STUDIES ON AGING OF THE BRAIN

Ishan Patro and Nisha Patro

Introduction

The human brain makes us different from the rest of the animals on the earth. Brain aging in terms of neurodegeneration and associated disorders contributes the most towards the functional decline that appears with age. In this article, an effort has been made to appraise the important contributions of Indian neuroscientists towards the understanding of ageing changes in the brain.

Chromatin structure, gene expression and regulation

Kanungo and his colleagues have been carrying out research since 1965 on biochemical and molecular changes that occur in the brain of rats and mice during physiological aging. In particular, their studies have focused on the changes in enzymes, isoenzymes and proteins, their induction, structure of chromatin and covalent modifications of chromosomal proteins, and receptors for neurotransmitters and steroid hormones as a function of age. They have shown that the M4 isoenzyme of lactic dehydrogenase (LDH) decreases with age in the brain making the tissue more oxygen dependent. The levels of cholineacetyltransferase (CAT) and acetylcholinesterase (AChE) decrease in the old. Both CAT and AChE can be induced by administration of the steroid hormone, 17b-estradiol, to old rats. This finding was supported by the discovery of estradiol receptor in the brain [Kanungo et al, 1975]. The level of the receptor is significantly lower in the old [Asaithambi et al, 1997]. Furthermore, they found that AChE had a circadian rhythm. In the immature, it was high in the night and low in the day. In the adult, it was low in the day but high in the night when the rat remained active.

Since one of the causes of the decline in enzyme levels in the brain in old age could be the increasing condensation of the chromatin that houses the genes, they digested the chromatin of immature, adult and old rats by DNase I and resolved the DNA fragments by gel electrophoresis. DNase I cuts the double stranded DNA at 10bp intervals. Significantly, less 10bp DNA fragments are produced in old rats. This established that the DNA in the chromatin gets compacted with histones in old age, and represses transcription. This was further supported by the finding that when the chromatin of the brain of young, adult and old rats was incubated with labeled acetate, methionine and phosphate separately to covalently modify the histones, the degree of acetylation, methylation and phosphorylation, respectively of histones was far lower in the old. They further found that 17b-estradiol stimulated histone acetylation and transcription of RNA [Kanungo and Thakur, 1979].

Thakur and his colleagues have made significant contributions on chromatin structure, gene expression and aging of the brain of mice and Alzheimer's disease. They reported for the first time that acetylation of histones in chromatin of the brain

is lower in old, which is responsible for reduced expression of genes [Thakur, et al, 1978]. They showed that the expression of two forms of estrogen receptor (ER) a and ERâ is differentially influenced by age, sex and gonadal steroids in the cerebral cortex of the mouse, suggesting differences in ER-mediated functions [Thakur et al, 2005; Sharma and Thakur, 2006]. The level of androgen receptor (AR) decreases, but phosphorylation of AR increases with advancing age [Thakur et al, 2000]. The expression of AR gene and methylation of its promoter are inversely regulated by testosterone and estradiol in adult mice [Kumar and Thakur, 2004a]. Also the sDnase-I accessibility of AR promoter is reduced by sex steroids in the brain of adult male mice. Such changes contribute to alterations in steroid-mediated brain functions [Kumar and Thakur, 2004b].

Another important finding is that the expression of different isoforms of amyloid precursor protein (APP), which is known to be involved in Alzheimer's disease, is down regulated by estrogen in old mice [Thakur and Mani, 2005]. In the cerebral cortex of female and male mice, APP promoter methylation is higher in females and differentially regulated by sex steroids [Mani and Thakur, 2006]. Also, the expression of norbin, a neurite-outgrowth-related brain protein involved in memory, decreases in old female mice [Mani et al, 2001]. As the brain cortex is involved in cognitive functions which deteriorate with age, these findings provide an insight into the changes that occur in the chromatin, steroid receptor, and genes implicated in Alzheimer's disease during aging of the brain, and the role of steroid hormones in this process.

The neurotransmitter dopamine is important for neurotransmission, and the impairment of its availability due to loss of substantia nigra neurons causes Parkinson's disease (PD). Using RT-PCR and western blot techniques, they have recently shown that the level of D2 dopamine receptor is significantly lower in normal old rats. This may be the reason for the increasing occurrence of the symptoms of PD even in normal old people who do not have genetic disorders that cause PD.

DNA damage and repair

Subba Rao and his group have made significant contributions to the understanding of the role of DNA damage and repair in the aging brain. They recently used post mitotic rat brain neurons as a model system to test the hypothesis that the inherited DNA repair potential would have profound influence on the aging process of the individual. Both single and double strand breaks in DNA accumulate in neurons with age. There are 7,400 single strand breaks in the genomic DNA of an old neuron as compared with 3000 in young neuron. The number of double strand breaks also increases four-fold in the old as compared to young. Because the Base Excision Repair (BER) pathway affects the repair of neuronal DNA damage (eg. depurination/ depyrimidination, deamination, base damage, oxidative damage), model oligo duplexes were used to assess the BER pathway.

