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Akçahan GEPDİREMEN[1]
Cemal GÜNDOĞDU[2]
Sinan SÖNMEZ[3]
Dilek SADELER[1]
Halis SÜLEYMAN[1]
Mehmet Emin BÜYÜKOKUROĞLU[1]

Global Ischemia and Turn Preference; A Comparative Study of the Effects of Global Ischemic Insult in Rats

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Departments of [1]Pharmacology, [2]Pathology, [3]Genetics, Faculty of Medicine, Atatürk University, 25240, Erzurum-Turkey

Abstract: We examined the effect of global cerebral ischemia (Two vessel occlusion plus systemic hypotension method) for 15 minutes on the hippocampal CA1 and CA3 neurones in rats, and compared with turn prefence scores. 14 of 27 animals were ambidexter while 9 had left and 4 had right turn preference. The left turns were significantly different from rights (t:2,26 df:26 p:0,032). Male rats prefered 62,73% and females 56,88% left turns. Forty-eight hours following the global cerebral ischemic insult, animals were sacrificed and brains were examined by using light microscope. Hippocampal neurones of the right hemisphere were survived more in males while left side neurones did it in female rats. Most impressive result in this study was that the CA1 hippocampal neurones were better protected than CA3 neurones in cerebral ischemic insult (t:4,69 df:53 p:0.000). This significance was especially originated from right hemisphere (t:4,55 df:26 p:0,000). In ambidexter rat group, left CA3 was better protected than right CA3 (t:2,17 df:13 p:0,049). Right CA3 neurones were significantly survived better in right turn prefence dominant than ambidexter rats (t:2,22 df:16 p:0,041).

Key Words: Hippocampus, Global ischemia, Neuronal death, Turn preference, CA1, CA3.

Introduction

Lateral asymmetries in antigravity excitatory strength arise from an imbalance in vestibular functioning physiologically (1). Approximately two third of blindfolded human subjects rotated to the right during a walking and stepping test because of a left otolithic dominance. Turning biases in normal, evidently reflect the greater excitation of the contralateral vestibular organ (2). Asymmetric dopamine content in the basal ganglia of mammals correlate extremely well with both contralateral turn and a contralateral paw preference (3). These biochemical asymmetries contribute to the lateralization of many of the immune and other pathological disorders such as torticollis, scoliosis, hemiatrophy and hemiparkinsonism (4).

Surprisingly, little is known about the comparative neuronal resistance of male and female animals to ischemic insult. Berry et al, found that as little as 12% incidence of unilateral carotid occlusion induced neurological sequels in female gerbils versus 50% for males (5). This difference was attributed to the more common occurrence of anterior cerebral anastomotic links in females. Stupfel et al, has reported that female mice show less mortality from an acute hypoxic challenge than males (6). Lesser ischemic vulnerability has been found in female Mongolian gerbils by Hall et al, and the results have been attributed to the antioxidant effect of endogenous estrogen. The same authors have also found that 17-β estradiol was more potent as an inhibitor of lipid peroxidation in brain tissue than vitamin-E (7). On the other hand, neuronal protection in ischemic insult and it's relation with cerebral laterality has not been studied so far.

Ischemia sensitive neurones include; the hippocampal CA1 pyramidal neurones, the cerebellar purkinje cells, medium sized striatal neurones and pyramidal neurones in neocortical layers 3,5 and 6 (8,9,10). The hippocampus contains neurones selectively more vulnerable to ischemia and hypoxia compared with other brain regions (8). During the global ischemia, cerebral blood flow measurement after 5-15 minutes was found to reduce 15% of control in hippocampus. This was found one of the most drastic drops of cerebral blood flow, together with caudate putamen and cingulate cortex

among all of the brain regions measured in rats (11). We studied, the hippocampal CA1 and CA3 regions following 15 minutes of global ischemia plus systemic hypotension and reperfusion, in both hemispheres and compared sex and turn preference in rats to determine the details about the pathopysiologic concepts of global ischemia in rodents. The aim of this study was to compare hippocampal neuronal vulnerability in rats. Since differences between hemispheres might play a role in the outcome of neuronal vulnerability, the hemispheric dominance of the animals was established preischemia.

