Physiology & Behavior, Vol. 63, No. 2, pp. 165–169, 1998

Printed in the U.S.A.
0031-9384/98 $19.00 + .00

ELSEVIER

PII S0031-9384(97)00395-8

Effect of Tryptophan Restriction on Short-Term Memory

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Received 31 May 1996; Accepted 20 December 1996

GONZALEZ-BURGOS, I., M. I. PEREZ-VEGA, A. R. DEL ANGEL-MEZA, AND A. FERIA-VELASCO. *Effect of tryptophan restriction on short-term memory.* PHYSIOL BEHAV **63**(2) 165–169, 1998.—Several brain regions are involved in the learning process that is integrated from sensorial inputs. It is thereafter consolidated in short- (STM) or long-term memory. Serotonin is strongly related to both types of memory, and particularly, to STM, however, its regulatory role is still unclear. In this study, the effects of tryptophan (TRY) restriction on learning and STM were evaluated. Ten Sprague–Dawley female rats were fed with a TRY-restricted diet (0.15g/100g) starting from postnatal Day 21. At 21, 40, and 60 days of age, 5 trials per animal were carried out in a ''hard-floor''-Biel maze, after 24 h of water abstinence. The number of errors per trial were registered before reaching the goal. At both 40 and 60 days, experimental rats committed less errors than controls. Likewise, the TRY-restricted group learned the task from the second trial on, whereas controls did not solve it until the third trial. TRY restriction, and therefore brain serotonin reduction, could impair normal cholinergic activity in some areas such as the hippocampus and the cerebral cortex, where involvement in learning and memory is well documented. Morphological and neurochemical plastic events could also be related to the more efficient performance of the task by the TRY-restricted rats. © 1998 Elsevier Science Inc.

Serotonin Memory Learning Tryptophan Cognition Behavior

LEARNING is a neuropsychological process by which an organism aquires new information from the environment and retain it by triggering the memorization process (19). Short-term memory (STM) or working memory allows an organism to recall usefull information, to accomplish a given task by free association between stimuli and responses; it is restricted and weaken if attention is distracted, and it lasts 2 minutes after aquisition (37).

Sensory stimuli and its concomitant information are stored and causes changes in the neural systems involved in both perception and analysis (36,37).

Previous studies have established involvement of several brain regions such as the hippocampus (9,17,30) and frontal cerebral cortex (4,10,12,16) in both learning and memory processes. However, hippocampal activity has been related to spatial learning (1,35) and long-term memory (LTM) (23).

Serial ordering of sensory stimuli underlies in the frontal cortex (CTX), and this is crucial for temporary organization of motor actions (11,12,29) directed to accomplish some STM requested tasks (27) such as problem solving. Prefrontal efferences to premotor and motor cortices mediate recalling of previous events, its serial organization, and further establishment of motor responses patterns (16).

Cholinergic afferences from the septal area innervates the CTX and hippocampus. Pharmacological or surgical blockade of cholinergic activity leads to deficiencies in accomplishment of learning and memory tasks (5,18,25,36,37). Likewise, acetycholine levels are diminished in patients with some neurodegenerative disorders associated with the deterioration of memory such as Alzheimer's disease (6,16,29,31,32). Hence, a close relationship between learning, memory, and cholinergic activity has been postulated.

Presynaptic inhibition of cholinergic release by serotoninergic innervation of both the CTX and the hippocampus has been postulated (31,40) to control the adequate accomplishment of learning and memory as well as spatial orientation paradigms (22,25,31,40). Pharmacological blockade of serotoninergic activity has been shown by some authors to enhance learning and memory skills (21,31), whereas others have reported that serotonin (5-HT) agonists deteriorate such skills (22). Still others (22,31) have reported memory to improve when serotoninergic neurotransmision is incremented.

It is well known that brain 5-HT levels depend directly on the amount of tryptophan (TRY) supplied in the diet (8). Because deterioration of STM is long-lasting in nature and acute pharmacological studies are controversial, in the present study, a TRY-defficient diet was subchronically administered to rats in order to investigate the role of 5-HT on execution of a STM task.