Since pol b is an important component of BER machinery, a systematic study to assess the levels of this enzyme in isolated neuronal and glial cells from cerebral cortex of young, adult and old rat brain was undertaken. These studies revealed that with age there is considerable reduction in this polymerase activity in the neuronal cells. In glial cells also, the decrease with age was statistically significant [Raji et al, 2002]. These results support the earlier observations that in the whole rat brain both the content as well as the "catalytically active" molecules of DNA polymerase â decrease with age [Rao et al, 1994; Rao et al, 2000]. In the same study, using various relatively specific inhibitors for different DNA polymerases, it was also shown that the predominant DNA polymerase in neurons is pol â. This is in line with the earlier observations.

Both extension of a primer as well as 1 or 4 nucleotide gap repair are markedly reduced in aging neurons as compared to young. The extension activity could be restored by supplementing the neuronal extracts with pure DNA pol â, while the restoration of gap repair needed the addition of both pol â and DNA ligase. It thus appears that both pol â and DNA ligase are deficient in aging neurons. The extension activity was measured by subjecting the products to sequencing polyacrylamide gel electrophoresis. While the neuronal extracts of all ages were able to degrade the primer to shorter lengths, only feeble extension of the primer to the longer lengths was seen with 'young' extracts. In the case of 'adult' and 'old' extracts, extension was almost undetectable. It was possible to establish conditions to restore this lost primer extension activity even in neuronal extracts from "old" brain [Rao, et al, 2000]. The strategy consisted of supplementing the neuronal extracts with pure rat liver pol b after the mismatched base at the 3'end of the primer is removed in a pre-incubation period. During the extension, magnesium is replaced by manganese. During this pre-incubation period, the mismatched base at the 3'-end of the primer is removed by a 3'-5' exonuclease activity present in the brain extracts [Krishna et al, 2004; Krishna et al, 2005].

SubbaRao's group has also established a system to study the non homologous end joining (NHEJ) mode of DNA repair in neurons. The end joining of cohesive but not of blunt or non-matching ends, is reduced with age [Rao, 2003; Vyjayanti and Rao, 2006].

Neurotransmitters, diabetes and brain aging

Paulose & Co-workers have reported an age-associated susceptibility to diabetes and impairment of glucose tolerance. They have shown [Padayatti and Paulose, 1999] that there is differential regulation of noradrenergic receptor function in the pancreatic islets of streptozotocin (STZ)-diabetic rats as a function of age.

Also, the effects of ageing on NE, cAMP content and noradrenergic receptors in the brain stem of young and old STZ-diabetic rats was demonstrated. Together with changes occurring in the brain stem during ageing could lead to the development of impaired pancreatic insulin function [Jackson and Paulose, 1999].

They compared kinetic parameters of brain glutamate dehydrogenase (GDH) in the brainstem, cerebellum and cerebral cortex of three weeks and one year old Streptozotocin induced four day diabetic rats with respective controls, and suggested there may be an important regulatory role of the glutamate pathway in brain neural network disturbances and neuronal degeneration in diabetes as a function of age [Biju and Paulose, 1998].

Age-related alterations in neuronal electrical activity

Rameshwar Singh and colleagues have provided data on age-related alterations in neuronal electrical activity. They were investigating electroencephalographic and multiple-unit activity in brain regions of the ageing brain [Sharma et al, 1993; Sharma and Singh, 1996; Kaur et al, 1998; Roy and Singh, 1988; Kaur et al, 2001; Kaur et al, 2003a; Kaur et al, 2003b; Singh and Sharma, 2005]. These studies showed that spontaneous basal electrical activity declined in several brain regions as a consequence of the process of ageing. To elucidate the biochemical basis underlying the age-related electrical activity changes, relationships between ageing-related electrical activity decline and a number of age-related biochemical parameters (Na^+,K^+-ATPase, lipid peroxidation, multiple unit action potentials, antioxidative enzymes, lipofuscin etc) were investigated [Sharma et al, 1993; Sharma and Singh, 1996; Roy and Singh, 1988; Kaur et al 1998; Singh et al, 2006]. The data derived from these studies provided significant information concerning the biochemical basis of electrical activity decline.