Methods

Twelve male (188.71±6.71 g) and sixteen (157.29±3.56 g) female Wistar rats, were used in this study. Five or six rats were housed together, at 23-25˚C and 60% humidity with a 12/12 hour light/dark (light on 06:00, off at 18:00) cycle. Rats were given rat chow and tap water ad libitum and they were not handled for one week before the starting of the behavioural experiments. One day before the behavioural experiment, the animals were weighed and housed two per cage.

Prior to commencing the surgical intervention, all the animals were categorised in terms hemispheric dominance by measuring turn preference in an open field (12). Animals were lifted from their holding cages using two hands, placed firmly on either side of their body, held for 2-3 seconds and then released into the open field. The direction of immediate movement to the right or left was recorded. The data generated was noted for each animal. Turn preference in each rat was determined twice daily (8 a.m. and 4 p.m.) for 10 days. Despite it is a continuous distribution of degrees of right and left preference, the criterion of dominance is accepted in variable ranges between 90% (13) and 70% (14). In according to latter study, 14 or more of 20 turns to any side regarded as turn preference in our experiment.

In operational stage, rats were anaesthetised (25 mg/kg, IP thiopentone) and given adjunctive treatment with atropine (0.1 mg/kg, IP) to prevent the salivation through their respiratory system during the anaesthesia. For induce systemic hipotension, 10 mg/kg acetylcholine was administered intraperitoneally. Rectal temperature of the animal was rigorously maintained at the same level and any outranged score was taken out of the consideration; it was 26,9-37,4 ˚C. Following anaesthesia, animals were fastened to a surgery table, an anterior midline cervical incision was made, the left and right common carotid arteries were isolated from the vagus nerve and small homeostatic clips placed on them, in such a way that, blood flow was completely arrested for a period of 15 minutes. A wet, sterilised cloth was used to protect the surgical area from direct effect of the heating lamp. At the end of the ischemic period, the clips were removed and the patency of the carotid arteries checked by direct visualisation. Dissolved chloramphenicol sodium succinate flacon (Farmitalia Carlo Erba, Milan Italy) was sprayed onto the incision area after washing it with saline to prevent contamination during the postoperative period. The skin incision was closed with 3-0 surgical sutures. Polyvinylpyrrolidone-iodine complex was used in the final cleaning of the skin and the animals were allowed to survive for 48 hours. During the postoperative period, rectal body temperatures checked in every 4 hours and maintained between the same ranges as during the operation. In case of any change in body temperatures morehan 0,5˚C, the animal was excluded.

Forty-eight hours following surgery, animals were reanaesthetised with the previous dosages of thiopentone. The thorax was opened, the descending aorta was ligated and the brain was perfused with 0,9% saline until the solution returning from the right artrium is clear at a rate of 10 mls/min using a standard procedure via the ascending aorta, by a polyethylene catheter fixed to a hand-pump. This was immediately followed by perfusion with a solution containing 0,4% paraformaldehyde, 0,9% saline, 5% sucrose in 0,4 M phosphate buffer at pH 7,2-7,4 for 15 minutes. Brains were removed and stored in a 10% buffered formaldehyde for 72 hours. Three mm thick coronal section was cut and embedded in wax. Five microns sections were cut and stained with hematoxylin-eosyn (15). Slide labels were covered with tape to enable blind evaluation. CA1 and CA3 regions of the dorsal hippocampus were examined by using light microscopy and rated for neuronal damage by counting all normal appearing pyramidal cell, asidophilic degenerated neurones and gliosis. Finally, losses of the neuronal layers of the CA1 and CA3 regions were scored. At least 10 different microscopic areas were examined for CA1 and CA3 separately for both hemispheres and the mean scores were calculated. The results were translated as the percent of all the cell population counted for each localization.

