Effect of administration of ascorbic acid and dopamine D2 receptors agonist in the hippocampal CA1 area on spatial learning and memory in adult male rats

Esmaeilpour-Bezenjani, Kh.[1, 2] and Abbasnejad, M.[1*]

[1]Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran; [2]Kerman Neuroscience Research Center (KNRC), Kerman University of Medical Sciences, Kerman, Iran

*Correspondence: M. Abbasnejad, Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran. E-mail: mabbas@uk.ac.ir

Summary

Previous studies have shown that ascorbic acid (AA) plays a crucial role in mammalian brain as a vitamin and neuronal modulator. There is increasing evidence indicating that dopaminergic system and AA could affect learning and memory processes. In addition, vitamin C acts as a dopamine antagonist in the brain. The aim of the present study was to evaluate the intra-hippocampal co-administration of AA and bromocriptine (Br), a dopamine D2 receptor agonist, on spatial memory and learning. Adult male rats were trained in Morris Water Maze task. Intra-hippocampal injection was performed through an implanted cannula in the CA1 region. Animals were divided into 12 experimental groups (n=7) including: control, AA (6, 12, 24 and 48 µg/rat), Br (0.5, 1, 2 and 2.5 µg/rat), AA plus Br (24 and 2 µg/rat), AA and Br related sham. The results showed that injection of AA caused a significant decrease in both learning and spatial memory. However, administration of Br alone or concomitant with AA caused a significant increase in learning and spatial memory as compared to the control and AA groups. These findings indicate that AA could attenuate the effects of D2 dopamine receptors agonist on spatial memory and learning.

Key words: CA1, Ascorbic acid, Spatial learning and memory, D2 receptors, Rats

Introduction

Ascorbic acid (AA) is believed to be a neuromodulator in the CNS in addition to its anti-oxidant role (Dai *et al*., 2006; Fazeli *et al*., 2010). Ascorbate is transported and concentrated into the brain and neurons via the sodium-dependent vitamin C transporter 2 (SVCT2). So, brain depletion of ascorbate is a difficult procedure. Ascorbate is proposed as a neuron modulator of glutamatergic, dopaminergic, cholinergic, and GABAergic transmission and their related behaviors. AA can protect nerve cells against glutamate excitotoxicity (May, 2012), It also has a crucial role in immune system (Lotfollahzadeh *et al*., 2005).

It is reported that intraperitoneal (i.p.) injection of ascorbate (125 mg/kg) reverses age and scopolamine-induced memory deficits in the rat elevated plus-maze test (Parle and Dhingra, 2003). Chronic administration of ascorbate accompanied by vitamin E in aged rats also improves performance on a passive avoidance task (Arzi *et al*., 2004). In addition, repeated ascorbate treatments (60-120 mg/kg) could improve both acquisition and retention of passive avoidance task (Shahidi *et al*., 2008). In contrast, AA (1 g/kg, i.p.) significantly decreases the rate of conditioned avoidance response (CAR) in the shuttle box avoidance test (Desole *et al*., 1987). Furthermore, treatment with amphetamine increases the number of avoidance responses in the shuttle-box task blocked by ascorbate treatment (Fiona *et al*., 2009).

Dopamine has an important role in memory processes, especially in three inter-connected brain regions i.e., striatum, hippocampus and prefrontal cortex (Amico *et al*., 2007). The hippocampal dopamine receptors are involved in the modulation of

memory (Rezayof *et al*., 2007). It has been demonstrated that dopamine (DA) finely tunes prefrontal neuronal activity associated with working memory performance (Stuchlik *et al*., 2007). Intra-hippocampal administration of D2 but not D1 agonist and antagonist elicit significant effect on memory or learning processes (Amico *et al*., 2007). There are several investigations indicating the relationship between AA with dopaminergic system as well as glutamate (Rebec and Pierce, 1994; Rice, 2000). Specially, the relationship between dopaminergic system and AA has been an attractive subject for scientists. For example, vitamin C can act as a DA antagonist in the brain (Tolbert *et al*., 1992). One report indicates that AA reduces d-amphetamine-induced behavioral changes (Rebec and Pierce, 1994). In addition, AA can block the d-amphetamine-induced stereotypy and enhance the anti-dopamine effect of haloperidol in rats. It directly alters striatal dopamine binding sites and inhibits the binding of DA antagonists to DA receptors (Liu *et al*., 2000). Taken together, it seems that brain extracellular ascorbate level can be modulated by dopaminergic neuro-transmission and this modulation varies quantitatively in different dopamine-containing brain structures.

