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ANS

Annals of Neurosciences

Official Journal of Indian Academy of Neurosciences

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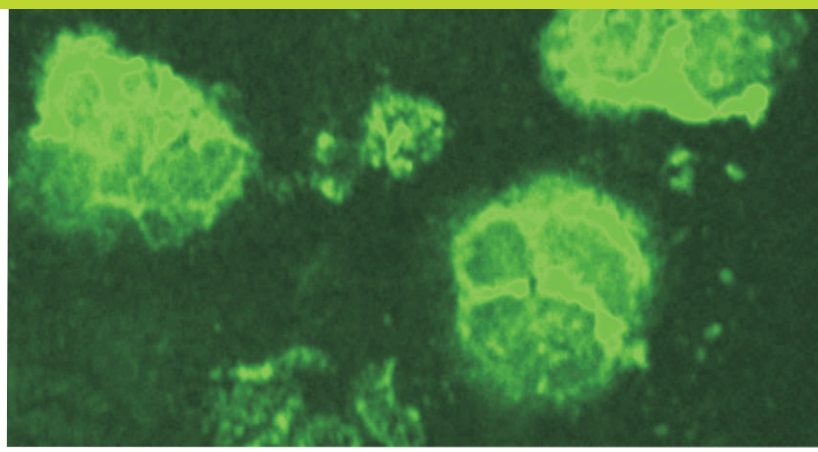

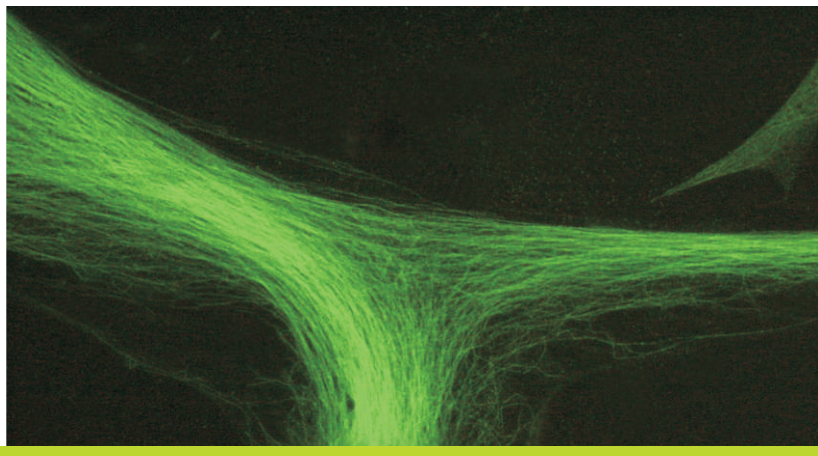

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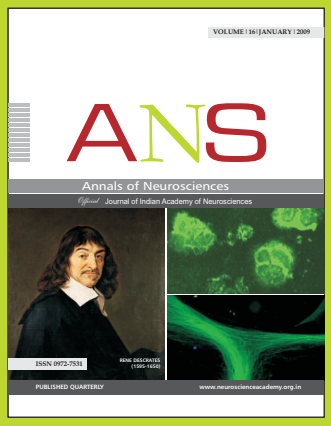
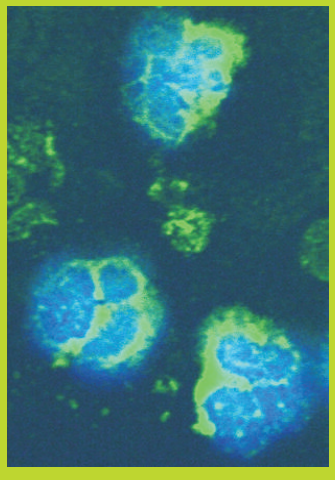
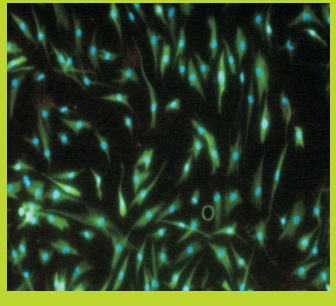


On Page 17, ANS reprints Rene Descartes' Classic manuscript published more than 350 years ago from the famous *Discourse on Method* *(with special permission)*



On Page 40, Parkin Immunostaning as seen under 40X



On Page 40, Neurons stained with Nestin/DAPI

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Case Report

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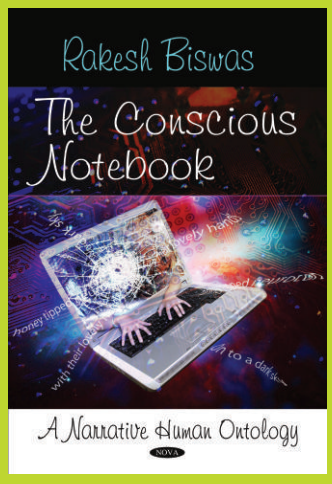
Molecular Shots

People and Views



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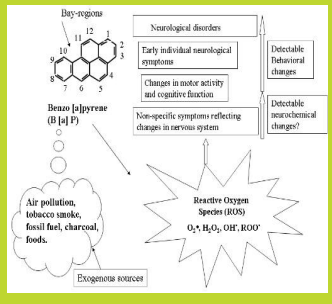
Book Review



On Page 25, Potential neurotoxicity of Banzo a pyrene

Editorial

Journal needs aggressive policy

I am honored to take over the Editorship of *Annals of Neurosciences (ANS)* for which I thank the Academy. I have held hectic consultations in order to implement ambitious plans that will enhance its quality, readership and to create a new model for communicating research results, commentaries and reviews. This will be done by expanding its sections such that there is multidimensional flow of scientific information. India, until recently, was riding a wave of economic prosperity and suddenly the scientific output has jumped to a new level never seen before. This could be partly due to enhanced R&D outlay by Indian govt and partly due to the returns from Biotechnology ventures. However, the growth of Indian journals or their citations has not matched those of International journals. Many argue that it is the reluctance of leading Indian scientists to communicate their results to Indian journals that is the cause of low impact factor of our journals, others believe that these journals lack professional competitiveness and business acumen. Prof Lakhotia ran a commentary in *Current Science* arguing that majority of leading Indian scientists communicate those articles to Indian journals that have been either been rejected from 'International journals' or they themselves view as 'second grade articles'. In contrast, the Chinese, Japanese and several other countries have built their own research around indigenous journals whose impact factors are constantly growing and they take pride in publishing in such journals. In a personal communication with Prof Siddiqui, the founding Director of NCBS, Bangalore it became clear that quality should not be compromised at any cost even if it involves reducing the issues. Although there is no immediate plan to do this, I wish to announce the new focus of ANS to be on Stem Cell research and Development with commitment to address regulatory issues, and highlight new ideas and controversies, both from bench and clinic. In the subsequent issues we will strive to challenge concepts long established and revisit the aspects long forgotten. We will also attempt to create a new order of thought leadership by introducing a section devoted to 'People and views' that may include biographies of accomplished scientists. There is also a proposal to include patient's views and their version of disease pathology, health economics and experience. This is intended to bridge the chasm between those who run the labs and those for whom the research is intended. We will be publishing the original articles, inviting reviews from successful scientists and clinical researchers, carry reprints of classics, book reviews and a section devoted to graduate students, a need that was visibly felt at the IAN meeting in Kochi. We are also planning to introduce another section on Molecular shots where high quality experimental pictures which will be published as a prelude for papers to follow. Indian and world policy in neuroscience needs special attention and keeping that in mind we plan to invite policy briefs that engage researchers in bringing policy change. With the tremendous spurt of research communications from this region, coupled with an equally fast growth of IT in today's world, the journal is set to enhance the citation of its articles at a pace never witnessed before. This issue features reprints from original manuscripts of Rene Descartes a famous scientist and philosopher, who defied skepticism and paved way for deductive science that we see today. Special permission has been obtained for this.

I have begun deliberations for the constitution of a new Editorial board that will facilitate capacity building, highlight innovative research and create new systems to work in a team such that the journal becomes a true beacon of scientific expression and achieves an impact factor that it deserves. I will announce the brand new team of board members in the next issue. These members will share Journal's short term goal to generate competitive Editorial policies, participate in periodic board meetings, put up new mechanisms for peer review and acceptance of manuscripts. An independent portal linked to IAN web page coupled with reallocation of portfolios and increasing readership of the journal are among some of the other priorities. The long term goal of the journal would be to become the most cited journal in India and Asia. We would be shortly announcing awards for best reviewers and best contribution to journal clubs to encourage young scientists. This would be based on the decision of the committee that would be shortly formed. I solicit your support and advice in raising this journal to new heights.

Akshay Anand
Editor in Chief

India's national biotechnology development strategy – a policy mired with controversies

For many years, the country has witnessed funding decisions from Department of Biotechnology (DBT), the premier national funding agency, without a vision document in place. A draft for biotechnology policy was hurriedly framed recently in two swift meetings of the Expert Committee constituted for the purpose; one was the inaugural meeting, followed by another meeting. There was one or the other sub committee meeting in addition. Surprisingly, no minutes of the meetings were circulated to the members of Expert Committee and suddenly the draft "National Biotechnology Development Strategy" appeared on the DBT website with a stipulated 6 week period for comments. The 6 week period for the public to comment on a report dealing with a complex and technical subject like biotechnology, is less than adequate. Posting a draft on a website for six weeks and calling that public participation is a sham. Headed by a Pediatrician turned Secretary of DBT, such decision on crucial Biotechnology matters is not entirely unexpected. Combined with the level of Internet connections in Indian cities, it is surprising which 'public' provided inputs to finalization of a policy which is mired with controversial overtones. When the "National Biotechnology Development Strategy" was finalized, it claimed that the document was the result of intensive consultations !