Following 20 observations of turn preference throughout 10 days, up to 13 turn to any side regarded as contralateral cerebral dominance, when others are ambidexterity. In according with this assumption, differences in mean cells densities between CA1 and CA3, right and left, male and female and regarding their turn

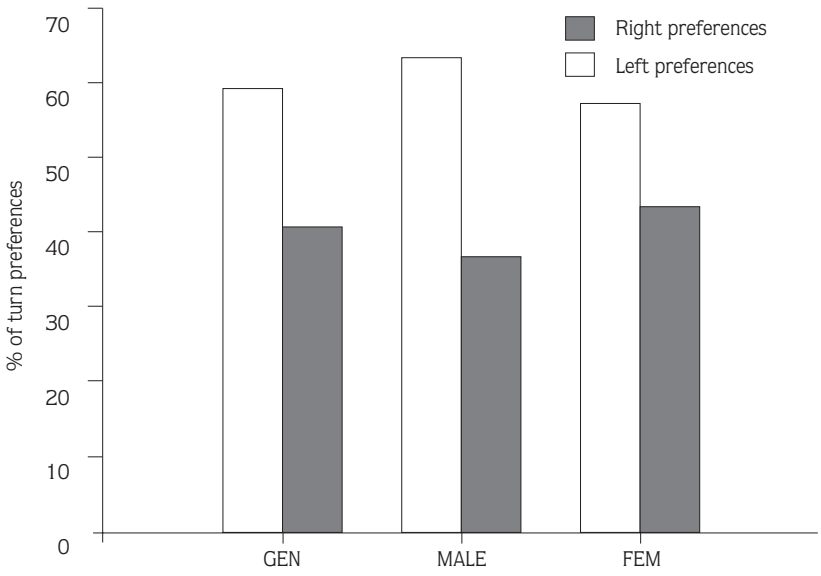


Figure 1. Bar graph represents turn preferences in rats as percent regarding their sexes. Statistical significance was found between right and left turn preferences in all groups tested (p<0.05).

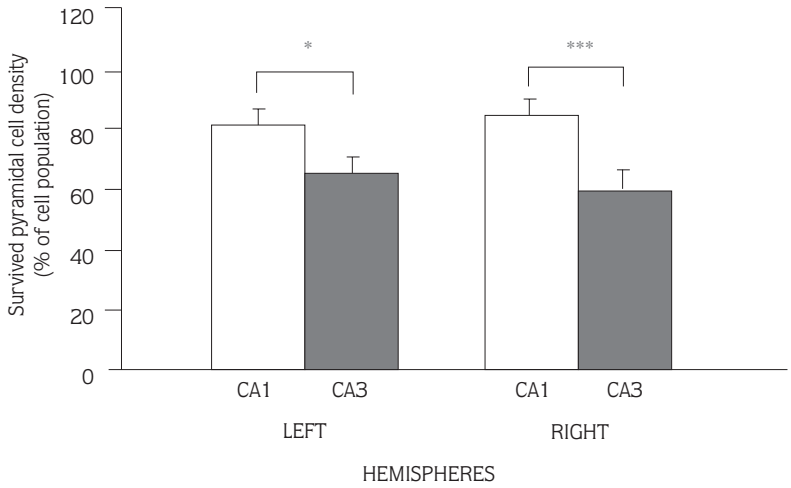


Figure 2. Bar graph represents survived pyramidal cell density as percent of cell population. *p:0,032 ***p:0,000.

preference were analysed using ANOVA and two tailed paired-t test, with p<0,05 being required for significance.

Results

14 of 27 animals were ambidexter, while 9 prefered left and 4 prefered right turn (contralateral hemispheric dominance). These results have been compared, taking into consideration the sex and cell counts of CA1 and CA3 regions of the dorsal hippocampus. Left turn preference was significantly dominant to right preference in two tailed paired-t test (df:26, t:2.26 and p:0.032) in rats. Male rats showed 62,73% and female rats showed 56,88% turn preference to left (59,26% in general, figure 1). Both CA1 and CA3 in right hemisphere were protected better in males while CA1 and CA3 neurones in left hemisphere survived in higher rates in females.

The most impressive result in our study was the very significant difference between CA1 and CA3 regions of the dorsal hippocampus. CA1 was more resistant area than CA3 in the animals tested. The total difference between CA1 and CA3 cell loss was exist (t:4.69, df:53, p:0.000), because there was lesser damage in right CA1 field than other sectors (t:4.55 df:26 p:0.000). There was a slight difference between the left CA1 and CA3 fields (t:2.37, df:26, p:0.026 Figure 2).