Since dopamine and ascorbic acid display a reciprocal interaction in some physiological activities and have critical effects on learning and memory processes, we decided to investigate the effect of intra-hippocampal co-administration of AA and D2 receptor agonist on learning and memory in Morris Water Maze task (MWM).

Materials and Methods

Adult male Wistar rats (n=84), weighing 250-300 g, were obtained from Pasteur Institute of Iran. Animals were housed four per cage at room temperature ($24\pm1°C$) under a 12-hour light/dark cycle. Food and water were available *ad libitum* throughout the experiment. The protocol was approved by the Animal Experimentation Ethic Committee of Neuroscience Research Center (EC/KNRC/87-5). All animals were handled (5 min/day) by the experimenter for seven consecutive days before the beginning of the experiments. Rats were randomly allocated into 12 experimental groups, each comprising 7 animals.

Surgery

Rats were anesthetized with ketamine hydrochloride (50 mg/kg i.p.) plus xylazine (5 mg/kg i.p.), (Netherlands Alfasan Co.) and placed in a stereotaxic apparatus (Stoeling, USA). Bilateral guide cannulae were placed 1 mm above the intended site of injection according to the atlas of Paxinos and Watson (1986). Stereotaxic coordinates were AP: -3.80 mm from Bregma, ML: ±2.2 from the midline and DV: -3.2 mm from the skull surface. Two screws were inserted into the skull and cannulae, fixed with dental cement. Stainless steel styles (23-gauge) were inserted into the guide cannulae to keep them free of debris. The rats had a one week recovery period after surgery.

Drugs

Ascorbic acid was purchased from Sigma Chemical Co., (dissolved in 0.9% sterile saline solution), Bromocriptine (Tocris Cookson) was dissolved in saline, propyleneglycol and ethanol solution.

Intracerebral injections were administered through guide cannulas (23-gauge) using injection needles (27-gauge) connected with polyethylene tube to 1-µl Hamilton microsyringes (1 µl/min/each side). The injection needle was inserted 1 mm beyond the tip of the cannula. All injections were done 30 min before test sessions in each experimental day.

Behavioral assessment

The learning and memory was assessed in a water tank as described by (Morris *et al*., 1982). The water maze was a black circular tank with 136 cm diameter and 60 cm height. The tank was filled with water at $20\pm1°C$ to a depth of 25 cm. Extra maze cues consisted of geometric shapes on the walls, posters, shelves, etc.; there were no intra maze cues. The maze was divided into four quadrants. A hidden circular platform (diameter: 10 cm) was located in the center of the South-West (SW) quadrant which was submerged 1.5 cm below the surface of the

water. A tracking system was used to measure the escape latency, the traveled distance of each rat, the percent of distance, and also the time in each quadrant.

Each rat received a block of four trials during five daily sessions. During the first 4 days, the platform, situated in the center of the South-West quadrant, was submerged 1.5 cm below the surface of water and therefore invisible, for testing spatial learning. On day 5 the platform was elevated above the water level and covered with a piece of aluminum foil and placed in the center of the southeast quadrant, and used for the assessment of sensory motor towards a visible platform. As soon as the rat had climbed onto the escape platform or when 90 s had elapsed each trial was terminated. Rats were allowed to stay on the platform for 20 s. The next trial was started if the rat did not find the platform within 90 s. Rats were put on the platform by the experimenter and allowed to stay there for 20 s. All tests were conducted between 09:00 and 13:00 h.

