to improve learning and memory. For example, posttraining intrahippocampal injection of the dopaminergic agonists apomorphine and ergometrine improved retention in a brightness discrimination task (21). But in our study, the 50 and 75 mg/kg groups performed poorly in the T-maze and passive avoidance tests. This could be due to interaction between the dopaminergic and cholinergic systems, particularly in the striatum. The striatum also has been shown to be involved in passive avoidance behavior and spatial learning (35,44). It has been suggested that striatal acetylcholine release is under tonic dopaminergic control through $D_2$ receptors (38). Stimulation of dopamine receptors has been shown to reduce the release of striatal acetylcholine (27). Perturbances of both catecholaminergic and cholinergic systems has been shown to result in behavioral deficits in animals that are similar to the cognitive impairments seen in patients with Alzheimer's disease (11). Thus, the impairments of learning and memory seen in our study could also be due to interactions of dopaminergic system with other neurotransmitter systems.

The increase in DA after DPH treatment is uniformly seen in all the doses except the 5 mg/kg group. The consequential effect of this increase DA on learning and memory may be marginal. However, changes in 5-HT are bimodal and appear to be better correlated with learning and memory. At lower (subtherapeutic) doses DPH does not cause any change in 5-HT turnover. At the dose affecting learning and memory 5-HT is increased, thereby validating the correlation between 5-HT and learning and memory. Possibly DPH might act primarily on the 5-HT neuronal system. Overall, changes in AChE activity and monoamines are modest considering the prominent role ascribed to the cholinergic and monoaminergic system in learning and memory (6,34). Pharmacological studies suggest that interactions of the cholinergic system with monoaminergic or other systems may be critical in memory (11,22). In this view, small changes in monoaminergic or cholinergic function could have synergistic effects with other neural systems in modulating memory function.

The results presented here highlight the impairment in learning and memory after chronic administration of therapeutic doses of DPH (i.e., 50 and 75 mg/kg). There is a reduction in the acetylcholine esterase activity in the striatum and hippocampus and an increase in the 5-HT levels in the hippocampus. Possibly these changes might play a role in the impairment of learning and memory.

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