Many argue that atleast one year should be set aside for comments and public consultations during which a genuine effort should be made to involve the people in this democratic country. This elicits healthy debate about risks of biotechnology applications against its potential benefits. At a time we are trying to develop a policy, it is imperative that a transparent and consultative opinion making is generated. This is particularly relevant when there is insufficient understanding and experience in research institutions and industry about how carefully Biotechnology and its products needs to be implemented. In a situation where violations are rampant the draft policy is the only public tool to strengthen the regulatory system. A striking feature of the current draft report is the total exclusion of NGOs from any aspect of decision making or implementation of biotechnology. Public participation in decision making must be ensured by including civil society in consultation committees. For a country as young as India, the large number of youth must also be given a say in how they see the future of technologies and their adoption. It is important that the regulatory structures and bodies must have civil society members on board, especially the IBSC. Many countries follow this practice. Excluding the public from decision making constitutes undemocratic governance which is also a violation of the Cartagena protocol on Biosafety. The Biosafety Protocol, to which India is a signatory, requires that the public be consulted in decision making matters related to GMOs (genetically modified organisms). On the practical front, excluding NGOs deprives the government of a valuable and critical source of information and analysis, since civil society usually has better and quicker access to information and developments in the field of Agbiotech than government departments in India.

The draft report in question makes an assertion that the existing regulatory system is sound and adequate. It also states that "there is consensus" that existing legislation is efficient. India's regulatory system for biotechnology has been criticized by a large number of stakeholders as being *ad hoc* and lacking technical skills as it does not possess adequate provisions to deal with violations and unexpected situations. The uncontrolled spread of Bt cotton and the failure of the regulatory system to deal with it is sufficient proof of the inherent inefficiencies. A consultative process leading to a thorough overhaul of the regulatory system is crucial if India is to adopt GM technology with any degree of responsibility and maturity. The Policy must take into account socio economic concerns in addition to evidence based science while conducting risk assessment. This is provided for in the Convention on Biological Diversity and the Biosafety Protocol. The Precautionary Principle, especially relating to the centers of origin for crop plants, socio-economic concerns relating to small farmers and consumers and the right of the public to participate in decision making must form an integral part of our biotechnology policy.

India is a biodiversity rich region from where major crops like rice have originated. It is therefore an important center of origin, where unique genetic wealth and diversity is found. There is global concern on GM crops being grown in their centers of origin and diversity because of the threat to this unique gene pool from contamination by foreign genes. This may have implications for human health which include neurological systems as reported from animal studies.

Such contamination has already been found in Mexico's corn and the authorities there are scrambling to resolve the problem. The draft biotech report is silent on this crucial issue of particular relevance to India since rice is the staple food of almost half of mankind. A decision on GM rice should be held in abeyance till sufficient data is collected on gene flow and its impact in the traits that are being used. A review of existing literature shows that GM rice will be problematic in rice growing areas. A public consultation should be held on this highly critical subject, which should include a cost, risk-benefit analysis.

Biosafety and risk assessment needs to be thoroughly revisited. The draft makes recommendations that are irresponsible and potentially dangerous to the environment and human health. Far from adopting a precautionary approach, as advocated even by international treaties like the Biosafety Protocol, the report makes a departure from the established principles of biosafety and risk assessment that are practiced in other countries. The draft strategy document recommends

that "an event that has already undergone extensive biosafety tests should not be treated as a new event even if it is in a changed background…." Further, the biotech policy document states that even if the foreign gene has been changed and modified and then inserted into a new host plant, there is no requirement to conduct tests for allergenicity and toxicity if there is 'no significant modifications in protein conformation'. This is contrary to scientific principles of toxicity testing and may be a dangerous precedent for others to emulate. Complete biosafety testing must be required if the gene is altered in any way. Nothing in such context can be considered 'insignificant' because a change that is not radical can have radical outcomes. Especially with respect to allergens and toxins in crop plants, it is well understood that the creation of new allergens and toxins is a complex affair influenced by multiple factors. Acknowledging this, the WHO and FAO are compiling an atlas of the known allergens in food plants so that these can be monitored when genetically modified plants are created and used as food. It is surprising that the national strategy report makes a recommendation of dispensing with testing for the presence of allergens and toxins.

There is too much unprofessional interference by the DBT in all aspects of the biotechnology strategy documents. The policy and its implementation must be entrusted to a competent autonomous body with the DBT playing a supporting role. The draft says that DBT is of the view that there is no reason for the regulatory bodies like the Genetic Engineering Approval Committee (GEAC) to ask for large scale field trials. This need not be the normal practice, since ICAR will take the responsibility for the trials but the GEAC or whatever the revised regulatory body is ultimately called, must not be deprived of that right. It is the GEAC that takes the ultimate decision to grant or refuse permission for the commercial release of a variety. They must continue to have the right to order a large scale trial if they are uncertain about the results. The Policy must take a clear position on crops and traits that are permissible and those that are not. GM crops that could have harmful social or economic consequences for farmers and consumers, those that are frivolous and those that will displace labor and impact rural livelihoods must be banned in this country. Herbicide tolerance should not be used in this country for it brings no significant advantages but destroys sources of supplementary nutrition and underutilized food sources, it would destroy vegetation that is used as supplementary fodder for livestock and it would destroy flora used as medicinal plants, in addition to taking away a significant source of wages, especially for rural women.

Those in the government who speak for the GM industry have an amusing justification about selective policy for the public sector while sparing the private sector. The reticence in government circles to spare the private sector to use GM technologies will hurt rural families and the nation, needs examination at the highest level; the people responsible for such decisions need to be identified and made accountable. A national policy applies to all sectors and its goal has to be to achieve the greatest public good, not create concessions for the private sector. In the case of the seed legislation called the Protection of Plant Variety and Farmers Rights Act, the decision was taken that the Terminator technology would not be allowed in India and the law expressly forbids the use of this technology by the private and public sector alike. The Food and Feed Safety testing regimes that are currently ad hoc must be brought up to the highest international standards. The ANZFA (Australia and NZ Food Agency) would be a good model to follow, adapted to Indian needs.

GM foods should not be allowed at present, not until sufficiently high standards of testing and adequate quality control are put in place and data is publicly available. The recent report of the Monsanto study on the organ damage and compromised immune system of rats fed with GM corn, should be an eye opener. The biotech policy must place the highest emphasis on ethical practices. We need to take a position on what we consider ethical science and what would not be considered permissible. Transparency, precautionary principle and public interest must constitute the cornerstone of our national policy, implemented with the highest levels of technical competence. There must be sufficient time for an open discussion for people to understand the contents of the document and express their views on it. This draft policy is in need of a comprehensive revision if it is to contribute to a safe and equitable development of this sector.

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Dr Sahai was a member of the Expert Committee set up to draft the National Biotechnology Policy. Her criticism of the policy process is well documented from the time the Expert Committee was constituted.

Notes of a biology watcher

This issue of the *Annals of Neurosciences* marks a turning point in its coverage in many notable directions. This is the first issue to be edited by Dr. Akshay Anand of Department of Neurology from premier Post Graduate Institute of Medical Education and Research, an assignment he undertook with the enthusiasm and dedication required from when the goal of a new editor in chief is to change fundamental elements of communication that a specific scientific journal provides. Dr Anand discussed his invitation with me at length and in detail and I encouraged him to not only accept this challenge but to do so eagerly and with pre-determined goals. These we discussed at some length, as he formulated the basic outline that the Journal shall assume in its quest to represent an innovative forum of communication of the etiology and therapy of diseases of the brain and retina, while trying to learn just how the brain functions.

The *Annals of Neurosciences* will not be content to stop there. Indeed, the Journal intends to take a new look at unanswered questions of neuroscience that have been once explored and now ignored. In this endeavor, the Journal could re-print copies of classic papers that once shaped the field, comment upon them and ask what we have learned since. Annals of Neurosciences can further communicate the state of the art of the field today, focusing on neural stem cells and their potential, as well as new techniques to assess neural function. These techniques, including functional MRI and advanced imaging may allow us to gain a better understanding of the role of stem cells in neural repair and hence the possible facilitation of neural repair by administration of endogenous stem cells. The stem cells that effect regeneration of neurons and connections of the central nervous system have only recently been identified. In the adult brain, their numbers are limited[1]. Many believe that expanding these cells in culture may overcome this problem. Others believe that cultured expansion leads to compromised ability of stem cells to regenerate tissues and organ systems and feel this approach while attractive is shortsighted.