Finally neuronal survival was compared between right and left sides and in subgroups with respect to turn preference. In ambidexter rat group, left CA3 was better protected than right CA3 (t:2.17, df:13, p:0.049). Also, right CA3 neurones were significantly better protected in right turn preference dominant rats than ambidexters (t:2.22, df:16, p:0.041 figure). Despite it is insignificant

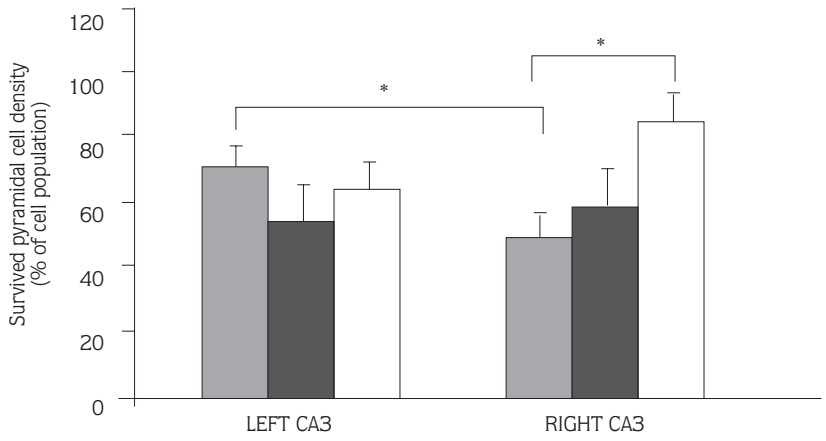
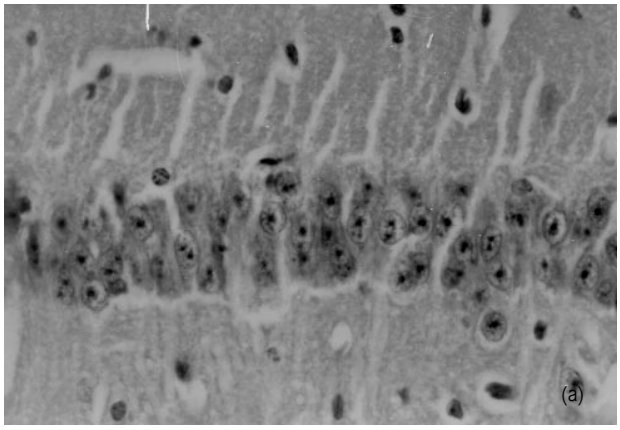


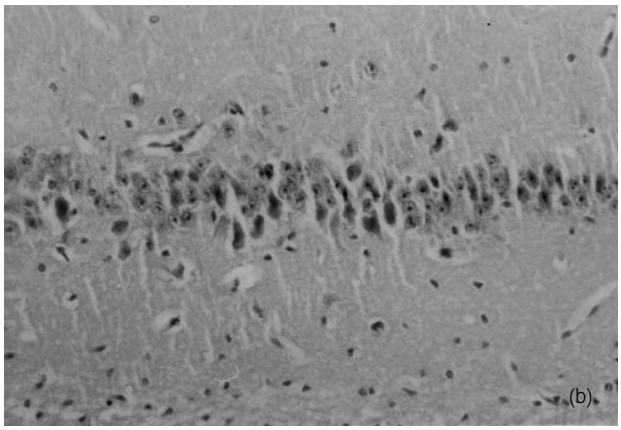
Figure 3. Comparison of the left and right CA3 of the hippocampus in ambidexter, right and left turn preferences rats. *p<0,05 (Between left and right CA3 of ambidexter rats and between ambidexter and right preference rat's CA3 area of hippocampus)

Ambidexter

Left turn,

Right turn preference rats.





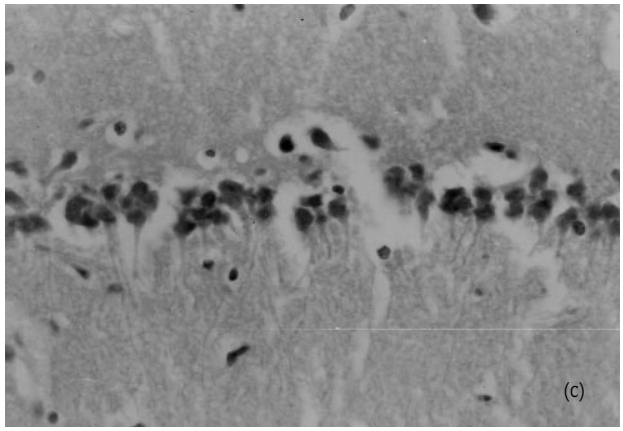


Figure 4a,b,c.

statistically, left CA3 was better protected than right CA3 in ambidexters (t:1.93, df:26, p:0.064). The photomicrographs of intact, slightly ischemic and excessive ischemic areas of the CA1 and CA3 are presented in figure 4 a,b and c.