A series of studies were done to investigate the antiageing action of some drugs and chemical compounds thought to be candidate antiageing pharmacological agents [Kaur et al, 2001; Kaur et al, 2003a; Kaur et al, 2003b; Sinha and Sharma, 2005]. A particular emphasis has been laid on the effects of these drugs on the electrical activity of the ageing brain [Singh and Sharma, 2005]. Compounds such as centrophenoxine, L-deprenyl, Acetyl-L-carnitine, Diethylhydroxyamine that have antioxidative effects were found to reverse age-related decline in electrical activity [Sharma et al, 1993; Kaur et al, 2001; Kaur et al, 2003a; Kaur et al, 2003b]. Antipsychotic drug chlorpromazine was also found to counter some ageing-related parameters [Roy et al, 1984; Gopal et al, 2000]. Studies have also focused on mechanisms by which environmental chemicals such as aluminium are likely to enhance the ageing process [Kaur et al, 2003a]. Sharma and colleagues have reported on the anti-aging effects of herbal drugs [Jyoti and Sharma, 2006; Bala et al, 2006]. The aluminium-induced accelerated ageing was found to be reversed by *Bacopa monniera* [Jyoti and Sharma, 2006]. Compounds of herbal origin were, thus, found to have significant anti-ageing pharmacological effects: Curcumin (derived from turmeric; [Bala et al, 2006]); *Bacopa monniera* (derived from Brahmi; [Jyoti and Sharma, 2006]). Baquer and colleagues have reported the effects of hormone replacement therapy in ageing rat brain and have provided significant data related to the beneficial affects of estrogen and estradiol in normalizing age-related neuronal markers like lipofuscin, malondialdehyde and acetylcholine esterase activity in

different age groups of naturally menopausal rats [Moorthy et al, 2005a]. Co-administration of estrogen and estradiol was found to be more effective in beneficially modulating the activities of antioxidant enzymes [Moorthy et al, 2005b] and carbohydrate metabolizing enzymes, and the status of lipid profile [Moorthy et al, 2004] in naturally menopausal rats from different age groups.

Aging of neural transplants

Tandon, Gopinath and associates (All India Institute of Medical Sciences, New Delhi) studied the behaviour of fetal substantia nigra of 12th gestation day transplanted into the anterior eye chamber completely isolated from the host neural tissue [Shetty et al, 1991a], lateral ventricle [Shetty et al, 1991b] partially accessible to the host brain tissue, and into the intact and lesioned striatum [Gopinath et al, 1996] with easy access to host tissue. Neonatal rats were also used as hosts for transplantation into the intact striatum (Sable et al, 1997).

In the anterior eye chamber (by the end of 30 days) the transplant achieved a maximum size filling up the chamber. Thereafter, the size remained unchanged, however by the end of one year the tissue became very thin. Striatal transplants in the adult were comparatively small in size, whereas in neonates, transplants were often seen to occupy large area pushing the host striatum towards the periphery obliterating the ventricle.

Neurons in all the transplants showed mature phenotypical characteristics of adult nigral neurons by one month. Synapses developed in all the transplants. In the anterior eye chamber, in addition, juxtaposed double neurons were frequent. Neuronal surface, particularly of large neurons appeared ruffled with irregular thickening. Neuronal processes when compared to fibres in the host striatum appeared thicker. Processes could be traced to the adjacent host tissue both from striatal and ventricular transplants. Tyrosine hydroxylase positive neurons were also seen in the host tissue close to the transplants. Neurons and their processes transplanted in neonates appeared normal.

By 6 months many of the large neurons (in the transplant) started accumulating lysosomes and the density of organelles started declining. Empty areas devoid of organelles were seen in the neuronal cytoplasm. Such changes were also observed in the synapses inside the transplants. Towards the end of one year polylysosomes started accumulating in the neurons. Simultaneously glial population increased and reactive astroglia were also seen. Gomori-positive glia encountered in ageing brain were also present. These changes were dramatic by the end of two years of transplantation with only a few neurons left in the transplant. These changes were noted earliest in the anterior chamber followed by in the ventricle and intact striatum. Changes were very slow to appear in neonates and only a few neurons were affected by the end of one year the neonatal transplants.

All the changes observed in the transplants including the accumulation of lipofuscin are also seen to occur in the ageing brains. It is not clear if there is neuronal loss as age advances due to heavy accumulation of the ageing pigment. Such changes appearing in the transplants first in the eye indicate that synaptic connections among the neurons in the transplant are unable to maintain normal neuronal activity. Even with some of the contacts established between the transplanted neurons in the ventricle and striatum and host striatum, ageing changes appear, showing thereby that contacts are not adequate or proper for maintaining the transplanted neurons (Gopinath et al, 1991).

Transplanted neurons in the neonates might have more target sites available on the striatal neurons, and hence the changes are not as obvious as observed in the adult hosts. Also the neonatal brain appears to be providing an environment conducive for proper connections between the transplanted and host neurons, unlike in the adult brain.