While reasonable estimates and guesses have been made, the phenotype of a neural stem cell that can reverse the devastating effects of a brain injury, ischemia or disease remains unknown. The participation of the few cells that possess characteristics of stem cells in certain areas of the brain is of no known significance in maintenance of function of the brain after birth. No one knows if stem cells play any role at all in the stunning recovery some patients experience after stroke or trauma. And no one knows if stem cells supplied exogenously by intravascular, intra-articular or local infusion can reverse or abate the damage sustained when neurons are disrupted and their function compromised when the barrier that separates the blood from the action cells of the brain is disrupted. The ability of the peripheral neurons to "regenerate" as they extend from an intact cell body axonal pseudopods that connect to target tissue and retain functional integrity engenders hope that similar responses in the central nervous system may reverse the devastation wrought by injury to the brain. Indeed the Journal --under the auspices of Dr Akshay Anand--- shall explore these issues and shall do so in a manner that will encourage innovative approaches to this highly publicized problem.

The *Annals of Neurosciences, Dr. Anand tell me,* however, shall not merely be a neural stem cell journal. The Journal shall entertain all aspects of neuroscience. In particular, *Annals of Neurosciences* shall communicate the work of the *Indian Academy of Neurosciences*. In doing so, the Journal should endeavor to supplement these reports with invited comprehensive reviews of items of particular interest and techniques of promise. This journal could focus on the resurrection of specific investigations into questions long ago abandoned, as their answers were thought to be impossible to reach. With new techniques, many of the basic questions of the past investigators who pondered pondering, thought about thinking and dreamt of the basis of dreams can be addressed. Many of these issues can now be re-evaluated ----if only in comment. For this purpose, one section of Journal could reprint classic works of the past and another section such as *Issues In Neurosciences*---- shall afford a platform for all to discuss these works and issues of modern research, including stem cell therapy. The journal can initiate its trek into unanswered questions of past investigators by reprinting portions of the classic work of Carl S. Lashley who searched for the "engram" from 1920 till 1950[2], never finding the elusive link thought to convert short term memory to ones stable, came up with a number of brilliant alternate hypotheses. These were presented right around the time that JT Bonner and colleagues were trying to convince the world that memories could be transferred by RNA extracts, a subject that immunologists endorsed a few years later. Immune memory now seems not to be transferred by RNA; no engram has yet been found and it is unclear if the primitive animals Bonner used in his experiments of memory transfer experiments, widely reported in the journal Science and other high profile journals of the time, could even possess a memory, or be taught to respond. But then arose the lowly Prion.

Does the engram thus not exist? Absence of evidence proves nothing; short term memories are converted to long term, stable memories that are not easily lost, but can be. Does the engram that Dr Lashley never found explain the conversion of short to long term memory and the etiology of amnesia vs. retrograde amnesia? Dr Anand has picked this subject as an example of one that fits the goals he sees for the revitalized Journal. Later issues could discuss dreams, thought, policies and the unique ability of the human to contemplate its own existence. Ah ha …"I think, therefore I am", uttered Rene Descartes over 300 years ago atop a clear mountain of the pristine earth. His colleagues-- astounded by the beautiful simplicity of his answer to a question that encompassed their minds for years -- were immediately relieved and

Rene's answer which held for quite a long time. At least until 1966, when Justin Hayworth of a group of psychologists from Birmingham, England, a group referred to as the "The Moody Blues" added a few words to Dr. Descartes conclusion…..

"I think, therefore I am, I think".

Finally rendering Rene's declaration accurate..... probably.

Annals of Neuroscience has a necessary and challenging task ahead and I think that Dr Anand and his selected team shall meet the demands of that task in a manner that promises to change fundamental concepts of communication in science. I know he shall give it all effort he has to give and I am confident that his efforts shall be rewarded by innovative thought, ideas and conclusions where we try -- with much futility -- to understand the nature of the thing that defines our actions, even those actions we take to understand this object we have come to refer to as a brain.

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President
Florida Foundation for Development Research

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Effect of ethanolic extract of *H. perforatum* on oxidative stress induced by cerebral ischemia-reperfusion in rats

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KEY WORDS

Neuroprotection
Oxidative stress
Reperfusion injury
H. perforatum
Transient cerebral ischemia.

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ABSTRACT

Restoration of blood flow to an ischemic brain region is associated with generation of reactive oxygen species. In Ayurveda, the medicinal properties of *Hypericum perforatum Linn* have been attributed to its anxiolytic, antioxidant, antidepressant and nootropic properties. The present study investigates the effect of standardized extract of *H. perforatum* on acute cerebral ischemia-reperfusion in rats. Acute cerebral ischemia-repersfusion (30 min occlusion of bilateral common carotid arteries followed by 45 min reperfusion) in Charles Foster (C.F.) strain rats was produced following standard technique. Effect of *H. perforatum* on lipid peroxidation, superoxide dismutase (SOD) activity, ascorbic acid, cyclic AMP level and total tissue sulfhydryl (T-SH) group in forebrain region in acute cerebral ischemia-reperfusion were evaluated. *H. perforatum* pre-treatment (100 mg/kg p.o. for 7 days) attenuated the reperfusion induced biochemical alterations. The results suggest protective role of *H. perforatum* in cerebral ischemia-reperfusion injury..

Introduction

A number of herbal drugs have been evaluated for their possible role in neurodegenerative disorders and cognitive functions. *Hypericum perforatum* (HP) or St. John's wort known as *Bassant* in Ayurveda (the classical Indian system of medicine), has been used for centuries, for a variety of diseases[1]. Ethanolic extract of *H. perforatum* is reported to have antioxidant, anti-inflammatory[2] and antidepressant[3] properties. Standaridized extract of *H. perforadum* is known to possess anxiolytic[4] and nootropic activity on the basis of neurotransmitter receptor mechanism[4,5].

Earlier investigations have indicated that *H. perforatum* contains many bioactive constituents; phenyl propanoids, flavonal glycosides, biflavones, oligomeric proanthocyanidins, xanthones, naphodianthrones and prenylated phloroglucinols[6]. The presence of many polyphenolic compounds in this herb suggests that they could have important antioxidant, anti-inflammatory properties[2]. The polyphenols have the ability of penetrate the blood brain barrier and act as potential neuroprotective agent. Recently, hyperforin, a prenylated phloroglucinol present in this plant, has been targeted as the major component responsible for the antidepressant activity of *H. perforatum*[7] and inhibition of the uptake of several neurotransmitters *in vitro*[8].

A majority of the present day disease are reported to be due to shift in the balance of pro-oxidant and antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of free radicals caused by excessive oxidative stress or due to their poor scavenging in the body caused by gradual decline in antioxidant defense mechanism[9]. Oxidative free radicals play an important role in cerebral ischemia as well as reperfusion injury which is a distinct entity from the primary ischemia injury. This study was designed to assess the neuroprotective activity of standardized extract of *H. perforatum* on acute cerebral ischemia-reperfusion.

Methods

Drug and reagents

1, 1, 3, 3-Tetraethoxypropane (TEP), (Merck, Germany), Thiobarbituric acid (TBA), NADH, nitroblue tetrazolium (NBT) and phenazine methosulfate (PMS) (Sigma, USA) were used. All other chemicals and reagents were of the highest analytical grades available.

The plant was collected during August from the company garden, Saharanpur, India. A 50% ethanolic extract (yield 26.75% w/w, standardized for 4.5-5% hyperforin, HPLC) of the dried overground parts (leaves, flowers and stem) of the plant, as administered orally as a 0.3% carboxymethyl cellulose (CMC) suspension, in dose of 100 mg/kg. p.o. once daily. The choice of particular dose was made according to our initial pilot experimental results[5].

Animals

After approval of Institutional Ethical Committee, the present study was conducted on inbred CF male albino rats weighing 250-300g, obtained from the central animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. They were kept in the departmental animal house in colony cages at an ambient temperature of 25±2°C and 45-55% relative humidity with 10:14 h light: dark cycles. They had free access to standard rodent pellet diet and drinking water. The food was withdrawn 18-24h before the surgical procedure, however, water was allowed *ad libitum.* Principles of laboratory animal care (NIH Publication No. 86-23, revised 1985) guidelines were followed throughout the experiments.

Experimental Procedure

Surgical Procedure

Surgical technique for induction of cerebral ischemia by bilateral common carotid artery occlusion (BCCAO) was adapted from earlier published method of Iwasaki *et al*[13]. Rats were anaesthetized by ketamine (100 mg kg^{-1}, i.p). After a midline skin incision in the neck, both common carotid arteries were identified and isolated carefully from accompanying vagosympathetic nerve.

Acute ischemia-reperfusion injury was produced by blocking bilateral common carotid arteries (BCCA) for 30 min (lifting arteries with the help of thread) and reperfusion for 45 min was allowed by releasing the thread. Body temperature was maintained at about 37°C. This protocol was adopted on the basis of earlier reports from our laboratory[14] and elsewhere[15].

Study Design

The animals were divided into four groups of six animals each. First group served as sham-operated control (underwent all surgical procedure except BCCAO). In second group, *H. perforatum* was administered to sham-operated animals to determine effect of drug *per se.* Third group of animals underwent 30 min BCCAO and 45 min reperfusion. In the fourth group (treatment) *H. perforatum* 100 $mgk^{-1}d^{-1}$, p.0 for 7 days, was administered before subjecting animals to ischemia-reperfusion.