In order to determine the placement of micro-injector tips the rat brains were removed and stored in 20% formalin solution for 48 h. Histological sections (150 µm thick) were prepared using a Vibrotome (WPI, USA), and stained with cresyl violet. Sections were examined under a light microscope. Animals were included for data analysis only if both needle placements were located within the CA1 region, regardless of their memory performance.

Rats were randomly assigned to the following groups (n=7): ascorbate-treated group (6, 12, 24 and 48 µg ascorbate/rat was given); bromocriptine-treated group (0.5, 1, 2 and 2.5 µg bromocriptine/rat was given); AA plus Br (24 and 2 µg/rat). Saline and vehicles were injected to the animals in the control group.

Statistical analysis

The mean data obtained from hidden platform tests during four training days was analysed by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. In all comparisons, $P<0.05$ was used as the criterion for statistical significance.

Results

Figure 1 shows intrahippocampal injection of ascorbic acid at doses of 12 and 24 µg/rat significantly increased escape latency as compared with control during the 4 days of training in the water maze with the hidden platform (F=4.895, $P<0.01$). Platform remove trials (day 5) related results showed that there was a significant difference of performance among the ascorbic acid (24 µg/rat) and control groups with regard to the percentage of time spent in the target quadrant (12 µg/rat: $P<0.05$ and 24 µg/rat: $P<0.01$) (F=5.756) (Fig. 2).

Intrahippocampal injection of bromocriptine related results show no significant difference in the escape latency between groups during hidden platform trials (days 1-4) (Fig. 3) and also in percentage of time spent in the target quadrant during platform remove trials (day 5) (Fig. 4).

Figure 5 shows the results obtained from the ascorbic acid plus bromocriptine injection and group receiving vehicle (propylenglycol+saline). A significant difference was generally noticed in the escape latency between (AA, 24 µg/rat + BR, 2 µg/rat) and (BR, 2 µg/rat: $P<0.05$), between (BR, 2 µg/rat: $P<0.01$) and control during hidden platform trails (Days 1–4) (Fig. 5).

A significant difference was generally found in the percentage of time spent in the target quadrant (AA 24 µg/rat + BR 2 µg/rat), (AA 24 µg/rat) and (BR 2 µg/rat) ($P<0.05$, F=1.28) during platform remove trials (day 5) (Fig. 6).

Discussion

The primary finding of the present study was that intrahippocampal AA treatment decreased traveling distance as well as time spent in the target quadrant and increased traveling escape latency. As it was expected D2 agonist, Bromocriptine, could increase traveling distance and time spent in the target quadrant and had a decreasing effect on escape latency. Administration of AA prior to bromocriptine injection attenuated the observed effect, but does not eliminate it.

It seems AA may act like a dopamine antagonist in hippocampus. No record is available regarding the effect of intrahippocampal injection of AA on
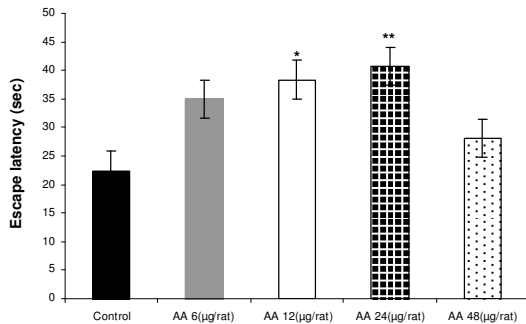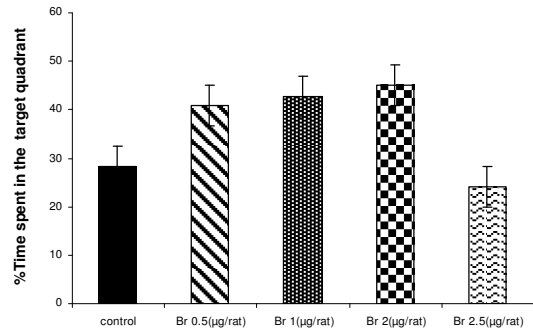