Oxidative stress, lipid peroxidation and antioxidants

Alteration in lipid-peroxidation products such as lipid peroxides, and lipid hydroperoxides with age, and under the influence of antioxidants and neuroprotective agents have been studied by several laboratories. Studies indicate that regional differences in the lipid peroxidation- induced damage in brain occurs, and accumulation of lipid peroxides and lipid peroxidation end products like the age-pigment takes place with aging but the susceptibility to lipid peroxidation decreases at old age along with a decline in antioxidative defense of the brain itself (Gupta & Hasan, 1988; Gupta et al, 1991; Sahoo & Chainy, 1997; Vohra et al, 2001). Drugs like chloropromazine, phenytoin, citiolone, centrophenoxine (Singhal, 1996), L-carnitine (Rani & Panneerselvam, 2002) grape seed extract (Balu et al, 2005), Maharshi Amrit Kalash (Vohra et al, 1999), acetylhomocystine thiolactone (Naqvi & Hasan, 1992) prevented formation of thiobarbiturate reactive substances and resulted in successful rejuvenation of the antioxidative enzyme system.

Studies on brain aging in rats have indicated linear decreases in glucose content in regions such as the cerebral cortex and medulla oblongata. Data obtained from studies on swim training of young, middle-aged and old rats show an age-related biochemical response in the cerebral cortex (Anitha & Asha-Devi, 1997). Data obtained have also indicated that the detoxification mechanisms decline with age and that vitamin E is effective in retarding the storage of lipid peroxidation products such as lipofuscin (LF) *in vitro* (Kan et al, 1991). However, further studies showed that although LF accumulates in the primary cortical cultures, there is no interference with the activity of certain key enzymes like creatinine phosphokinase (Asha-Devi et al, 1998). An age-related deficit in antioxidant enzymes was demonstrated in the cerebral cortex and hippocampus. This deficit could be overcome through supplementation of vitamin E with swim training, thus suggesting a rationale for looking at these markers of oxidative stress in several age-associated

neuronal diseases (Asha Devi & Ravi Kiran, 2004). Studies had doses effective in up-regulating the antioxidant defense mechanisms by attenuating both lipid peroxidation and protein oxidation. The changes observed in the cholinergic system, however, indicated an increase in the acetylcholine concentration with a moderate reduction in acetylcholine esterase activity, suggesting further that PA may have a potential role in enhancing cognition in older rats (Asha Devi et al, 2006). Based on the studies in animal models it can be suggested that aerobic exercise training when initiated at an early age and continued for rest of the life may prevent dementia, improve mental faculties and cognitive ability in the old age.

Currently, all these laboratories are focusing on questions pertaining to the applicability of various antioxidants for overcoming the age-related oxidative stress and neuronal death. These studies are also of great interest and provide important information on antioxidant intervention during aging of the brain.

Age-pigment and brain aging

The age-pigment lipofuscin accumulates in neurons and other post-mitotic cells, intrinsically and progressively, with age and in disease conditions. The deleterious effects of lipofuscin have been frequently emphasized. The chemical nature of its accumulation during spontaneous deposition and under experimental conditions were characterized in situ by Patro and associates and proposed the concept of 'pre-lipofuscin' (Patro et al, 1987a) and classified the pigment as 'pre-lipofucin', 'immature lipofuscin' and 'mature lipofuscin' (Patro et al, 1989).

With this 'unified hypothesis' of lipofuscin formation in mind Patro and associates screened several experimental conditions like crowding and restrain stress (Patro et al., 1987b; Chaudhary et al., 1995), heavy metals (Patro et al., 1992), lysosomal enzyme inhibitors (Sharma et al., 1987a) and nutritional status of the animals (Sharma et al., 1987b) for developing suitable models for age-pigment research. Most of these experimental conditions triggered accelerated lipofuscin accumulation, which could be reversed by lipofuscinolytic agents.

In the senile brain, age-pigment accumulation was one of the most prominent changes recorded in most areas of the brain. Getting the lead from Hasan and associates (Glees & Hasan, 1976), Sharma and associates tried several neuroprotectants for their possible influence on the age-pigment accumulation (Patro & Sharma, 1984; Patro et al, 1988; Patro et al, 1991). Initially centrophenoxine was the drug of choice and subsequently Patro and associates made a comparative study of 4 drugs known for their antioxidative and / or neuroprotective effects and found their lipofuscinolytic efficiency (after a 3-month treatment) in the order: encephabol > centrophenoxine > chlorpromazine > dimethyl-aminoethanol (Patro, 1990; Patro et al, 1991; Patro et al, 2002). Roy et al, 1983 provided information about the mechanism by which centrophenoxine might inhibit the formation of lipofuscin. They showed biochemically that the drug elevated the activity of

antioxidant enzymes and lowered the levels of lipid peroxidaion in the brain regions.