Biochemical analysis

At the end of experiments animals were sacrificed by decapitation and frontoparietal part of cerebral cortex from both the hemispheres were separated. After rinsing with ice-cold normal saline the brain tissue were transferred to the appropriate homogenizing medium and analyzed for the biochemical parameters of the oxidant-antioxidant status i.e. thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) activity, tissue total sulfhydryl (T-SH) level, ascorbic acid and cyclic AMP. All the procedures on the brain samples were performed on ice or ice bath and sample were kept at -20°C. For all biochemical parameter studies, frontoparietal part of cerebral cortex of both the hemispheres was analysed.

Lipid peroxidation

Estimation of lipid peroxidation was done by measuring the lipid peroxidation product TBARS (Thio Barbituric Acid Reactive Substances) following the method of Ohkawa *et al*[16]. TEP was used as an external standard, and the level of lipid peroxidation was expressed as nanomoles TBARS mg^{-1} of protein.

Superoxide dismutase (SOD)

SOD was estimated by adopting the procedure of Kakkar *et al*[17] and results are expressed in milliunits mg^{-1} of protein.

Total tissue sulfhydryl groups (T-SH)

Total T-SH in brain was measured according to the method of Sedlack and Lindsay[18]. The level of T-SH was expressed as moles of SH 100^{-1} g of wet tissue weight.

Ascorbic acid

Ascorbic acid levels were determined by the method of Omaye *et al*[19] and the results are expressed in terms of mg/100g wet weight.

Estimation of brain total protein

The protein content of brain tissue was estimated using the method of Lowry *et al*[20].

Cyclic AMP estimation

Cyclic AMP estimation of frontoparietal part of forebrain was done by ELISA using EIATM cyclic AMP kit (Assay Designs Inc., USA). This kit uses a polyclonal antibody to cyclic AMP which binds, in a competitive manner with the cyclic AMP. Results were expressed as nmol of cyclic AMP per g (wet weight) of tissue.

Statistical Analysis

Statistical analysis was performed by applying one-way Analysis of Variance (ANOVA) followed by post hoc Tukey Test for biochemical parameters. A p-value of <0.05 was considered statistically significant.

Results

Acute BCCAO for 30 min followed by 45 min reperfusion induced increase in lipid peroxidation (TBARS), (2.0 fold), superoxide dismutase (SOD), (2.1 fold) activity and fall in T-SH levels (43% decrease). *H. perforatum* pretreatment attenuated enhanced TBARS level ($p < 0.01$) and SOD activity ($p < 0.01$) as well as prevented the consumption of T-SH significantly ($p < 0.01$) following cerebral ischemia reperfusion injury. *H. prforatum*

Table-1 : Effect of *H. perforatum* (100 mg/kg p.o. x 7 days) on biochemical parameters of oxidative stress in rat forebrain following cerebral ischemia-reperfusion injury (30 min BCCAO followed by 45 min reperfusion).

Groups	TBARS (nmol/mg protein)	SOD (milliunits/mg protein)	T-SH (x 10^{-5} M/mg protein)	Ascorbic Acid (mg/100g wet weight)
Sham-operated control	1.98±0.41	303.48±88.00	3.78±0.48	9.16±3.18
Per Se	1.93±0.37	336.71±99.66	3.71±0.50	8.33±2.65
Ischemia-reperfusion	4.10±0.57	666.80±175.56	2.16±0.30	7.50±2.43
Treatment	2.37 ±0.44	402.55±90.69	3.40±0.27	7.00±2.36

All data is expressed as mean ± SD, n=6 in each group. Sham-operated control and treatment groups are compared with ischemia-reperfusion group. *H. perforatum* per se is compared with sham-operated control group. Superscript indicates p-value <0.01. Statistical analysis was done by one-way ANOVA followed by Tukey test.

Groups	Cyclic AMP (nmol/g)
Sham-operated contro	I8.50±1.89
Per se	9.83±1.33
Ischemia-reperfusion	18.33±4.17[b]
Treatment	39.36±9.67[a]

Table 2 : Effect of *H perforatum* (100 mg/kg p.o. x 7 days) on level of cyclic AMP in frontoparietal region of rat brain following cerebral ischemia-reperfusion injury (30 min BCCAO followed by 45 min reperfusion)

All data is expressed as mean ± S.D., n=6 in each group. Sham-operated control and treatment groups are compared with ischemia-reperfusion group. Superscripts [a]and[b] p indicate p-value <0.01 and < 0.05 respectively. Statistical analysis was done by one-way ANOVA followed by Tukey test.

perse had no significant effect on any of these biochemical parameters (Table 1). Ischemia followed by reperfusion increased cyclic AMP level significantly as compared to that in sham-operated animals ($p < 0.05$). *H. perforatum* pretreatment of ischemia reperfused animals led to a significant rise in cyclic AMP level compared to ischemia reperfusion group ($p < 0.01$) (Table 2). Ascoric acid levels, however, did not show any change after reperfusion injury and/or *H. perforatum* pretreatment. Thus total ascorbic acid levels appear unaffected during reperfusion injury (Table-1).

Discussion

The study confirms the previous reports that cerebral post-ischemic reperfusion is associated with generation of free radicals[15,21]. The analysis of biochemical parameters show that BCCAO for 30 min followed by 45 min reperfusion causes ischemia-reperfusion injury. Increased generation of free radicals initiates lipid peroxidation and this reflected as increased level of TBARS[22]. Polymorphonuclear leukocytes are known to be involved in cerebral reperfusion injury. Leukocyte accumulation has been noted in brain after cerebral ischemia[23]. These activated neutrophils are a source of free radicals, especially superoxide anion[10]. The increased SOD activity is an indication that brain's antioxidant machinery is activated in response to excessive generation of free radicals[24]. Enhanced SOD activity catalyzes the conversion of superoxide anion to hydrogen peroxide and molecular oxygen. Hydrogen peroxide, the product of this reaction, is more toxic than the oxygen derived free radicals and requires to be scavenged further by tissue thiols (glutathione redox pathway) and catalase[25]. A fall in GSH (a non protein sulfhydryl) during cerebral reperfusion injury is well reported[26] and reduced level of T-SH reflects consumption of tissue thiols. Sulfhydryl compounds are among the most important endogenous antioxidants. They have role in maintenance of cellular proteins and lipids in their functional states. When these are consumed, the toxic effects of oxidative insult are exacerbated resulting in increased membrane and cell damage[27]. The data reveals that *H. perforatum* could antagonize ischemia-reperfusion injury induces rise in TBARS level. Similarly, *H. perforatum* reverses ischemia reperfusion induced change in SOD and T-SH. These findings are in agreement with earlier reported antioxidant and neuroprotective properties of *H. perforatum*[2,12,28,29]. Reperfusion injury did not produce any significant change in ascorbic acid level. Possibly, reperfusion injury increases the ascorbate levels (reduced form of ascorbic acid) without altering the total ascorbic acid levels. This finding receives direct support from an earlier investigation that also suggests lack of change in total ascorbic acid levels with a decrease in ascorbate levels secondary to cerebral reperfusion injury[30].

The study also revealed significant increase in cyclic AMP level in brain (frontoparietal region). Following ischemia-reperfusion injury, cyclic AMP is known to increase in striatum[31], neocortex and hippocampus[32] and in cerebral cortex[33]. The increase in cyclic AMP levels following such injury has been implicated in reversing stroke induced vasospasm in central vessles[11]. Increased level of cycle AMP is known to inhibit release of excitatory amino acid like glutamate through modulation of adenosine[34]. *H. perforatum* pretreatment enhanced cyclic AMP concentration in ischemia-reperfused animals. It is quite tempting to posulate that part of the beneficial effect of *H. perforatum* might be due to its effect on cyclic AMP.

Several studies have identified and isolated the active principle of *H. perforatum*. Recently, hyperforin, the fluoroglucinol derivative of *H. perforatum* has gained attention, as it represents the major constituents responsible for modulation in neurotransmitter levels in the brain of rodents[35]. Hunt *et al* (2001) demonstrated that there is a free radical scavenging effect of *H. perforatum* extract and postulated that this effect could be related to an active constituent. Likewise the beneficial effects of *H. perforatum* on acute cerebral ischemia-reperfusion could also be attributed to its bioactive constituents.

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Acute electrical stimulation of ventral tegmental area improves depressive behavior

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Background

Depression is a common and devastating neuropsychiatric disorder, affect approximately 5% of the population and a better understanding of its pathophysiology is needed to improve diagnosis, treatment and prevention. Research has revealed some promising approaches that hold considerable therapeutic promise for the treatment of a range of neuropsychiatric disorders which include deep-brain stimulation (DBS), chronic administration of BDNF in mediating antidepressant effects and stimulation of mesolimbic dopamine (DA) system originating in the ventral tegmental area (VTA). Ventral tegmental area (VTA) neuronal activity plays an important role in reward-related learning and motivation. It is the origin of dopaminergic neuron cell bodies in the mesolimbic system and represents a reasonable site for intervention through DBS.