Fig. 1: The effects of ascorbic acid (AA) on the mean escape latency during the 4 days of training in a water maze with the hidden platform. Bars indicate mean±SEM (n=7). * P<0.05, and ** P<0.01 vs. control
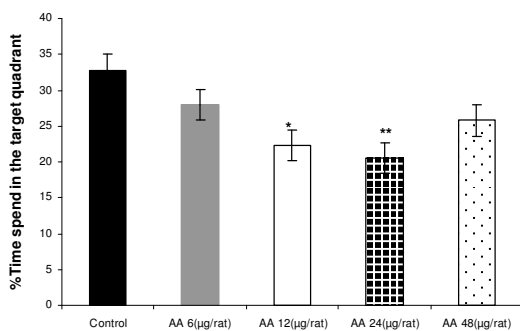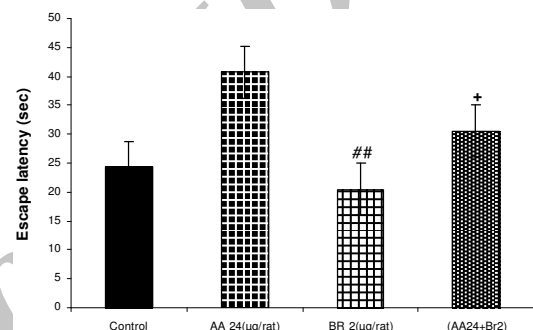

Fig. 2: The effects of ascorbic acid (AA) on percent time spent in the target quadrant during the day 5 of training in a water maze with the removed platform. Error bars indicate ±SEM. * P<0.05 vs. control, and ** P<0.01 vs. control. n=7, and AA: Ascorbic acid
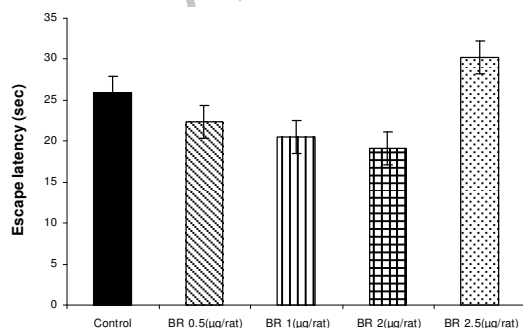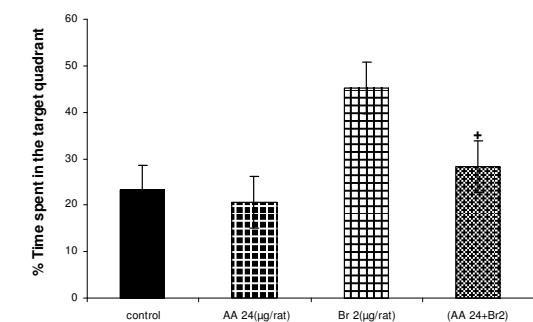

Fig. 3: The effects of bromocriptine during water maze training. Mean escape latency during 4 days of training in a water maze with the hidden platform. Error bars indicate ±SEM. n=7, and BR: Bromocriptine

Fig. 4: The effects of bromocriptine during water maze training. Percentage of time spent in the target quadrant during day 5 of training in a water maze with the removed platform. Error bars indicate ±SEM

Fig. 5: The effect of co-administration ascorbic acid plus bromocriptine during MWM training. Mean escape latency during the 4 days of training in a water maze with the hidden platform. Error bars indicate ±SEM. \#\# P<0.01 vs. AA and control, + P<0.05 vs. BR and control. n=7, and BR: Bromocriptine, AA: Ascorbic acid