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TABLE 1
COMPOSITION OF DIETS (g/100g DIET)

Components	Control	Experimental
Chow	98.0	—
Gelatine	—	27.0
Tryptophan	—	0.15
Vegetal oil	2.0	5.0
Vegetal fat	—	8.0
Glucose	—	19.0
Sacarose	—	20.0
Dextrin	—	7.0
Mineral mixture*	—	2.0
Vitamin mixture*	—	2.0
Non-nutritive fiber*	—	10.0
Amount of protein (%)	23.0	23.0
Kcal/100g	400.0	393.0

* Tecklad (cat. No. 170760, 40060, and 160390, respectively).

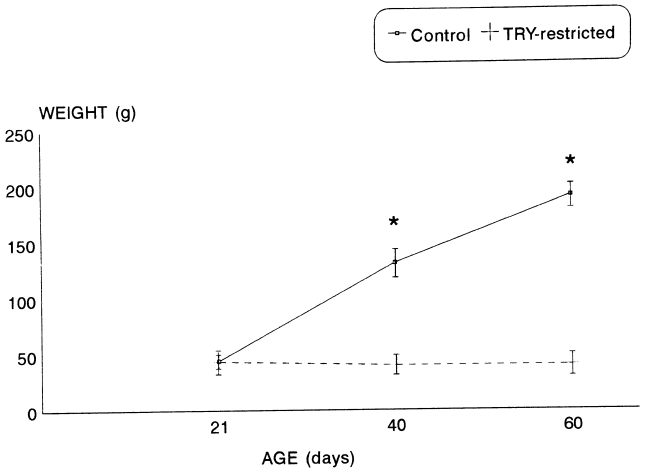

FIG. 2. Body weight from control and from tryptophan-restricted rats. Mean +/− SD. Asterisks represent significant difference at $p < 0.0001$.

MATERIAL AND METHODS

Normal, 21-day-old female Sprague–Dawley rats were used. A control group ($n = 10$) was fed with conventional food for rodents, and an experimental group ($n = 10$) was fed a grenetin-based TRY-defficient diet [0.15 g/100 g (Table 1)]. In both cases, food and water were at free access. Both groups were maintained under regular 12 h × 12 h dark-light cycle (light: 0700 h; darkness: 1900 h).

Body weight was registered at 21, 40, and 60 days of age, and at these same ages, all rats were exposed to the ''hard-floor''-Biel maze [(39); see Fig. 1], the walls of which were white-colored and 20 cm in height. After 24 h of water abstinence at 0100 +/− 2 h and under red light illumination (6), each rat was placed at the entrance of the maze and the number of errors was registered before reaching the goal, which consisted of a water-filled bottle. Once the animal had drunk a few drops of water, the bottle was immediately withdrawn, and the rat was returned to a ''waiting cage.'' An ''error'' was considered to be committed when the animals' head and forelimbs entered any of the impasses of the maze (Fig. 1). Five trials per animal were carried out at 30 s intervals.

Data was statistically analyzed by the Student *t*-test both for body and brain weight, by the Mann-Whitney *u*-test for intergroup comparison, and by the Wilcoxon matched-pairs signed-ranks test for intragroup comparison. The number of errors committed during the first trial, versus each of the following until the fifth trial, were registered and compared.

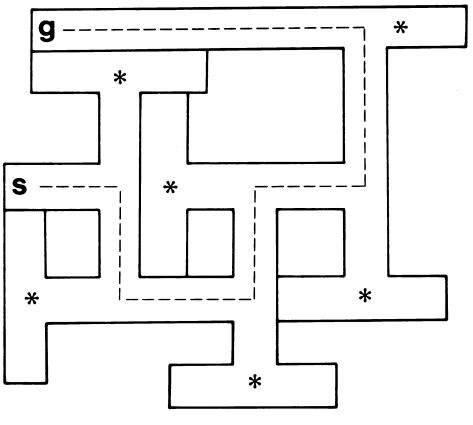

FIG. 1. Diagram of the Biel-maze; s = start; the animals were challenged by trial to solve the maze starting from this point. g = goal, where the water-filled bottle was placed. Dotted line = correct way leading to the goal. Asterisks = impasses, where, if the animals' head and forelimbs entered, and ''error'' was registered.