Study Design

In this study, the Friedman *et al* have tested a new method for intervention in depressive disorders, based on DBS of the VTA, as a source of incentive motivation and hedonia, in comparison to chemical antidepressants which may require several weeks to produce their clinical effects. They used acute electrical stimulation (AES), a modified version of DBS, which applies short-term low frequency programmed stimulation instead of continual DBS. They have tested this method on the male Flinder Sensitive Line (FSL) rats (genetic animal model of depression) as well as SD rats (as controls) and compared it to conventional antidepressant treatments.

In first set of experiment Friedman *et al* demonstrated the VTA neuronal firing and estimated the AES pattern. They used single unit recordings from VTA neurons of SD and FSL rats by inserting recording electrode streotaxically and quantified the firing pattern of dopaminergic cells. They found that VTA of SD rats has the capability of firing burst with large amount of spikes, whereas FSL rats rarely showing this pattern. Thus they picked a defined electrical template from the recorded and analyzed SD VTA cell-firing data and applied this pattern of stimulation, shaped to mimic the firing pattern of the normal rat, in the VTA of the FSL rats.

The authors then explored the normalization of FSL depressive like behavior after AES. The effect of AES on depressive-like behavioral expressions was monitored by a battery of five behavioral tests. Rats were first trained for behavioral tests and baseline was recorded. Depressive like behavior was again tested two weeks after implantation of bipolar electrode into the VTA of FSL and control rats, followed by AES of FSL through electrode and again monitored for same behavior paradigm. Treatment with AES resulted in improved grooming, eating and drinking as compared to non treated FSL rats. The level of depression was measured by immobility in the swim test and they found that immobility was higher in depressive rats than in controls. AES not only attenuated the degree of immobility in FSLs but also showed a significant faster onset and long-term effect compared to other treatment. They demonstrated this by stimulating FSL rat brain in a nonspecific region which resulted in no effect on depressive behavior. Then they confirmed whether AES to the VTA may have acted as an antidepressant, or it may have had a stimulant effect. They measured the locomotor activity of rats in open field test. Naive FSL rats have lower locomotor activity than the control SD rats, and treatment with AES did not improve locomotor activity supporting the rationale AES has an antidepressant, and not stimulant function.

The results of sucrose self-administration test (measure of anhedonia) clearly demonstrated that FSL rats exhibited anhedonic-like behavior, measured by a low number of presses on the active lever as compared to controls. But AES treatment improved self administration task in AES-treated FSL rats. They validated whether this disrupted behavior was due to cognition deficit or their high level of anhedonia in FSL rats. They confirmed this by measuring water self-administration

following water deprivation in FSL and control rats and found that naive FSL rats did not show significant differences in active lever responses for water reinforcement as compared to controls which confirmed the effects of DBS on hedonic motivation without involvement of cognitive deficit or any other impairment in physical performance.

In a social interaction test FSL rats showed submissive behavior before treatment in comparison to SD rats, whereas following AES treatment, they found inverse relationship. In novelty exploration behavior FSL rats show a low level of 'interest' in the new object, whereas AES treatment normalized object exploration.

In last set of experiments, Friedman and colleagues investigated BDNF expression in different brain regions following AES by real-time PCR and western blot analysis. Surprisingly, AES significantly normalizes BDNF mRNA levels in the PFC, and also in the Nucleus accumbens. However, in the VTA, no differences were found between groups which indicates that BDNF transcription levels are correlated with depressive-like behavior and are not a result of neuronal lesion or electrical stimulus. On the basis of those results, they suggest a significant therapeutic effect of AES on depressive behavior, in addition to the functional role of BDNF in mediating the antidepressant effects of AES.

Implications

As with any new paradigm Friedman *et al* data revealed that AES has several advantages, specifically, fast onset of action, a selective effect within specific brain sites, and greater efficacy in correcting depressive behavior. Specifically, they showed that AES exerts its effect immediately within one short treatment session, whereas antidepressants or ECT require between 7 and 14 treatment sessions. The remedial effect of AES persists for up to 1 month after a single stimulation. A possible limitation confronting the future implementation of this procedure would be the sensitive location of the VTA. They raised interesting question for future research which shall determine whether this is a viable and practical mode of treatment in humans. But in light of the current findings it holds potential to become possible treatment of major depression.

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RENE DESCARTES (1595-1650)

"I am thinking therefore I exist."
from the *Discourse on Method*

Rene Descartes was a famous French scientist, mathematician and philosopher. He was the first major philosopher in the modern era to make a serious effort to defeat skepticism. His views about knowledge and certainty, as well as his views about the relationship between mind and body have been very influential over the last three centuries. Descartes was born at La Haye (now called Descartes), and educated at the Jesuit College of La Flche between 1606 and 1614. Descartes later claimed that his education gave him little of substance and that only mathematics had given him certain knowledge. In 1618 he went to Holland to serve in the army of Prince Maurice of Nassau, in travelled to Germany with that army. On the night of Nov ember 10, he had a series of dreams which he interpreted as signs that he would found a universal science. His first substantial work was the *Regulae* or *Rules for the Direction of Mind* written in 1628-9 but not published until 1701. This work shows Descartes interest in method which he shared with many sixteenth and seventeenth century scientists. Descartes was clearly convinced that the discovery of the proper method is the key to scientific advance. In November 1628 Descartes was in Paris, where he made himself famous in a confrontation with Chandoux. Chandoux claimed that science could only be based on probablities. This view reflected the dominance in French intellectual circles of Renaissance skepticism. This skptical view was rooted in the religious crisis in Europe resulting from the Protestant Reformation and had been deepened by the publication of the works of Sextus Empiricus and reflections on disagreements between classical authors. It was strengthend again by considerations about the differences in culture between New World cultures and that of Europe, and by the debates over the new Copernican system. Descartes attacked this view, claiming only that certainty could serve as a basis for knowledge, and that he himself had a method for attaining such certainty. In the same year Descartes moved to Holland where he remained with only brief interruptions until 1649.

In Holland Descartes produced a scientific work called *Le Monde* or *The World* which he was about to publish in 1634. At the point, however, he learned that Galileo had been condemned by the Church for teaching Copernicanism. Descarte s' book was Copernican to the core, and he therefore had it supressed. In 1638 Descartes published a book containing three essays on mathematical and scientific subjects and the *Discourse on Method.* These works were written in French (rather th an Latin) and were aimed at the educated world rather than simply academics.

Descartes' death in Stockholm of pneumonia, has regularly been attributed to the rigours of the Swedish climate and the fact that Descartes was sometimes required to give the Queen lessons as early as five in the morning. However unpleasant these conditions may have been, it seems plain that Descartes acquired his fatal malady as a result of nursing his friend the French ambassador (who had pneumonia) back to health.

Descartes Time Line

1596 31 March: born at La Haye near Tours

1616 Descartes takes doctor of law at University of Poitiers

1619 He travels in Germany; on 10 November in Ulm has dream of a unified scientific system based on mathematics

1628 (or 29) He composes *Rules for the Direction of Mind* (which was first published in 1701);

1628 In November Descartes distinguished himself in a confrontation with Chandoux, who claimed that all science is based on probability while Descartes claimed that only certainty could be the basis of human knowledge and that he had a method for attaining certainty. Following this, Descartes was encouraged by Cardinal Berulle to develop his system.

1633 The condemnation of Galileo leads Descartes to abandon plans to publish *The World*

1635 The birth of Descartes' natural daughter, named Francine, baptized 7 August (died 1640)

1637 Descartes publishes *Discourse on Method*, with *Optics*, *Meterology* and the *Geometry*

1641 *Meditations on First Philosophy* published together with the first six sets of *Objections and Replies*

1642 Second edition of the *Meditations* published along with all seven sets of objections and replies. Descartes meets Princess Elisabeth of Bohemia

1643 Cartesian philosophy condemned at the University of Utrecht; Descartes' long correspondence with the Princess Elisabeth of Bohemia begins.

1644 Visits France: *The Principles of Philosophy* published.

1647 Descartes is awarded a pension by the King of France; he publishes *Comments on a Certain Broadsheet*; begins work on *The Description of the Human Body*

1648 He is interviewed by Frans Burman at Egmond-Binnen which leads to the publication of *Conversations with Burman*

1649 He goes to Sweden on invitation of Queen Christina; the *Passions of the Soul* published

1650 11 February: dies in Stockholm

Discourse on the method of rightly conducting the reason, and seeking truth in the sciences

Rene Descartes

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Prefatory Note by the Author

If this Discourse appear too long to be read at once, it may be divided into six Parts: and, in the first, will be found various considerations touching the Sciences; in the second, the principal rules of the Method which the Author has discovered, in the third, certain of the rules of Morals which he has deduced from this Method; in the fourth, the reasonings by which he establishes the existence of God and of the Human Soul, which are the foundations of his Metaphysic; in the fifth, the order of the Physical questions which he has investigated, and, in particular, the explication of the motion of the heart and of some other difficulties pertaining to Medicine, as also the difference between the soul of man and that of the brutes; and, in the last, what the Author believes to be required in order to greater advancement in the investigation of Nature than has yet been made, with the reasons that have induced him to write.