Fig. 6: The effect of co-administration ascorbic acid plus bromocriptine during MWM training. Percentage of time spent in the target quadrant during day 5 of training in a water maze with the removed platform. Error bars indicate ±SEM. + P<0.05 vs. AA and Br. n=7, Br: Bromocriptine, and AA: Ascorbic acid

cognitive indices in rats. It is considered that

AA is not only a simple antioxidant but also a neuromodulator in CNS (Dai *et al*., 2006). In addition, it can interfere with glutamatergic, dopaminergic, cholinergic and GABAergic transmission and related behaviors (Fiona *et al*., 2009). These neurotransmitter systems have a basic and crucial role in learning and memory processes (Myhrer, 2003). It has been reported that peripheral administration of AA decreases the learning and memory in the shuttle box avoidance test (Desole *et al*., 1987), while acute peripheral AA injection has no significant effect on passive avoidance learning in rats. Short and long-term supplementation with AA can facilitate the acquisition and retrieval processes of passive avoidance learning and memory in rats (Shahidi *et al*., 2008). Intrastriatal ascorbate oxidase (AAO) inhibits behavior-related neuronal activity in the striatum but AAO administration into dorsal hippocampus fails to alter behavioral activation (George and Zhongrui, 2001). The results of this study are different from other studies which suggested that AA could repair or prevent memory deficits (Parle and Dhingra, 2003; Monteiro *et al*., 2005; Delwing *et al*., 2006; Landmark, 2006). This can be explained by the age of the animals under study as intact young rats were used in the present study, but aged rats were tested in other studies.

It has been reported that AA-related changes in learning and memory depend on the method of administration (Shahidi *et al*., 2008), animal age and period of injection (Arzi *et al*., 2004). In addition, numerous studies have shown that the anti-impairment effects of AA on learning and memory are due to its antioxidant effects (Reis *et al*., 2002; Cho *et al*., 2003; Castagne *et al*., 2004). This issue is in contradiction with our results that show AA has a decreasing effect on learning and memory indices in Morris Water Maze task.

Therefore, it is possible that ascorbic acid-induced learning and memory impairment is due to its neurotransmitter and neuromodulatory properties. It has been shown that local application of AA interferes with the response of neurons to dopamine and glutamate (Rebec and Pierce, 1994). Injection of dopamine D2 receptor antagonist into the hippocampus impairs memory performance and is ameliorated by an agonist (Fujishiro *et al*., 2005). On the other hand, there is behavioral evidence supporting the anti-dopaminergic action of AA (Rebec and Pierce, 1994). Our results showed that a combination of AA with bromocriptine attenuated the increasing effects of bromocriptine on learning and memory indices in MWM task. In the shuttle-box task, amphetamine, as dopamine agonist, increases the number of avoidance responses blocked by AA (Fiona *et al*., 2009). AA blocks the D-amphetamine-induced stereotypy and also enhances the anti-dopamine effect of haloperidol (Liu *et al*., 2000), also there is some evidence to show that AA is able to modulate the effect of DA in the mammalian forebrain. Moreover, it inhibits binding of both DA agonists and antagonists in a dose-dependent manner (Tolbert *et al*., 1992). One of the earliest observations about the interaction of AA with the dopaminergic system is that ascorbic acid could completely inhibit the DA-stimulated adenylate cyclase activity in rats (Thomas and Zemp, 1997) AA may act in part via cholinergic signaling because its intraperitoneal administration attenuates scopolamine-induced cognitive deficits in young mice (Harrison *et al*., 2008). In addition, AA may interfere with nicotine-induced place preference and behavioral sensitization in mice (Sahraei *et al*., 2007).

The cooperation of AA and glutamate is important for neuron metabolism so that ascorbic acid participates as a metabolic switch modulates neural metabolism between resting and activation periods (Fiona *et al*., 2009). Furthermore, glutamate stimulates AA release from astrocyte in the CNS (Maite *et al*., 2009). More recent studies relating ascorbate modulation of glutamate dynamics with changes in rat behavior show that such modulation is complex, because it depends on the sites in the brain, level of behavioral activity and level of extracellular ascorbate (Fiona *et al*., 2009). There is a significant interaction between DA and glutamate in CA1, for example, DA in the CA1 evokes a protein synthesis-dependent LTP that requires synergistic NMDA-receptor activation and protein synthesis (Navakkode *et al*., 2010).