Discussion

While some authors claimed that rodents and primates such as monkey, chick, baboon, rat and mice, show cerebral asymmetry, just like humans, in their brain morphology and functions (16), some others say that the central nervous system is symmetric bilaterally. Findings in literature indicate asymmetry in some animal species, and has been claimed to find by chance or by asymmetric environment (17). For example, left-handed rats may prefer right if they bring a proper environment (18).

In rats, there is evidence of different rates of development between the hypothalamic regions of the two sides, as judged from the differing effects of sex hormones placed on each side (19). Glick, Jerussi and Zimmenberg, have found differences between the quantity of dopamine in rats in the left and right pathways to the basal ganglia (20). Animals were found to turn spontaneously more often in the direction opposite to the side with more dopamine. Rats were said to turn more often to the right, but gerbils turned equally

to the left or right (21). Testosterone was believed to delay the growth of the left cerebral hemisphere in-utero, and this effect is greater in males because the foetal testes secrete testosterone (22). The serum testosterone levels were found to be negatively linearly correlated with the degree of the right hand preferences, as well as the foot and eye preference in humans (23). In our study, left turn preference was found dominant in females (56,88%) and especially in males (62,73%). This can be explained by the undeveloped left hemisphere due to the testosterone in rats. Annett has reported 53% ambidexterity of the paw preference test in rats, which is compatible with our results (16). But they are contradicted by the results of Glick et al, mentioned above (20).

Here we have discussed rats hippocampal neuronal resistance, after global cerebral ischemic insult, taking into account their turn preference, sexes and side hemispheres, comparatively. We have observed that the CA3 region of the hippocampus is less protected than the CA1. The similar results have been obtained with systemic kainic acid neurotoxicity studies in our laboratories (unpublished data), which is very similar to the results of Balchen et al (24) and in dose dependent kainic acid induced neurotoxicity in hippocampus by Werner et al (25). But nearly all of the global ischemic studies are differ from this data in gerbils (26), cultured cerebellar rat neurones (27), rats (28) and rabbits (29). Werner et al reports that the higher vulnerability of CA3 pyramidal neurones than CA1 might be explained by the putative high affinity kainate receptor existence predominant in hippocampal CA3 cells (25). Domoic and kainic acids are the most potent neurotoxic agents in brain tissues, and in our study, global ischemic insult might involve kainic acid receptors predominantly in CA3. Or these receptors might exist in higher concentration in CA3 and so the activation of them could be the result of greater neuronal cell death in that part of hippocampus. On the other hand, glutamate is another important neuromediator that mediates in cerebral ischemic injuries. The CA3 sublocalization of hippocampus is found to have a rich glutamatergic network (30). By this reason, it is possible to have occured more degeneration in CA3 than CA1, in our study.

No statistical significance was found between turn preference and hippocampal cell degeneration in both side. This is compatible with the results of Witt (17) and Collins (18) whose indicate no asymmetry in central nervous system in animals.