RESULTS

Body Weight

All 21-day-old rats from both groups weighed 44.1 ± 5.6 g. The body weight of the experimental animals was signifficantly lower than that of the control group when the rats were 40 and 60 days old (Fig. 2).

Brain Weight

At 21-days-old, there were no significant differences between groups. However, at both 40 and 60 days of age, TRY-restricted rats showed lesser brain weight gain than control animals (Fig. 3).

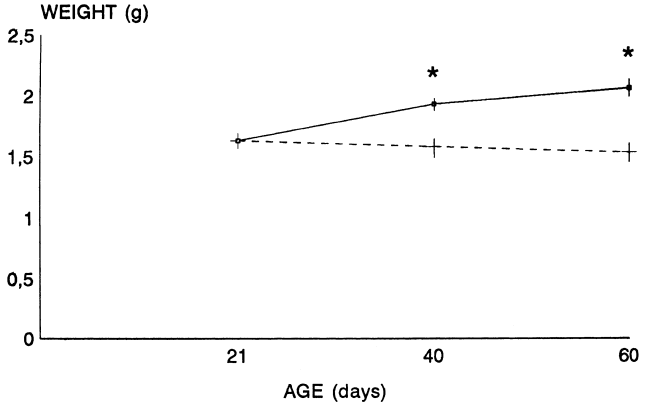

FIG. 3. Brain weight gain curves from control and experimental animals. Mean +/− S.D. Asterisk represents significant differences at $p < 0.0001$.

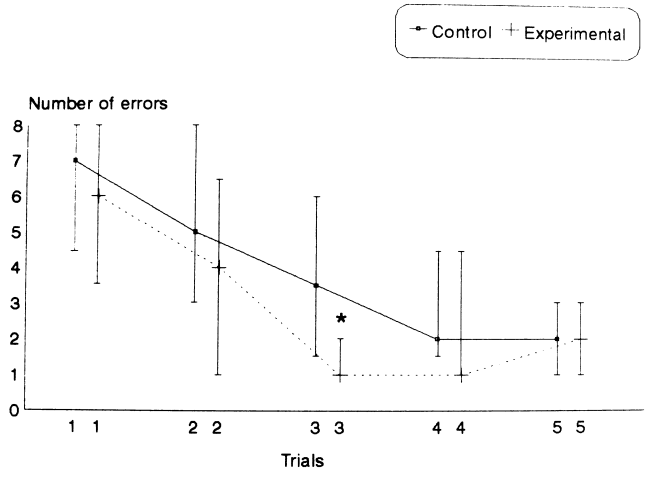

FIG. 4. Intergroup comparison related to the number of errors committed per trial at 21 days of age by the animals from both groups. Median +/− quartils. Asterisk represents the statistical difference at $p < 0.05$.

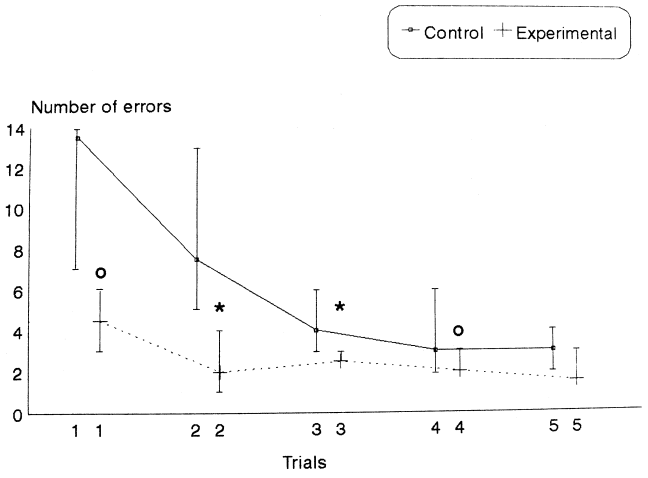

FIG. 6. Intergroup comparison at 40 days of age. Starting from the first trial, there were statistical differences until the fourth one between both groups. Median +/− quartils. *, $p < 0.01$; ○, $p < 0.025$.