Our earlier studies and reports from other laboratories have indicated a possible involvement of lipofuscin in the age-associated deterioration of the CNS and loss of neurons (Patro & Patro, 1992). In a study the possible increased survival of neurons subsequent to removal of lipofuscin was explored in 3 groups of Swiss mice treated with citiolone (8 mg/ Kg/ day, i.p.), centrophenoxine (100 mg/ Kg/ day, i.p) and phenytoin (8 mg/ Kg/ day, i.p for 7 days, followed by 40 mg/ Kg/ day, i.p in divided doses of 10 hrs interval) separately for 1-3 months, at an age when lipofuscin accumulation is faster in the CNS. A combination method for studying lipofuscin and cytological changes (Patro et al, 1996) was employed along with standard estimations of TBA reactive substances and protein carbonyl content in the brain. Total neuron count in various nuclei in the brainstem and spinal cord revealed a 10-32 % neuron loss. With 40-59 % lipofuscin removal at adult-hood, the neuron loss at senility was as low as 6- 22%. This supported the proposition extended by Patro and associates that removal and/or restriction of lipofuscin accumulation can help in increased survival of a significant population of neurons in senile brain (Patro et al, 1994; Patro, 2000; Patro et al, 2002).

Microglia in neurodegeneration

Patro and Patro (Patro et al, 2005; Patro & Patro, 2004) developed a model in which a double stranded RNA (Poly I:C) infusion into the brain resulted in microglial activation as well as release of cytokines and inflammatory antigens which induced the apoptotic death of neurons and astrocytes. Poly I:C does not cause neuronal damage. They also proposed a “Dual autotoxic loop hypothesis” for the role of microglia and astrocytes in neurodegeneration similar to the age-associated neurodegenerative disorders. This model is being further investigated to induce degeneration of neurons and for studying the changes in the neuronal stem cells and in the genesis of cellular components in the hippocampus. In the peripheral (sciatic) nerve injury model we observed that suppression of inflammatory response in microglia following injury with the help of FK506, an immunosuppressant immunofilin ligand, helped neuroprotection as well as significantly improved motor coordination (Saxena et al, 2007; Patro et al, 2008). FK506 however did not influence microglial proliferation.

The other important aspect under investigation has been the role of microglia in ageing brain and the aging of microglia per se. In the senile brain, a great heterogeneity in the morphology of glial cells has been recorded. Microglia in the senile brain display classical ramified morphology as well as hypertrophy (with enlarged and swollen cytoplasmic processes similar to activated microglia). Some microglia simply look abnormal and dystrophic. Such dystrophic microglia are scattered and present an unhealthy look. It is even difficult at times to distinguish

between hypertrophied and activated microglia (Patro, unpublished data). More such aspects are under active investigation.

References

Anitha V, Asha-Devi S: Responses of aging brain to physical exercise: changes in the substrates of energy metabolism. Ind J Exp Biology. (1997) 35, 280-285.

Asaithambi A, Mukherjee S, Thakur MK: Expression of 112 kDa estrogen receptor in mouse brain cortex and its autoregulation with age. Biochem Biophys Res Commun. (1997) 231, 683- 685.

Asha Devi S, Kan S, Kawashima S: Studies on creatine phosphor-kinase in primary culture of dissociated cerebral cortical cells. Zoo Sci. (1998) 5, 1284.

Asha Devi S, Ravi Kiran T: Aging Rat Brain: Responses of antioxidant enzymes of the cerebral cortex and hippocampus to exercise and vitamin E. Neurobiol of Aging. (2004) 25, 501-508.

Asha Devi S, Jolitha AB, Ishii N: Grape seed proantho-cyanidin extract (GSPE) and antioxidant defense in the brain if adult rats. Med Sci Monitor. (2006) 12, 124-129.

Bala K, Tripathy BC, Sharma D: Neuroprotective and antiageing effects of curcumin in aged rat brain regions. Biogerontology. (2006) 7, 81-89.

Balu M, Sangeetha P, Haripria D, Panneerselvam C: Rejuvination of antioxidant system in central nervous system of aged rats by grape seed extract. Neurosci Lett. (2005) 383, 295-300.

Biju MP, Paulose CS: Brain glutamate dehydrogenase changes in streptozotocin diabetic rats as a function of age. Biochemistry and Molecular Biology International. (1998) 44, 1-7.

Chaudhary A, Sharma SP, James TJ: Lipofuscin accumulation in neurons with restraint stress. Gerontology. (1995) 41, 229-237.

Glees P, Hasan M: Lipofuscin in neuronal aging and diseases. Norm. Pathol. Anat. (Stuttg). (1976) 32, 1-68.

Gopal V, Sriram AV, Sharma D, Singh R: Glutathione-s-transferase in the ageing rat brain cerebrum and the effect of Chlorpromazine. Gerontology. (2000) 46, 7-11.

Gopinath G, Shetty AK, Tandon PN: Ageing changes in the transplants of fetal substantia nigra grafted to striatum of adult rat. Neuroscience. (1991) 40, 429-443.