PART 1

Good sense is, of all things among men, the most equally distributed; for every one thinks himself so abundantly provided with it, that those even who are the most difficult to satisfy in everything else, do not usually desire a larger measure of this quality than they already possess. And in this it is not likely that all are mistaken the conviction is rather to be held as testifying that the power of judging aright and of distinguishing truth from error, which is properly what is called good sense or reason, is by nature equal in all men; and that the diversity of our opinions, consequently, does not arise from some being endowed with a larger share of reason than others, but solely from this, that we conduct our thoughts along different ways, and do not fix our attention on the same objects..

For to be possessed of a vigorous mind is not enough; the prime requisite is rightly to apply it. The greatest minds, as they are capable of the highest excellences, are open likewise to the greatest aberrations; and those who travel very slowly may yet make far greater progress, provided they keep always to the straight road, than those who, while they run, forsake it.

For myself, I have never fancied my mind to be in any respect more perfect than those of the generality; on the contrary, I have often wished that I were equal to some others in promptitude of thought, or in clearness and distinctness of imagination, or in fullness and readiness of memory and besides these, I know of no other qualities that contribute to the perfection of the mind; for as to the reason or sense, inasmuch as it is that alone which constitutes us men, and distinguishes us from the brutes, I am disposed to believe that it is to be found complete in each individual; and on this point to adopt the common opinion of philosophers, who say that the difference of greater and less holds only among the accidents, and not among the forms or natures of individuals of the same species. I will not hesitate, however, to avow my belief that it has been my singular good fortune to have very early in life fallen in with certain tracks which have conducted me to considerations and maxims, of which I have formed a method that gives me the means, as I think, of gradually augmenting my knowledge, and of raising it by little and little to the highest point which the mediocrity of my talents and the brief duration of my life will permit me to reach. For I have already reaped from it such fruits that, although I have been accustomed to think lowly enough of myself, and although when I look with the eye of a philosopher at the varied courses and pursuits of mankind at large, I find scarcely one which does not appear in vain and useless, I nevertheless derive the highest satisfaction from the progress I conceive myself to have already made in the search after truth, and cannot help entertaining such expectations of the future as to believe that if, among the occupations of men as men, there is any one really excellent and important, it is that which I have chosen.

After all, it is possible I may be mistaken; and it is but a little copper and glass, perhaps, that I take for gold and diamonds. I know how very liable we are to delusion in what relates to ourselves, and also how much the judgments of our friends are to be suspected when given in our favor. But I shall endeavor in this discourse to describe the paths I have followed, and to delineate my life as in a picture, in order that each one may also be able to judge of them for himself, and that in the general opinion entertained of them, as gathered from current report, I myself may have a new help towards instruction to be added to those I have been in the habit of employing. My present design, then, is not to teach the method which each ought to follow for the right conduct of his reason, but solely to describe the way in which I have endeavored to conduct my own. They who set themselves to give precepts must of course regard themselves as possessed of greater skill than those to whom they prescribe; and if they err in the slightest particular, they subject themselves to censure. But as this tract is put forth merely as a history, or, if you will, as a tale, in which, amid some examples worthy of imitation, there will be found, perhaps, as many more which it were advisable not to follow, I hope it will prove useful to some without being hurtful to any, and that my openness will find some favor with all.

From my childhood, I have been familiar with letters; and as I was given to believe that by their help a clear and certain knowledge of all that is useful in life might be acquired, I was ardently desirous of instruction. But as soon as I had finished the entire course of study, at the close of which it is customary to be admitted into the order of the learned, I completely changed my opinion. For I found myself involved in so many doubts and errors, that I was convinced I had advanced no farther in all my attempts at learning, than the discovery at every turn of my own ignorance. And yet I was studying in one of the most celebrated schools in Europe, in which I thought there must be learned men, if such were anywhere to be found. I had been taught all that others learned there; and not contented with the sciences

actually taught us, I had, in addition, read all the books that had fallen into my hands, treating of such branches as are esteemed the most curious and rare. I knew the judgment which others had formed of me; and I did not find that I was considered inferior to my fellows, although there were among them some who were already marked out to fill the places of our instructors. And, in fine, our age appeared to me as flourishing, and as fertile in powerful minds as any preceding one. I was thus led to take the liberty of judging of all other men by myself, and of concluding that there was no science in existence that was of such a nature as I had previously been given to believe.

I still continued, however, to hold in esteem the studies of the schools. I was aware that the languages taught in them are necessary to the understanding of the writings of the ancients; that the grace of fable stirs the mind; that the memorable deeds of history elevate it; and, if read with discretion, aid in forming the judgment; that the perusal of all excellent books is, as it were, to interview with the noblest men of past ages, who have written them, and even a studied interview, in which are discovered to us only their choicest thoughts; that eloquence has incomparable force and beauty; that poesy has its ravishing graces and delights; that in the mathematics there are many refined discoveries eminently suited to gratify the inquisitive, as well as further all the arts an lessen the labour of man; that numerous highly useful precepts and exhortations to virtue are contained in treatises on morals; that theology points out the path to heaven; that philosophy affords the means of discoursing with an appearance of truth on all matters, and commands the admiration of the more simple; that jurisprudence, medicine, and the other sciences, secure for their cultivators honors and riches; and, in fine, that it is useful to bestow some attention upon all, even upon those abounding the most in superstition and error, that we may be in a position to determine their real value, and guard against being deceived.

But I believed that I had already given sufficient time to languages, and likewise to the reading of the writings of the ancients, to their histories and fables. For to hold converse with those of other ages and to travel, are almost the same thing. It is useful to know something of the manners of different nations, that we may be enabled to form a more correct judgment regarding our own, and be prevented from thinking that everything contrary to our customs is ridiculous and irrational, a conclusion usually come to by those whose experience has been limited to their own country. On the other hand, when too much time is occupied in traveling, we become strangers to our native country; and the over curious in the customs of the past are generally ignorant of those of the present. Besides, fictitious narratives lead us to imagine the possibility of many events that are impossible; and even the most faithful histories, if they do not wholly misrepresent matters, or exaggerate their importance to render the account of them more worthy of perusal, omit, at least, almost always the meanest and least striking of the attendant circumstances; hence it happens that the remainder does not represent the truth, and that such as regulate their conduct by examples drawn from this source, are apt to fall into the extravagances of the knight-errants of romance, and to entertain projects that exceed their powers.

I esteemed eloquence highly, and was in raptures with poesy; but I thought that both were gifts of nature rather than fruits of study. Those in whom the faculty of reason is predominant, and who most skillfully dispose their thoughts with a view to render them clear and intelligible, are always the best able to persuade others of the truth of what they lay down, though they should speak only in the language of Lower Brittany, and be wholly ignorant of the rules of rhetoric; and those whose minds are stored with the most agreeable fancies, and who can give expression to them with the greatest embellishment and harmony, are still the best poets, though unacquainted with the art of poetry.

I was especially delighted with the mathematics, on account of the certitude and evidence of their reasonings; but I had not as yet a precise knowledge of their true use; and thinking that they but contributed to the advancement of the mechanical arts, I was astonished that foundations, so strong and solid, should have had no loftier superstructure reared on them. On the other hand, I compared the disquisitions of the ancient moralists to very towering and magnificent palaces with no better foundation than sand and mud: they laud the virtues very highly, and exhibit them as estimable far above anything on earth; but they give us no adequate criterion of virtue, and frequently that which they designate with so fine a name is but apathy, or pride, or despair, or parricide.

I revered our theology, and aspired as much as any one to reach heaven: but being given assuredly to understand that the way is not less open to the most ignorant than to the most learned, and that the revealed truths which lead to heaven are above our comprehension, I did not presume to subject them to the impotency of my reason; and I thought that in order competently to undertake their examination, there was need of some special help from heaven, and of being more than man.

Of philosophy I will say nothing, except that when I saw that it had been cultivated for many ages by the most distinguished men, and that yet there is not a single matter within its sphere which is not still in dispute, and nothing, therefore, which is above doubt, I did not presume to anticipate that my success would be greater in it than that of others; and further, when I considered the number of conflicting opinions touching a single matter that may be upheld by learned men, while there can be but one true, I reckoned as well-nigh false all that was only probable.

As to the other sciences, inasmuch as these borrow their principles from philosophy, I judged that no solid superstructures could be reared on foundations so infirm; and neither the honor nor the gain held out by them was sufficient to determine me to their cultivation: for I was not, thank Heaven, in a condition which compelled me to make merchandise of science for the bettering of my fortune; and though I might not profess to scorn glory as a cynic, I yet made very slight account of that honor which I hoped to acquire only through fictitious titles. And, in fine, of false sciences I thought I knew the worth sufficiently to escape being deceived by the professions of an alchemist, the predictions of an astrologer, the impostures of a magician, or by the artifices and boasting of any of those who profess to know things of which they are ignorant.

For these reasons, as soon as my age permitted me to pass from under the control of my instructors, I entire y abandoned the study of letters, and resolved no longer to seek any other science

than the knowledge of myself, or of the great book of the world. I spent the remainder of my youth in traveling, in visiting courts and armies, in holding intercourse with men of different dispositions and ranks, in collecting varied experience, in proving myself in the different situations into which fortune threw me, and, above all, in making such reflection on the matter of my experience as to secure my improvement. For it occurred to me that I should find much more truth in the reasonings of each individual with reference to the affairs in which he is personally interested, and the issue of which must presently punish him if he has judged amiss, than in those conducted by a man of letters in his study, regarding speculative matters that are of no practical moment, and followed by no consequences to himself, farther, perhaps, than that they foster his vanity the better the more remote they are from common sense; requiring, as they must in this case, the exercise of greater ingenuity and art to render them probable. In addition, I had always a most earnest desire to know how to distinguish the true from the false, in order that I might be able clearly to discriminate the right path in life, and proceed in it with confidence.