Behavioral Tasks; 21-Days-Old

Intergroup analysis. There were no differences in any of the trials between both groups, except for the third trial in which the TRY-restricted animals committed less errors than those in the control group (Fig. 4.).

Intragroup analysis. The number of errors committed by the rats from both groups was signifficantly less from the third trial (Fig. 5).

Behavioral Tasks; 40-Days-Old

Intergroup analysis. Beginning from the first trial, the animals from the experimental group committed less errors than the control group in all trials except the fifth, in which there were no significant differences in the data from either group (Fig. 6).

Intragroup analysis. The TRY-restricted rats committed less errors from the second trial onward, whereas the control group did it from the third trial onward (Fig. 7).

Behavioral Tasks; 60-Days-Old

Intergroup analysis. Begining from the first trial until the fifth, the experimental animals committed less errors than the control group, with the exception of the third trial (Fig. 8).

Intragroup analysis. Both groups of animals diminished their number of errors from the third trial onward (Fig. 9).

DISCUSSION

In female rats, a higher activity of 5-HT synthesizing enzymes, a greater storage capacity for 5-HT in brain serotoninergic neurons, and enhanced 5-HT-dependent behaviors are typical (9).

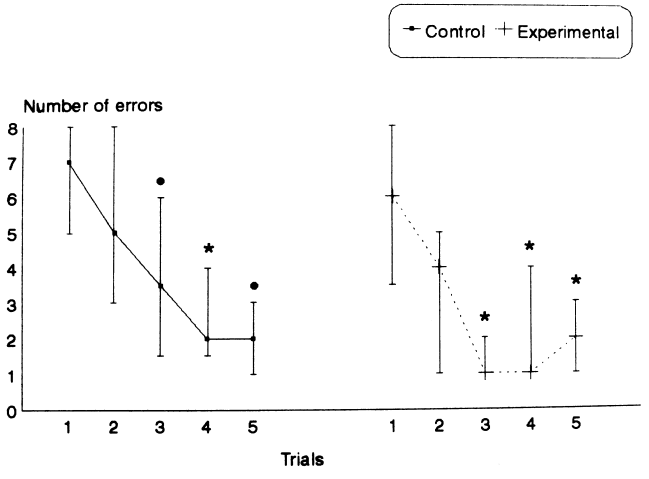

FIG. 5. Intragroup comparison between control and experimental 21-day-old rats. Both groups of rats committed less number of errors starting from the third trial compared to the first trial. Median +/− quartils. *, $p < 0.001$; ●, $p < 0.01$.

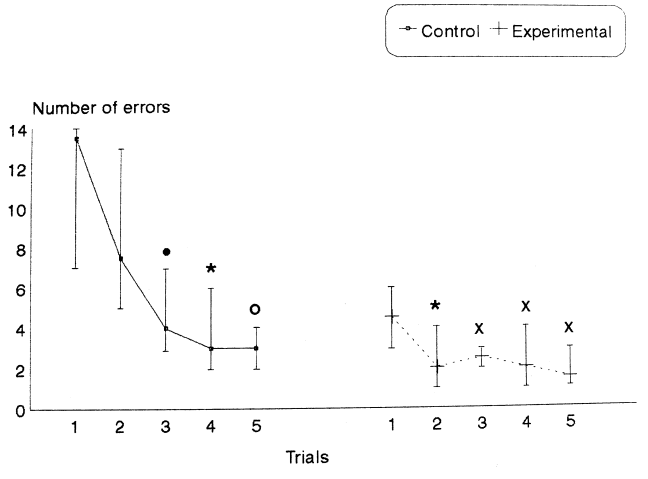

FIG. 7. Intragroup comparison at 40 days of age. Control rats commited less errors than the experimentals from the third trial, where tryptophan-defficient animals commited less errors starting from the second trial. Median +/− quartils. ○, $p < 0.0001$; ×, $p < 0.001$; *, $p < 0.01$; ●, $p < 0.05$.