Despite that no statistical significant results were found, there was a slight neuronal protection in females than males, both in CA1 and CA3 of hippocampus in the left hemisphere. Same neuroprotective effect was observed in right hemisphere, as well. Hall et al, found similar neuroprotective effects of internal estrogen in female gerbils in ischemic insult by unilateral carotid occlusion (7). The differences between male and female animals may originate from the antioxidant effect of internal estrogen, internal testosterone or, anastomotic links between different anatomic localisation in brain may provide extra resistance for two different sexes. The role of free oxygen radicals and lipid peroxydation in postischemic brain damage have been widely discussed (31, 32). While lipid peroxydation causes the formation of hydroperoxy (HPETE) and monoperoxy (HETE) fatty acids, leukotrienes, prostanoides, cyclic endoperoxydes via phospholipase A and phospholipase C pathways, toxic oxygen radicals such as H_2O_2, O_2, OH^- and single oxygen have been formed as side products in specific brain areas (33). One of the most fundamental differences between male and female animals is the presence of the hormone 17-β-estradiol in the latter. Estradiol has been demonstrated to have lipid antioxidant properties (34). Thus, in our study, it is conceivable that the increased postischemic neuronal survival observed in females in left hippocampus, may be due to a protective antioxidant effect of internal estrogen. Reperfusion may trigger the formation of toxic free oxygen radicals such as peroxynitrite ($ONOO^-$) and peroxynitrous acids. These toxic products cause an increase of ATP consumption. In this way, glucose utilisation from neuronal cells and ion transportation blocked and, membrane potential may not provide. The defect in cell permeability leads to increased Ca^{++} influx, and this precipitates cell death (33). The internal free oxygen radical scavengers such as glutation peroxidase or superoxide dismutase are being depleted during ischemia and reperfusion. In comparison, CA1 and CA3 of hippocampus, internal free oxygen radical scavengers might be less depleted or more generated in CA1, and may exist more in left side in female rats and right side in males in global ischemia. For a further discussion of the role of free radicals and lipid peroxidation in postischemic brain damage, the reader is referred to several reviews (31, 32).

The Haber-Weiss reaction, which causes the formation of toxic free radicals, is known to be catalysed by iron and copper (35). Larger contents of stored and functional iron and copper may also cause further degeneration in left side of male rats and right hemispheres in female rats. Testing the compounds, such as desferroxamine in ischemic insult, will help to sort out the role of this hypothesis. The ratio of HDL/LDL is decreased by

testosterone and increased by estrogen. This leads the increased risk of atherosclerosis and neuronal degeneration due to atherosclerotic brain vessels in males.

The severity of the neuronal degeneration of either

References

1. Previc FH. A general theory concerning the prenatal origins of cerebral lateralization in humans. Physiological Review, 98: 299-334, 1991.
2. Ghez C. Posture. In: Principles of Neural Sciences (Eds: Kondel ER, Schwartz JH and Jessel TM) Third Edition, Elsevier Sci Pub Co Inc, New York, p: 596-607, 1991.
3. Glick SD, Shaphiro RM. Functional and neurochemical mechanisms of cerebral lateralization in rats. In: Glick S.D. (Ed.) Cerebral lateralization in nonhuman species, Academic Press, San Diego, p: 157-183, 1985.
4. Geschwind N, Galaburda A. Cerebral lateralization. Biological mechanizms, associations, and pathology: III. A hypotesis and a program for research. Archives of Neurology, 42: 634-654, 1985.
5. Berry K, Wisniewsky JM, Svarzbein L, Baez S. On the relationship of brain vasculature to production of neurological deficit and morphological changes following acute unilateral common carotid artery ligation in gerbils. J Neurol Sci. 25: 75-92, 1975.
6. Stupfel M, Valleron AJ, Demeestere M, Masse H. Hypoxia survival variations in male and female mice as functions of chronological and environmental factors. Aviat Space Environ Med. 49: 1087-1092, 1978.
7. Hall ED, Pazara KE, Linseman KL. Sex differences in postischemic neuronal necrosis in gerbils. Journal of Cerebral Blood Flow and Metabolism, 11: 292-298, 1991.
8. Graham DI. Hypoxia and vascular disorders. In: Greenfield's Neuropathology (Eds: Adams J.H., Corsellis J., Duchen L.W.). Edward Aronld Publishers Ltd, London, p: 153-268, 1992.
9. Plum F, Pulsinelli W. Cerebral metabolism and hypoxic ischemic brain injury. In: Diseases of the nervous system (Eds: Asbury A, McKhann G, and McDonald A.), WB Saunders, Phladelphia, p: 1086, 1986.
10. Smith ML, Auer RN, Siesjö BK. The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. Acta Neuropathol (Berl). 64: 319-332, 1984.
11. Kagström E, Smith ML, Siesjö BK. Rec irculation in the rat brain following incomplete ischemia. J Cereb Blood Flow Metab, 3: 183-192, 1983.
12. Bradbury AJ, Costall B, Domeney AM, Naylor RJ. Laterality of dopamine function and neuroleptic action in the amygdala in the rat. Neuropharmacology, 24: 1163-1170, 1985.
13. Cole J. Paw preference in cats related to hand preference in animals and men. Journal of Comparative and Phisiological Psychology, 48: 137-140, 1955.
14. Lehman RAW: The handedness of Rhesus monkeys. I.Distribution Neuropsychologia, 16: 33-42, 1978.
15. Gilbert JJ. Central Nervous System Pathology. In. Pratical Surgical Pathology (Eds: Karcıoğlu ZA and Someren A.) The Collamoreress, D.C. Health Company, Lexington, Massachusets Toronto, p: 994-1043, 1985.
16. Annett M. Left, right hand and brain: the right shift theory. Lawrence Erlbaum Associates Ltd, London, Hilldale, New Jersey, p: 78, 1985.
17. Witt PN. Lateralization and the invertebrate nervous system; discussion. in; Evolution and the lateralization of the brain (ed: Dimond SJ, Blizard DA) New York Academy of Sciences 1977.
18. Collins RL. Origins of the cells of asymmetry: Mendelian and non-Mendelian model of inheritance. In: Evolution and Lateralization of the Brain (ed: Dimond SJ, Blizard DA) New York, New York Academy of Sciences, 1977.
19. Nordeen EJ, Yahr P. Hemispheric assymetries in the behavioral and hormonal effects of sexually differentiating mammalian brain. Science, 218: 391-393, 1982.
20. Glick SD, Jerussi TP, Zimmenberg B. Behavioral and neuropharmacological correlates of nigrostriatal asymmetry in rats. In: Lateralization on the nervous system (eds: Harnad S, Doty RV, Goldtein L, Laynes J, Krauthamer G.) New York Academic Press, New York, 1977.
21. Williams M. Laterality of suckling by foals. Paper presented at conference of the Europaischen Vereinung. Tierzucht, Freiburg, 1972.
22. Geschwind N, Beham P. Left-handednees: association with immune disease, migraine, and developmental learning disorder. Proc Natl Aca Sci USA. 79: 157-165, 1990.
23. Tan Ü. Relation of testosterone and hands preference in right-handed young adults to sex and familial sinistrality. Intern J Neurosci.. 53: 157-165, 1990.
24. Balchen T, Berg M, Diemer NH. A