It is true that, while busied only in considering the manners of other men, I found here, too, scarce any ground for settled conviction, and remarked hardly less contradiction among them than in the opinions of the philosophers. So that the greatest advantage I derived from the study consisted in this, that, observing many things which, however extravagant and ridiculous to our apprehension, are yet by common consent received and approved by other great nations, I learned to entertain too decided a belief in regard to nothing of the truth of which I had been persuaded merely by example and custom; and thus I gradually extricated myself from many errors powerful enough to darken our natural intelligence, and incapacitate us in great measure from listening to reason. But after I had been occupied several years in thus studying the book of the world, and in essaying to gather some experience, I at length resolved to make myself an object of study, and to employ all the powers of my mind in choosing the paths I ought to follow, an undertaking which was accompanied with greater success than it would have been had I never quitted my country or my books.

PART 2

I was then in Germany, attracted thither by the wars in that country, which have not yet been brought to a termination; and as I was returning to the army from the coronation of the emperor, the setting in of winter arrested me in a locality where, as I found no society to interest me, and was besides fortunately undisturbed by any cares or passions, I remained the whole day in seclusion, with full opportunity to occupy my attention with my own thoughts. Of these one of the very first that occurred to me was, that there is seldom so much perfection in works composed of many separate parts, upon which different hands had been employed, as in those completed by a single master. Thus it is observable that the buildings which a single architect has planned and executed, are generally more elegant and commodious than those which several have attempted to improve, by making old walls serve for purposes for which they were not originally built. Thus also, those ancient cities which, from being at first only villages, have become, in course of time, large towns, are usually but ill laid out compared with the regularity constructed towns which a professional architect has freely planned on an open plain; so that although the several buildings of the former may often equal or surpass in beauty those of the latter, yet when one observes their indiscriminate juxtaposition, there a large one and here a small, and the consequent crookedness and irregularity of the streets, one is disposed to allege that chance rather than any human will guided by reason must have led to such an arrangement. And if we consider that nevertheless there have been at all times certain officers whose duty it was to see that private buildings contributed to public ornament, the difficulty of reaching high perfection with but the materials of others to operate on, will be readily acknowledged. In the same way I fancied that those nations which, starting from a semi-barbarous state and advancing to civilization by slow degrees, have had their laws successively determined, and, as it were, forced upon hem simply by experience of the hurtfulness of particular crimes and disputes, would by this process come to be possessed of less perfect institutions than those which, from the commencement of their association as communities, have followed the appointments of some wise legislator. It is thus quite certain that the constitution of the true religion, the ordinances of which are derived from God, must be incomparably superior to that of every other. And, to speak of human affairs, I believe that the pre-eminence of Sparta was due not to the goodness of each of its laws in particular, for many of these were very strange, and even opposed to good morals, but to the circumstance that, originated by a single individual, they all tended to a single end. In the same way I thought that the sciences contained in books (such of them at least as are made up of probable reasonings, without demonstrations), composed as they are of the opinions of many different individuals massed together, are farther removed from truth than the simple inferences which a man of good sense using his natural and unprejudiced judgment draws respecting the matters of his experience. And because we have all to pass through a state of infancy to manhood, and have been of necessity, for a length of time, governed by our desires and preceptors (whose dictates were frequently conflicting, while neither perhaps always counseled us for the best), I farther concluded that it is almost impossible that our judgments can be so correct or solid as they would have been, had our reason been mature from the moment of our birth, and had we always been guided by it alone.

It is true, however, that it is not customary to pull down all the houses of a town with the single design of rebuilding them differently, and thereby rendering the streets more handsome; but it often happens that a private individual takes down his own with the view of erecting it anew, and that people are even sometimes constrained to this when their houses are in danger of falling from age, or when the foundations are insecure. With this before me by way of example, I was persuaded that it would indeed be preposterous for a private individual to think of reforming a state by fundamentally changing it throughout, and overturning it in order to set it up amended; and the same I thought was true of any similar project for reforming the body of the sciences, or the order of teaching them established in the schools: but as for the opinions which up to that time I had embraced, I thought that I could not do better than resolve at once to sweep them wholly away, that I might afterwards be in a position to admit either others more correct, or even perhaps the

same when they had undergone the scrutiny of reason. I firmly believed that in this way I should much better succeed in the conduct of my life, than if I built only upon old foundations, and leaned upon principles which, in my youth, I had taken upon trust. For although I recognized various difficulties in this undertaking, these were not, however, without remedy, nor once to be compared with such as attend the slightest reformation in public affairs. Large bodies, if once overthrown, are with great difficulty set up again, or even kept erect when once seriously shaken, and the fall of such is always disastrous. Then if there are any imperfections in the constitutions of states (and that many such exist the diversity of constitutions is alone sufficient to assure us), custom has without doubt materially smoothed their inconveniences, and has even managed to steer altogether clear of, or insensibly corrected a number which sagacity could not have provided against with equal effect; and, in fine, the defects are almost always more tolerable than the change necessary for their removal; in the same manner that highways which wind among mountains, by being much frequented, become gradually so smooth and commodious, that it is much better to follow them than to seek a straighter path by climbing over the tops of rocks and descending to the bottoms of precipices.

Hence it is that I cannot in any degree approve of those restless and busy meddlers who, called neither by birth nor fortune to take part in the management of public affairs, are yet always projecting reforms; and if I thought that this tract contained aught which might justify the suspicion that I was a victim of such folly, I would by no means permit its publication. I have never contemplated anything higher than the reformation of my own opinions, and basing them on a foundation wholly my own. And although my own satisfaction with my work has led me to present here a draft of it, I do not by any means therefore recommend to every one else to make a similar attempt. Those whom God has endowed with a larger measure of genius will entertain, perhaps, designs still more exalted; but for the many I am much afraid lest even the present undertaking be more than they can safely venture to imitate. The single design to strip one's self of all past beliefs is one that ought not to be taken by every one.

The majority of men is composed of two classes, for neither of which would this be at all a befitting resolution: in the first place, of those who with more than a due confidence in their own powers, are precipitate in their judgments and want the patience requisite for orderly and circumspect thinking; whence it happens, that if men of this class once take the liberty to doubt of their accustomed opinions, and quit the beaten highway, they will never be able to thread the byway that would lead them by a shorter course, and will lose themselves and continue to wander for life; in the second place, of those who, possessed of sufficient sense or modesty to determine that there are others who excel them in the power of discriminating between truth and error, and by whom they may be instructed, ought rather to content themselves with the opinions of such than trust for more correct to their own reason.

For my own part, I should doubtless have belonged to the latter class, had I received instruction from but one master, or had I never known the diversities of opinion that from time immemorial have prevailed among men of the greatest learning. But I had become aware, even so early as during my college life, that no opinion, however absurd and incredible, can be imagined, which has not been maintained by some on of the philosophers; and afterwards in the course of my travels I remarked that all those whose opinions are decidedly repugnant to ours are not in that account barbarians and savages, but on the contrary that many of these nations make an equally good, if not better, use of their reason than we do. I took into account also the very different character which a person brought up from infancy in France or Germany exhibits, from that which, with the same mind originally, this individual would have possessed had he lived always among the Chinese or with savages, and the circumstance that in dress itself the fashion which pleased us ten years ago, and which may again, perhaps, be received into favor before ten years have gone, appears to us at this moment extravagant and ridiculous. I was thus led to infer that the ground of our opinions is far more custom and example than any certain knowledge. And, finally, although such be the ground of our opinions, I remarked that a plurality of suffrages is no guarantee of truth where it is at all of difficult discovery, as in such cases it is much more likely that it will be found by one than by many. I could, however, select from the crowd no one whose opinions seemed worthy of preference, and thus I found myself constrained, as it were, to use my own reason in the conduct of my life.

But like one walking alone and in the dark, I resolved to proceed so slowly and with such circumspection, that if I did not advance far, I would at least guard against falling. I did not even choose to dismiss summarily any of the opinions that had crept into my belief without having been introduced by reason, but first of all took sufficient time carefully to satisfy myself of the general nature of the task I was setting myself, and ascertain the true method by which to arrive at the knowledge of whatever lay within the compass of my powers.