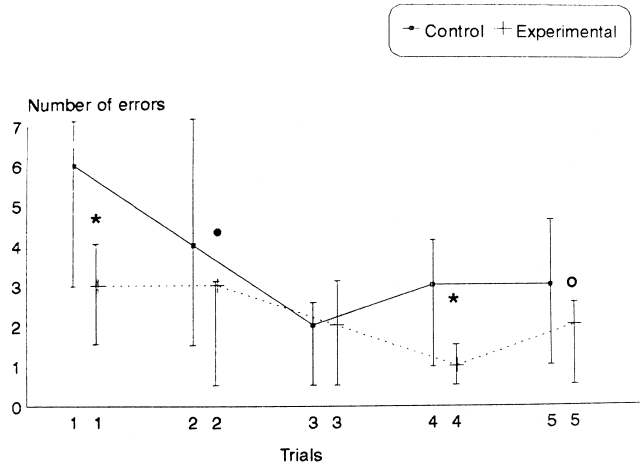

FIG. 8. At 60 days of age, intergroup comparison showed that experimental animals committed less errors than controls at all trials with the exception of the thirth trial. Median +/− quartils. *, $p < 0.01$; ○, $p < 0.025$; ●, $p < 0.05$.

Therefore, because this was a behavioral study, only female rats were used. Among other metabolic roles, TRY stimulates hepatic protein synthesis (27), which reaches its maximal peak before weaning, and represents only 1.5% of the total amino acid content of total protein (28). Thus, weight difference between control and experimental groups at 40 and 60 days of age were likely due to a generalized growth retardation, possibly due to a suppression of growth hormone (GH) release mediated by the diminished levels of TRY and the 5-HT (8), taking into account the regulatory role of 5-HT in the release of GH (2,9). In this sense, it has been demonstrated that TRY stimulates the secretion of GH, which is episodically secreted from the rat at 22-days of age (33).

It is well documented that early malnutrition leads to deficits in several cognitive processes such as learning and memory (3,15). However, in the present study, TRY-restricted animals showed a higher capability to solve the Biel-maze than normal rats. TRY restriction began at weaning, at which stage, cell differentiation of the nervous system is completed and the synaptogenesis of serotoninergic pathways has been established. Therefore, the lower brain weight observed in the TRY-restricted animals could be due to cell hypotrophy rather than to a reduction in cell density. Further, protein and caloric content used in both diets were equivalent, and the protein source of the experimental was of nutritionally high quality (18). Likewise, there were no differences in the quantity of food ingested by both groups (data not shown). It is well known that TRY is a precursor of 5-HT and that brain 5-HT synthesis depends on blood TRY levels (8). It has been reported that a 75% TRY-restriction diet produces a decrease in both 5-HT and 5-hydroxyindol acetic acid concentration in several brain regions without affecting other neurotransmitter systems (37). In the present study, we restricted the TRY content in the diet to 50% of the normal requirement. Therefore, a direct relationship between low brain 5-HT concentration induced by TRY restriction and later behavioral observations is postulated.

At 21 days of age, both groups behaved similarly, as was expected. It could be rendered as a similar learning pattern between groups and, therefore, that the behavioral paradigm was methodologically reliable.

At 40 days of age, both inter- and intragroup analysis suggest that TRY-restricted animals learned more quickly than controls, and the task accomplishment by experimental rats was more efficient. Analysis of data in the intragroup suggests that learning ability by control rats was lower than that of experimentals, because the formers always committed more errors, with the exception of the fifth trial. In this trial, they reached a learning level corresponding to that reached by the TRY-restricted rats at the second trial. During the second trial, the number of errors committed by the experimental group was less than seen in controls, and these were also invariable.