Gopinath G, Sailaja K, Tandon PN: Long-term nigral transplants in rat striatum: an electron microscopic study. Int. J. Dev Neuroscience. (1996) 14, 453-460.

Gupta A, Hasan M: Age-related changes in lipid profiles and lipid peroxidation in CNS following restraint stress. Indian J Med Res. (1988) 87, 624-630.

Gupta A, Hasan M, Chandar R, Kapoor NK: Age-related elevation of lipid peroxidation products: diminution of superoxide dismutase activity in the central nervous system of rats. Gerontology. (1991) 37, 305-309.

Jackson J, Paulose CS: Enhancement of (m-methoxy 3H) MDL100907 binding to 5HT2A receptors in cerebral cortex and brainstem of streptozotocin induced diabetic rats. Mol.Cell Biochem. (1999) 199, 81-85.

Jyoti A, Sharma D: Neuroprotective role of *Bacopa monniera* extract against aluminum-induced oxidative stress in the hippocampus of rat brain. Neurotoxicology. (2006) 27, 451-457.

Kan S, Asha-Devi S, Kawashima S: Effect of vitamin E on the accumulation of fluorescent material in cultured cerebral cortical cells of mice. Exp Gerontol. (1991) 26, 365-374.

Kanungo MS, Patnaik SK, Koul O: Decrease in the level of 17b-estradiol receptor in the brain of rats during aging. Nature. (1975) 253, 366-367.

Kanungo MS, Thakur MK: Effects of oestradiol on covalent modifications of chromosomal proteins and transcription of chromatin of the brain of rats of various ages. J Steroid Biochem. (1979) 1, 879-888.

Kaur J, Sharma D, Singh R: Regional effects of ageing on Na+, K(+) - ATPase activity in rat brain and correlation with multiple unit action potentials and lipid peroxidation. Indian J Biochem Biophy. (1998) 35, 364-371.

Kaur J, Sharma D, Singh R: Acetyl-L-carnitine enhances Na+, K+-ATPase, glutathione- S- transferase and multiple unit activity, and reduces lipid peroxidation and lipofuscin concentration in aged rat brain regions. Neurosci Letters. (2001) 301, 1-4.

Kaur J, Sharma D, Singh S, Singh R: Aluminum-induced enhancement of Ageing-related parameters in rat brain regions. Indian J Biochem. Biophys. (2003a) 40, 330-339.

Kaur J, Sharma D, Singh S, Singh R: Neurostimulatory and antioxidative effects of L- deprenyl in the aging rat brain. Biogerontology. (2003b) 4, 105-111.

Krishna TH, Nemkal A, Rao KS: The presence of 3'-5' exonuclease activity in rat brain neurons and its role in template-driven extension of 3'-mismatched primers by DNA-polymerase â in aging neurons. Neurochem Res. (2004) 29, 761-770.

Krishna TH, Mahipal S, Sudhakar A, Sugimoto H, Kalluri R, Rao, KS: Reduced DNA gap repair in aging rat neuronal extracts and its restoration by DNA polymerase â and DNA-ligase. Journal of Neurochemistry. (2005) 92, 818-823.

Kumar RC, Thakur MK: Androgen receptor mRNA expression is inversely regulated by testosterone and estradiol in adult mouse brain. Neurobiol Aging. (2004a) 25, 925-933.

Kumar RC, Thakur MK: Sex steroids reduce DNase I accessibility of androgen receptor promoter in adult male mice brain. Mol Brain Res. (2004b) 131, 1-7.

Mani ST, Kumar RC, Thakur MK: Age and sex-specific expression of norbin in the mouse brain cortex. Neurosci Lett. (2001) 308, 57-59.

Mani ST, Thakur MK: In the cerebral cortex of female and male mice, amyloid precursor protein (APP) promoter methylation is higher in females and differentially regulated by sex steroids. Brain Res. (2006) 1067, 43-47.

Moorthy K, Umesh CS Yadav, Mantha AK, Cowsik SM, Sharma D, Basir SF, Baquer NZ: Effect of Estradiol and Progesterone treatment on lipid profile in naturally menopausal rats from different age groups. Biogerontology. (2004) 5, 411-419.

Moorthy K, Umesh CS, Yadav, Siddiqui MR, Mantha AK, Basir SF, Sharma D, Cowsik SM, Baquer NZ: Effect of hormone replacement therapy in normalizing age-related neuronal markers in different age groups of naturally menopausal rats. Biogerontology. (2005a) 6, 345-356.

Moorthy K, Sharma D, Basir SF, Baquer NZ: Administration of estradiol and progesterone modulate the activities of antioxidant enzyme and aminotransferases in naturally menopausal rats. Exp Gerontol. (2005b) 40, 295-302.

Naqvi SM, Hasan M: Acetylhomocysteine thiolactone protects against phosphamidon-induced alteration of regional superoxide dismutase activity in the central nervous system and its correlation with alter lipid peroxidation. Indian J Exp Biol. (1992) 30, 850-852.