paradox after systemic kainate injection in rats: Lesser damage of hippocampal CA1 neurons after higher doses. Neurosci Letters. 163: 151-154, 1993.

25. Werner P, Voight M, Keinanen K. Cloning of a putative high-affinity kainate receptor expressed predominantly in hippocampal CA3 cells. Nature, 351 (6329): 742-4, 1991.

26. Ohotsuki T, Matsumoto M, Kuwabara K. Influence of oxidative stress on induced tolerance to ischemia in gerbil hippocampal neurons. Brain Res. 599 (2): 246-52, 1992.

27. Lysko PG, Lysko KA, Yue TL. Neuroprotective effects of carvedilol a new antihypertensive in cultured rat cerebellar neurons and in gerbil global brain ischemia. Stroke, 23 (11): 1630-5, 1992.

28. Endoh M, Pulsinelli WA, Wagner JA. Transient global ischemia induces dynamic changes in the expression of bFGF and FGF receptor. Brain Res Mol Brain Res. 22 (1-4): 76-88, 1994.

29. Rasool N, Faroqui M, Rubinstein EH. Lidocain accelerates neuroelectrical recovery after incomplete global ischemia in rabbits. Stroke. 21 (6): 929-35, 1990.

30. Illievic VM, Zornow MH, Choi KT. Effects of hypothermic metabolic suppressions on hippocampal glutamate concentrations after transient global cerebral ischemia. Anesth Analg. 78 (5): 905-11, 1994.

31. Braughler JM, Hall ED. Central nervous system trauma and stroke: I.Biochemical considerations for oxygen radical formation and lipid peroxydation. Free Rad Biol Med. 6: 289-301, 1989.

32. Siesjö BK, Agarth CD, Bengstsson F. Free radicals and brain damage. Cerebrovasc Brain Metab Rev. 1: 165-211, 1989.

33. Lipton AS, Choi YB, Zhuo HP. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature, 364: 626-32 (1993).

34. Niki E. Antioxidants in relation to lipid peroxidation. Chem Phys Lipids. 44: 227-53, 1987.

35. Lefkowitz RJ, Hoffman BB, Taylor P. Neurohumoral transmission: The autonomic and somatic motor nervous systems. In: The Pharmacological Basis