At 60 days of age, the curve tendencies observed from both groups were very similar, and the number of errors were significantly different from the third trial onward. However, errors committed by the experimental group diminished up through the fourth trial, whereas it was observed in controls from the third trial onward.

The first trial in the Biel maze was solved hazardly by the animals because they had not been in contact with it previously. In contrast, to accomplish the subsequent trials, the animals had to learn correctly the passage through the maze in a short-term fashion, by virtue of the CTX functional activity (29). Likewise, in order to choose the correct alternative for turning in the maze in subsequent trials, the animals had to resort to their so-called spontaneous alternation ability (SA), which consists of the ability to inhibit useless behaviors after not finding significant stimuli in a firstly visited arm of a T-maze (34). The paradigm used in the present study challenged the animal to turn either to the right or to the left side of the maze, each time the rat had to choose an alternative to turning.

Inhibitory regulation of acetylcholine (Ach) release leads to the suppression of such useles behaviors (26), and serotoninergic presynaptic terminals have been described on both hippocampal and corticofrontal cholinergic fibers (22) modulating the Ach release (31,40). We have suggested that the increase in the percentage of SA in TRY-restricted rats (13), as well as better performance observed in the Morris water maze at 40 days of age (manuscript in preparation), could be mediated by a subregulatory activity of 5-HT by virtue of its decreased synthesis caused by the low availability of TRY, its precursor (8). Thus, the major efficiency in the task accomplishment by 40 and 60-day-old rats could be related to an abnormal regulation of cholinergic release by

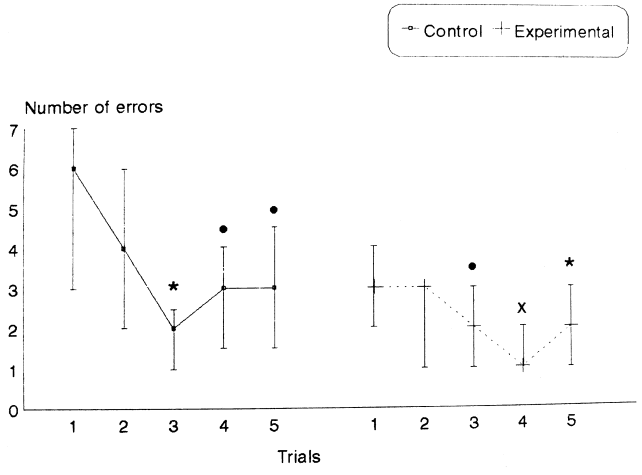

FIG. 9. Intragroup comparison at 60 days of age. Animals from both groups committed less errors from the third trial onward. Median +/− quartils. ×, $p < 0.001$; *, $p < 0.01$; ●, $p < 0.05$.

serotoninergic terminals in CTX and perhaps in the hippocampus as well. Psychopharmacological experiments are needed to challenge this hypothesis.

Another converging mechanism to explain the better performance in the task accomplishment by experimental rats could be mediated by cytoarchitectural changes in cortical neurons. The CTX has been related to the organization of STM (11,16). In previous studies, we have reported an increment in dendritic spine density as well as modifications in the dendritic arborization pattern of CTX third-layer pyramidal neurons using this same model of TRY-restriction (14), leading us to conclude that dendritic spines represent the anatomical substrate underlying the learning and memory processes. This is in agreement with what has been postulated by other authors (24,37), and this could be related to possible modifications in the neuronal firing pattern (5); therefore, efferent information from prefrontal cortex to premotor and finally to motor cortex, underlying planned and thereafter voluntary motor actions, could be abnormally transmitted. In this sense, further psychopharmacological studies could help to demonstrate such hypothesis.