AA entry into the neurons and within the cell can inhibit glucose consumption and stimulate lactate transport (Maite *et al.*, 2009).

The above mentioned documents suggest that different neurotransmitters or neuromodulators might be involved in intrahippocampal AA-induced reduction in learning and memory. However, further investigation is necessary to evaluate the exact mechanism(s) underlying the effect of AA in the lowering of learning and memory. Taken together, this study showed that the central injection of AA caused a significant decrease in both learning and spatial memory and attenuates the positive effects of D2 dopamine receptors agonist on spatial memory and learning in rats.

References

Amico, F; Spowart-Manning, L; Anwyl, R and Rowan, MJ (2007). Performance and task-dependent effects of the dopamine D1/D5 receptor agonist SKF 38393 on learning and memory in the rat. Eur. J. Pharmacol., 577: 71-77.

Arzi, A; Hemmati, AA and Razian, A (2004). Effect of vitamins C and E on cognitive function in mouse. J. Pharmacol. Res., 49: 249-252.

Castagne, V; Rougemont, M; Cuenod, M and Do, KQ (2004). Low brain glutathione and ascorbic acid associated with dopamine uptake inhibition during rat's development induce long-term cognitive deficit: relevance to schizophrenia. J. Neurobiol. Dis., 15: 93-105.

Cho, J; Kang, JS; Long, PH; Jing, J; Back, Y and Chung, KS (2003). Antioxidant and memory enhancing effects of purple sweet potato anthocyanin and cordyceps mushroom extract. Arch. Pharm. Res., 26: 821-825.

Dai, F; Yang, J; Gu, PF; Hou, Y and Wu, CF (2006). Effect of drug-induced ascorbic acid release in the striatum and the nucleus accumbens in hippocampus-lesioned rats. J. Brain Res., 1125: 163-170.

Delwing, D; Bavaresco, CS; Monteiro, SC; Matte, C; Netto, CA and Wyse, AT (2006). Alpha-Tocopherol and ascorbic acid prevent memory deficits provoked by chronic hyperprolinemia in rats. J. Behav. Brain Res., 168: 185-189.

Desole, MS; Anania, V; Esposito, G; Carboni, F; Senini, A and Miele, E (1987). Neurochemical and behavioural changes induced by ascorbic acid and D-amphetamine in the rat. J. Pharmacol. Res. Commun., 19: 441-450.

Fazeli, P; Zamiri, MJ; Farshad, A and Khalili, B (2010). Effects of vitamin C on testicular and seminal characteristics of Markhoz goats. Iranian J. Vet. Res., 32: 267-273.

Fiona, E; Harrison, J and May, M (2009). Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. J. Free Radical Biol. Med., 46: 719-730.

Fujishiro, H; Umegaki, H; Suzuki, Y; Oohara-Kurotani, S; Yamaguchi, Y and Iguchi, A (2005). Dopamine D2 receptor plays a role in memory function: implications of dopamine-acetylcholine interaction in the ventral hippocampus. J. Berl. Psychopharmacol., 182: 253-261.

George, V and Zhongrui, W (2001). Behavioral activation in rats requires endogenous ascorbate release in Striatum. J. Neurosci., 21: 668-675.

Harrison, FE; Yu, SS; Van Den Bossche, KL; Li, L; May, JM and McDonald, MP (2008). Elevated oxidative stress and sensorimotor deficits but normal cognition in mice that cannot synthesize ascorbic acid. J. Neurochem., 106: 1198-1208.

Landmark, K (2006). Could intake of vitamins C and E inhibit development of Alzheimer dementia? J. Tidsskr. Nor. Laegeforen., 126: 159-161.

Liu, W; Wu, CF; Huang, M and Xiao, K (2000). Opposite effects of sulpiride and SCH 23390 on ethanol-induced striatal ascorbic acid release in intact and 6-hydroxydopamine lesioned rats. J. Brain Res., 869: 31-38.