Padayatti PS, Paulose, CS: Alpha 2 Adrenergic and high affinity serotonergic receptor changes in the brainstem to streptozotocin-induced diabetic rats. Life Sci. (1999) 65, 403-414.

Patro IK, Sharma SP: Cytochemical interaction of nucleus and cytoplasm in the Purkinje cells of senile white rats under the influence of centrophenoxine. Expt Gerontol. (1984) 19, 241-252.

Patro IK, Sharma SP, Patro Nisha: Pre-lipofuscin: The concept and influence of centrophenoxine. Proc Nat Acad Sci (India). (1987a) 57, 105-108.

Patro IK, Sharma SP, Patro Nisha: Influence of crowding stress on neuronal aging. Age. (1987b) 10, 114.

Patro IK, Sharma SP, Patro Nisha: Neuronal lipofuscin: Its formation and reversibility. Indian Rev Life Sci. (1988) 8, 95-120.

Patro IK, Sharma SP, Patro Nisha: Formation and maturation of neuronal lipofuscin. Proc Nat Acad Sci (India). (1989) 59, 287-293.

Patro IK: Lipofuscinolysis by four neurotropic agents: A comparative study. In: Singh R, Singhal GS, (eds.) Perspective in Aging Research. (1990) 133-136.

Patro IK, Patro Nisha, Sharma SP, Chaudhary Asha: Lipofuscinolysis: An overview. Advances in Physiological Sciences, Manchanda SK, Selvamurthy W, Mohankumar V, (eds.) Macmillan (India) Co., New Delhi, 441-450. (1991).

Patro IK, Patro Nisha: Lipofuscin in aging brain- A selective reappraisal. Indian Rev Life sci. (1992) 12, 133-146.

Patro Nisha, Patro IK, Mathur R: Age-pigments as possible markers of environmental damage; $CoCl_2$ induced lipofuscin accumulation in myocardium. Geobios. (1992) 19, 231-234.

Patro IK, Bhardwaj SK, Patro Nisha, Singhal P: Relationship between lifespan potential, specific metabolic rate and lipofuscin accumulation in neurons. J. Neurochem. (1994) 63, 10.

Patro IK, Patro Nisha, Singhal P, Bhardwaj SK: Simultaneous demonstration of microscopic anatomy, Nissl, lipofuscin and cytological changes using a combination method and fluorescence technique. Acta Histochem Cytochem. (1996) 29, 193-197.

Patro IK: Experimental removal of lipofuscin restricts neuron loss in senile brain. Annals Neurosci. (2000) 7, 5.

Patro Nisha, Gupta Praveena, Patro IK: Lipofuscin in cell aging and cell death. Indian J Gerontol. (2002) 16, 45-68.

Patro Nisha, Patro IK: Effects of immune activator (Poly I:C) on the rat cerebral cortex. J Tissue Res. (2004) 3, 71-75.

Patro IK, Pathak Seema, Patro Nisha: Central response to peripheral nerve injury: Role of non-neuronal cells. In: Thakur MK, Prasad S. (eds.) Molecular and Cellular Neurobiology, Narosa Publishing House, New Delhi. (2005).

Patro, I., Saxena, K., Tiwari, S. Patro, N: FK506 helps motor coordination recovery following sciatic nerve transection in the young but not in the senile rats Int. J. Neuroprotec. & Neuroregeneration (In press). (2008).

Raji NS, Harikrishna T, Rao KS: DNA-polymerase a,b,d and e activities in isolated neuronal and astroglial cell fractions from developing and aging rat cerebral cortex. Int J Dev Neurosci. (2002) 20, 491-496.

Rani PJ, Panneerselvam C: Effect of L-carnitine on brain lipid peroxidation and antioxidant enzymes in old rats. J Gerontol. A Biol Sci Med Sci. (2002) 57, 134-137.

Rao KS, Vinay Kumar, D, Bhaskar, MS, Sripad, G: On the 'active' molecules of DNA-polymerase b in aging rat brain. Biochem Mol Biol Intl. (1994) 34, 287-294.

Rao KS, Annapurna V, Raji NS, Harikrishna T: Loss of base excision repair and its restoration by DNA Polymerase b in aging rat neurons. Mol Brain Res. (2000) 85, 251-259.

Rao KS: DNA-Repair and Brain Aging: The importance of Base Excision Repair and DNA polymerase â. Proc Indian Natn Sci Acad. (2003) 69, 141-156.

Roy D., Pathak D.N., Singh R: Effect of centrophenoxine on the antioxidative enzymes in various regions of the aging rat brain. Exp. Gerontol. (1983) 18, 185-197.