REFERENCES

1. Amsel, A. Hippocampal function in the rat: Cognitive mapping or vicarious trial and error? Hippocampus 3:251–256; 1993.
2. Anderson, I; Cowen, P. Neuroendocrine responses to L-tryptophan as an index of brain serotonin function: effect of weight loss. Adv. Exp. Med. Biol. 294:245–254; 1991.
3. Bedy, K. S. Spatial learning ability of rats undernourished during early postnatal life. Physiol. Behav. 51:1001–1007; 1992.
4. Broersen, L. M.; Heinsbroek, R. P.; de-Bruin, J. P.; Ulyings, H. B.; Oliver, B. The role of medial prefrontal cortex of rats in short-term memory functioning: Further support for involvement of cholinergic, rather than dopaminergic mechanisms. Brain Res. 674:221–229; 1995.
5. Calverley, R. K. S.; Jones, D. G. Contributions of dendritic spines and perforated synapses to synaptic plasticity. Brain Res. Rev. 15:215–249; 1990.
6. Cardinali, D. P.; Lavin, F.; Wurtman, R. J. Action spectra for effects of light on hydroxyindol-O-methyltransferases in rat pineal, retina and harderian gland. Endocrinology 91:877–886; 1972.
7. Decker, M. W.; Pelymounter, M. A.; Gallagher, M. Effects of training on a spatial memory task on high affinity choline uptake in hippocampus and cortex in young adult and aged rats. J. Neurosci. 8:90–91; 1988.
8. Del Angel, A. R.; Beas-Zárate, C.; Morales Villagrán, A. Effects of corn-feed and protein restriction on rat cerebellum and brain stem maturation. Nutr. Rep. Inter. 40:1199–1206; 1989.
9. Delgado, P. L.; Charney, D. S.; Price, L. H.; Landis, H.; Heninger, G. R. Neuroendocrine and behavioral effects of dietary tryptophan restriction in healthy subjects. Life Sci. 45:2323–2332; 1989.
10. Dunnett, S. B.; Wareham, A. T.; Torres, M. Cholinergic blockade in prefrontal cortex and hippocampus disrupts short-term memory in rats. Neuroreport 1:61–64; 1990.
11. Fuster, M. J. The prefrontal cortex and its relation to behavior. Prog. Brain Res. 87:201–211; 1991.
12. Fuster, M. J. Frontal lobes. Curr. Op. Neurobiol. 3:160–165; 1993.
13. González-Burgos, I.; Olvera-Cortés, E.; Del Angel-Meza, A. R.; Feria-Velasco, A. Serotonin involvement in the spontaneous alternation ability: A behavioral study in tryptophan restricted rats. Neurosci. Lett. 190:143–145; 1995.
14. Gonzáles-Burgos, T.; Del Angel-Meza, A. R.; Barajas-López, G.; Feria-Velasco, A. Tryptophan restriction causes long-term plastic changes in corticofrontal pyramidal neurons. Int. J. Devl. Neurosci. 14:673–679; 1996.
15. Goodlett, C. H. R.; Valentino, M. L.; Morgane, P. J.; Resnick, O. Spatial cue utilization in chronically malnourished rats: Task-specific learning deficits. Dev. Psychobiol. 19:1–15; 1986.
16. Granon, S.; Vidal, C.; Thinus-Blanc, C.; Changeux, J. P.; Poucet, B. Working memory, response selection, and effortful processing in rats with medial prefrontal lesions. Behav. Neurosci. 108:883–891; 1994.
17. Harrell, L. M.; Barlow, T. S.; Parson, D. Cholinergic neurons, learning, and recovery of function. Behav. Neurosci. 101:644–652; 1987.
18. Hernández, M. Valor nutritivo de los alimentos. Instituto Nacional de Nutrición; 1971:18.
19. Kametani, H.; Spangler, E. L.; Bresnahan, E. L.; Kobayashi, S.; Long, I. M.; Ingram, D. K. Impaired acquisition in a 14-unit t-maze following medial septal lesions in rats is correlated with lesions size and hippocampal acetycholinesterase staining. Physiol. Behav. 53:221–228; 1993.