Lotfollahzadeh, S; Mokhber Dezfouli, MR; Khazraii Nia, P and Tajik, P (2005). The effects of oral administration of ascorbic acid on serum γ-globulin concentrations of neonatal calves. Iranian J. Vet. Res., 12: 79-83.

Maite, A; Felipe, A; Sebastian, B and Ilona, I (2009). A metabolic switch in brain: glucose and lactate metabolism modulation by ascorbic acid. J. Neurochem., 110: 423-440.

May, JM (2012). Vitamin C transport and its role in the central nervous system. J. Subcell Biochem., 56: 85-103.

Monteiro, SC; Matte, C; Bavaresco, CS; Netto, CA and Wyse, AT (2005). Vitamins E and C pretreatment prevents ovariectomy-induced memory deficits in water maze. J. Neurobiol. Learn. Mem., 84: 192-199.

Morris, RGM; Garrud, P; Rawlins, JNP and O Keefe, J (1982). Place navigation impaired in rats with hippocampal lesion. J. Nature. 297: 681-683.

Myhrer, T (2003). Neurotransmitter systems involved in learning and memory in the rat: a meta-analysis base on studies of four behavioural tasks. J. Brain Res. Rev., 41: 268-287.

Navakkode, S; Sajikumar, S and Sacktor, TC (2010). Frey Protein kinase Mzeta is essential for the induction and maintenance of dopamine-induced long-term potentiation in apical CA1 dendrites. J. Learn Mem., 17: 605-611.

Parle, M and Dhingra, D (2003). Ascorbic acid: a promising memory-enhancer in mice. J. Pharmacol. Sci., 93: 129-135.

Paxinos, G and Watson, C (1986). *The rat brain in stereotaxic coordinates*. 2nd Edn., Orlando, Academic Press. PP: 139-142.

Rebec, GV and Pierce, RC (1994). A vitamin as neuromodulator: ascorbate release into the extracellular fluid of the brain regulates dopaminergic and glutamatergic transmission. J. Prog. Neurobiol., 43: 537-565.

Reis, EA; Zugno, AL; Franzon, R; Tagliari, B; Matte, C; Lammers, ML; Netto, CA and Wyse, AT (2002). Pretreatment with vitamins E and C prevent the impairment of memory caused by homocysteine administration in rats. J. Metab. Brain Dis., 17: 211-217.

Rezayof, A; Motevasseli, T; Rassouli, Y and Zarrindast, MR (2007). Dorsal hippocampal dopamine receptors are involved in mediating ethanol state-dependent memory. J. Life Sci., 80: 285-292.

Rice, ME (2000). Ascorbate regulation and its neuroprotective role in the brain. J. Trends Neurosci., 23: 209-216.

Sahraei, H; Aliabadi, AA; Zarrindast, MR; Ghoshooni, H; Nasiri, A; Barzegari-Sorkheh, AA; Yari, M; Zardooz, H; Hossein-Mardi, L; Faraji, N and Shams, J (2007). Ascorbic acid antagonizes nicotine-induced place preference and behavioral sensitization in mice. J. Eur. J. Pharmacol., 560: 42-48.

Shahidi, S; Komaki, A; Mahmoodi, M; Atrvash, N and Ghodrati, M (2008). Ascorbic acid supplementation could affect passive avoidance learning and memory in rat. J. Brain Res. Bull., 76: 109-113.

Stuchlik, A; Rehakova, L; Telensky, P and Vales, K (2007). Morris water maze learning in Long-Evans rats is differentially affected by blockade of D1-like and D2-like dopamine receptors. J. Neurosci. Let., 422: 169-174.

Thomas, TN and Zemp, JW (1997). Inhibition of dopamine-sensitive adenylate cyclase from rat brain striatal homogenates by ascorbic acid. J. Neurochem., 28: 663-665.

Tolbert, LC; Morrise, PE; Spollen, JJ and Ashe, SC (1992). Streospecific effect of ascorbic acid analogues of D1 and D2 agonist binding. J. Life Sci., 51: 921-930.