Roy D, Pathak D, Singh R: Effect of chlorpromazine on the activities of antioxidant enzymes and lipid peroxidation in the various regions of ageing rat brain. J Neurochem. (1984) 42, 628-633.

Roy D, Singh R: Age related changes in multiple unit activity of the rat brain parietal cortex and the effect of centrophenoxine. Exp. Gerontol. (1988) 23, 161-174.

Sable Vandana, Sailaja K, Gopinath G, Tandon PN: Fetal dopaminergic neurons transplanted to the normal striatum of neonatal or adult rats and to the denervated striatum of adult rats. J Neural Transplant and Plast. (1997) 6, 73-81.

Sahoo A, Chainy GB: Alterations in the activities of cerebral antioxidant enzymes of rat are related to aging. Int J Dev Neuroscience. (1997) 15, 939-948.

Saxena K., Patro N., Patro I: FK506 protects neurons following peripheral nerve injury via immunosuppression. Cell Mol Neurobiol. (2007) 27, 1049-1057.

Sharma SP, Patro IK, Patro Nisha: Removal of chloroquine induced lipofuscin by centrophenoxine. Age. (1987a) 10, 123.

Sharma SP, Gupta SK, Patro IK: Influence of centrophenoxine on the anterior horn cells of protein malnourished Wistar rats. Proc Nat Acad Sci (India). (1987b) 57, 247-249.

Sharma D, Maurya A, Singh R: Age-related decline in multiple unit action potentials of CA3 region of rat hippocampus: correlation with lipid peroxidation and lipofuscin concentration and the effect of centrophenoxine. Neurobiol Aging. (1993) 14, 319-330.

Sharma D, Singh R: Age-related decline in multiple unit action potentials of cerebral cortex correlates with the number of lipofuscin-containing neurons. Indian J Exp Biol. (1996) 34, 776-781.

Sharma PK, Thakur MK: Expression of estrogen receptor (ER) á and â in the mouse cerebral cortex: effect of age, sex and gonadal steroids. Neurobiol Aging. (2006) 27, 880-887.

Shetty AK, Banerjee Ranjita, Gopinath G, Tandon PN: Fetal nigral transplants in the anterior chamber of the adult rats: A long-term morphological study. Exp Neurol. (1991a) 111, 106-114.

Shetty A K, Gopinath G, Tandon PN: Fetal nigral transplants in the lateral ventricle of adult rats: A long-term morphological study. Dev Neuroscience. (1991b) 13, 1-10.

Singh R, Sharma D: Electrophysiological ageing of the brain and pharmacology of ageing. In: Thakur MK, Prasad Shukla (eds) Molecular and Cellular Neurobiology. Narosa Publishing House New Delhi, India. (2005) 135-151.

Singh R, Sharma D, Singh S, Kaur J: Biochemical correlates of electrophysiological ageing of the brain. Pain Updated: Mechanism and Effects Mathur R (ed.) Anamaya Publishers, New Delhi, India. (2006) 116-124.

Singhal P: Formation and removal of lipofuscin and its role in brainstem of aging mice. Ph.D. Thesis, Jiwaji University, Gwalior. (1996).

Sinha N, Sharma D: Antioxidative effects of exogenous administration of Dehydroepiendrosterone (DHEA) in the ageing rat brain regions. Indian J Exp Biol. (2005) 43, 420-424.

Thakur MK, Das R, Kanungo MS: Modulation of acetylation of chromosomal proteins of the brain of rats of various ages by epinephrine and estradiol. Biochem Biophys Res Commun. (1978) 81, 828-831.

Thakur MK, Asaithambi A, Mukherjee S: Synthesis and phosphorylation of androgen receptor of mouse brain cortex and their regulation by sex steroids during aging. Mol. Cell Biochem. (2000) 23, 95-101.

Thakur MK, Mani ST: Estradiol regulates APP mRNA alternative splicing in the mice brain cortex. Neurosci Lett. (2005) 381, 154-157.

Thakur MK, Sharma PK, Ghosh S: Estrogen effects in the brain: implications for aging and Alzheimer's disease. In: MK Thakur and S Prasad (eds), Molecular and Cellular Neurobiology. Narosa Publishing House Pvt. Ltd., New Delhi. (2005) 55-69.

Vohra BP, Sharma SP, Kansal VK: Maharshi Amrit Kalash rejuvenates aging central nervous system's antioxidant defense system: an in vivo study. Pharmacol Res. (1999) 40, 497-502.

Vohra BP, Sharma SP, Kansal VK: Age-dependent variations in mitochondrial and cytosolic antioxidant enzymes and lipid peroxidation in different regions of central nervous system of guinea pigs. Indian J Biochem Biophys. (2001) 38, 321-326.

Vyjayanti VN, Rao KS: DNA double strand break repair in brain: Reduced NHEJ activity in aging rat neurons. Neuroscience Letters. (2006) 393, 18-22.