20. Kandel, E. R.; Hawkins, R. D. The biological basis of learning and individuality. Sci. Am. September:53–60; 1992.
21. Lamberty, Y.; Gower, A. J. Cholinergic modulation of spatial learning in mice in a Morris-type water maze. Arch. Pharm. Ther. 309:5–19; 1991.
22. McEntee, W. J.; Crook, T. H. Serotonin, memory, and the aging brain. Psychopharmacology 103:143–149; 1991.
23. McNaughton, B. L.; Barnes, C. A.; Rao, G.; Baldwin, J.; Rasmussen, M. Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information. J. Neurosci. 6:563–571; 1986.
24. Mervis, R. F.; Pope, D.; Lewis, R.; Dvorak, R. M.; Williams, L. R. Exogenous nerve growth factor reverses age-related structural changes in neocortical neurons in the aging rat. A quantitative Golgi study. Ann. NY Acad. Sci. 640:95–101; 1991.
25. Normile, H. J.; Altman, H. J. Effects of the combined acetylcholinesterase inhibition and serotonergic receptor blockade on age-associated memory impairments in rats. Neurobiol. Aging. 13:735–740; 1992.
26. Olpe, H. R.; Klebs, K.; Kung, E.; Campiche, P.; Glatt, A.; Ortmann, R.; D'Amato, F.; Pozza, M. F.; Mondadori, C. Cholinomimetics induce O rhythm and reduce hippocampal pyramidal cell excitability. Eur. J. Pharmacol. 142:275–283; 1987.
27. Pazos, A.; Cortés, R.; Palacios, M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res. 346:231–249; 1985.
28. Peters, J. C. Tryptophan nutrition and metabolism: An overview. Adv. Exp. Med. Biol. 294:345–358; 1991.
29. Petrides, M. Functional specialization within the dorsolateral frontal cortex for serial order memory. Prog. R. Soc. Lond. Biol. 246:299–306; 1991.
30. Poucet, B.; Herrmann, T. Septum and medial frontal cortex contribution to spatial problem-solving. Behav. Brain Res. 37:269–280; 1990.
31. Richter-Levin, G.; Segal, M. The effects of serotonin deppletion and raphe grafts on hippocampal electrophysiology and behavior. J. Neurosci. 11:1585–1596; 1991.
32. Ridley, R. M.; Samson, N. A.; Baker, H. S.; Johnson, J. A. Visuospatial learning impairment following lesions of the cholinergic projection to the hippocampus. Brain Res. 456:71–87; 1988.
33. Rivest, R. W. Sexual maturation in female rats. Experientia 47:1027–1038; 1991.
34. Rodríguez, M.; Gómez, C.; Alonso, J.; Afonso, D. Laterality, alternation, and perseveration relationships on the T-maze test. Behav. Neurosci. 106:974–980; 1992.
35. Shen, Y.; Specht, S. M.; De Saint Ghislain, I.; Li, R. The hippocampus: A biological model for studing learning and memory. Prog. Neurobiol. 44:458–496; 1994.
36. Somjen, E. Neurofisiología; México. Panamericana; 1986:67–74.
37. Squire, L. R. Mechanisms of memory. Science 232:1612–1618; 1986.
38. Venero, J. L.; Herrera, A. J.; Machado, A.; Cano, J. Changes in neurotransmitter levels associated with the deficiency of some essential amino acids in the diet. Br. J. Nutr. 68:409–420; 1992.
39. Vorhes, Ch. V. Methods for assessing the adverse effects of foods and other chemicals on animal behavior. Nutr. Rep. Suppl. 44:185–192; 1986.
40. Yukihiro, N.; Yoshiaki, O.; Etsuko, S.; Makoto, O. Involvement of central cholinergic mechanisms in RU-24969-induced behavioral deficits. Pharmacol. Bichem. Behav. 38:441–446; 1991.