Among the branches of philosophy, I had, at an earlier period, given some attention to logic, and among those of the mathematics to geometrical analysis and algebra, -- three arts or sciences which ought, as I conceived, to contribute something to my design. But, on examination, I found that, as for logic, its syllogisms and the majority of its other precepts are of avail- rather in the communication of what we already know, or even as the art of Lully, in speaking without judgment of things of which we are ignorant, than in the investigation of the unknown; and although this science contains indeed a number of correct and very excellent precepts, there are, nevertheless, so many others, and these either injurious or superfluous, mingled with the former, that it is almost quite as difficult to effect a severance of the true from the false as it is to extract a Diana or a Minerva from a rough block of marble. Then as to the analysis of the ancients and the algebra of the moderns, besides that they embrace only matters highly abstract, and, to appearance, of no use, the former is so exclusively restricted to the consideration of figures, that it can exercise the understanding only on condition of greatly fatiguing the imagination; and, in the latter, there is so complete a subjection to certain rules and formulas, that there results an art full of confusion and obscurity calculated to embarrass, instead of a science fitted to cultivate the mind. By these considerations I was induced to seek some other method which would comprise the advantages of the three and be exempt from their defects. And as a multitude of laws often only

hampers justice, so that a state is best governed when, with few laws, these are rigidly administered; in like manner, instead of the great number of precepts of which logic is composed, I believed that the four following would prove perfectly sufficient for me, provided I took the firm and unwavering resolution never in a single instance to fail in observing them.

The first was never to accept anything for true which I did not clearly know to be such; that is to say, carefully to avoid precipitancy and prejudice, and to comprise nothing more in my judgement than what was presented to my mind so clearly and distinctly as to exclude all ground of doubt. The second, to divide each of the difficulties under examination into as many parts as possible, and as might be necessary for its adequate solution. The third, to conduct my thoughts in such order that, by commencing with objects the simplest and easiest to know, I might ascend by little and little, and, as it were, step by step, to the knowledge of the more complex; assigning in thought a certain order even to those objects which in their own nature do not stand in a relation of antecedence and sequence.

And the last, in every case to make enumerations so complete, and reviews so general, that I might be assured that nothing was omitted.

The long chains of simple and easy reasonings by means of which geometers are accustomed to reach the conclusions of their most difficult demonstrations, had led me to imagine that all things, to the knowledge of which man is competent, are mutually connected in the same way, and that there is nothing so far removed from us as to be beyond our reach, or so hidden that we cannot discover it, provided only we abstain from accepting the false for the true, and always preserve in our thoughts the order necessary for the deduction of one truth from another. And I had little difficulty in determining the objects with which it was necessary to commence, for I was already persuaded that it must be with the simplest and easiest to know, and, considering that of all those who have hitherto sought truth in the sciences, the mathematicians alone have been able to find any demonstrations, that is, any certain and evident reasons, I did not doubt but that such must have been the rule of their investigations. I resolved to commence, therefore, with the examination of the simplest objects, not anticipating, however, from this any other advantage than that to be found in accustoming my mind to the love and nourishment of truth, and to a distaste for all such reasonings as were unsound. But I had no intention on that account of attempting to master all the particular sciences commonly denominated mathematics: but observing that, however different their objects, they all agree in considering only the various relations or proportions subsisting among those objects, I thought it best for my purpose to consider these proportions in the most general form possible, without referring them to any objects in particular, except such as would most facilitate the knowledge of them, and without by any means restricting them to these, that afterwards I might thus be the better able to apply them to every other class of objects to which they are legitimately applicable. Perceiving further, that in order to understand these relations I should sometimes have to consider them one by one and sometimes only to bear them in mind, or embrace them in the aggregate, I thought that, in order the better to consider them individually, I should view them as subsisting between straight lines, than which I could find no objects more simple, or capable of being more distinctly represented to my imagination and senses; and on the other hand, that in order to retain them in the memory or embrace an aggregate of many, I should express them by certain characters the briefest possible. In this way I believed that I could borrow all that was best both in geometrical analysis and in algebra, and correct all the defects of the one by help of the other.

And, in point of fact, the accurate observance of these few precepts gave me, I take the liberty of saying, such ease in unraveling all the questions embraced in these two sciences, that in the two or three months I devoted to their examination, not only did I reach solutions of questions I had formerly deemed exceedingly difficult but even as regards questions of the solution of which I continued ignorant, I was enabled, as it appeared to me, to determine the means whereby, and the extent to which a solution was possible; results attributable to the circumstance that I commenced with the simplest and most general truths, and that thus each truth discovered was a rule available in the discovery of subsequent ones. Nor in this perhaps shall I appear too vain, if it be considered that, as the truth on any particular point is one whoever apprehends the truth, knows all that on that point can be known. The child, for example, who has been instructed in the elements of arithmetic, and has made a particular addition, according to rule, may be assured that he has found, with respect to the sum of the numbers before him, and that in this instance is within the reach of human genius. Now, in conclusion, the method which teaches adherence to the true order, and an exact enumeration of all the conditions of the thing .sought includes all that gives certitude to the rules of arithmetic.

But the chief ground of my satisfaction with thus method, was the assurance I had of thereby exercising my reason in all matters, if not with absolute perfection, at least with the greatest attainable by me: besides, I was conscious that by its use my mind was becoming gradually habituated to clearer and more distinct conceptions of its objects; and I hoped also, from not having restricted this method to any particular matter, to apply it to the difficulties of the other sciences, with not less success than to those of algebra. I should not, however, on this account have ventured at once on the examination of all the difficulties of the sciences which presented themselves to me, for this would have been contrary to the order prescribed in the method, but observing that the knowledge of such is dependent on principles borrowed from philosophy, in which I found nothing certain, I thought it necessary first of all to endeavor to establish its principles. And because I observed, besides, that an inquiry of this kind was of all others of the greatest moment, and one in which precipitancy and anticipation in judgment were most to be dreaded, I thought that I ought not to approach it till I had reached a more mature age (being at that time but twenty-three), and had first of all employed much of my time in preparation for the work, as well by eradicating from my mind all the erroneous opinions I had up to that moment accepted, as by amassing variety of experience to afford materials for my reasonings, and by continually exercising myself in my chosen method with a view to increased skill in its application.

.....to be continued in next issue of ANS

Polycyclic aromatic hydrocarbons in air and their neurotoxic potency in association with oxidative stress : A brief perspective

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic organic chemicals widely distributed in the environment and in food stuffs. PAHs such as Benzo a pyrene, [B (a) P] essentially enters either through the ingestion of contaminated food and water or by the inhalation of particulates in the ambient air. The link between B (a) P metabolism and oxidative damage appears to be one of the key pleiotropic modulators which may be involved with several pathological processes because of its high affinity for lipid-rich tissues such as brain. B (a) P can enhance the generation of reactive oxygen species (ROS) by inducing cytochrome P450 enzymes and free radicals produced by B (a) P metabolism. This can alter physiological functions like neuronal development, differentiation, and signal transduction. Brain does not have a strong antioxidant defense system and has limited or poor ability to replace adult neurons. The molecular mechanism of oxygen derived species such as superoxide radicals, hydrogen peroxide, singlet oxygen and hydroxyl radicals produced by B (a) P metabolism and its implication in the etiology of wide array of neurological disorders remain elusive. ROS can be involved in the neuropathology of bipolar disorder, schizophrenia, and possible reduction in cognitive abilities among infants, and it is known to play janus like role of possessing both deleterious and beneficial effects. This review discusses the role of oxidative stress derived from B (a) P metabolism relating to neurological disorders, which can introduce new targets for the development of therapeutic interventions.

KEY WORDS : Polycyclic Aromatic Hydrocarbons, PAH; Benzo (a) pyrene , Reactive Oxygen Species (ROS), Oxidative stress, Behavioral Neurotoxicity

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, among which benzo (a) pyrene (B (a) P) is a PAH having five aromatic rings in a fused, honey comb-like structure. B (a) P is toxic to humans and laboratory animals and is generated through the burning of fossil fuels or wood, and is notably found in diesel exhaust particles, cigarette smoke, charcoal-cooked foods and industrial waste by products[1]. B (a) P is not manufactured and has no industrial use, but produced by industries involved in the production of aluminum, graphite, coal and asphalt and ubiquitously distributed throughout the environment. Human environmental exposure to B (a) P mainly occurs through cigarettes, ingestion of contaminated food and water[1]. Their levels in mainstream tobacco smoke are reported to be 20-40ng/cigarette[2], coke oven workers are exposed to about 42μg/m^3 B (a) P[3]. Indoor exposure to B (a) P from cooking oil fumes has been reported to be 20 μg/ m^3.[4] Lioy *et al*., estimated that B (a) P intake ranged from 20-800ng/m^3 in people living in the vicinity of hazardous waste sites contaminated by PAHs[5]. Similarly, B (a) P emitted from wood combustion in rural houses has been measured as high as 100μg/m^3.[6]

Benzo (a) pyrene is generally large, flat molecule built from a collection of fused benzene-like rings and have a relatively low solubility in water, since they are rich in carbon and are hydrophobic. It can pass easily through the cell membranes and travel quickly into the cells. Benzo (a) pyrene does not attack DNA directly, but forms an intermediate within cells, with a reactive epoxide ring, for e.g. the 9, 10-epoxide (BPDE) that damages cellular macromolecules like proteins, lipids and DNA (7). The bay region diol epoxides of PAHs are widely accepted as the ultimate carcinogenic forms of PAHs through their covalent binding to DNA. If this were so, one would expect that they would be carcinogenic at lower concentrations than the parental hydrocarbon. The formation and accumulation of B (a) P diol epoxide (BPDE)-DNA adducts, as represented by the common BPDE, are considered a critical early event in the initiation of carcinogenesis[8]. In addition, the radical cationic forms of B (a) P may be involved in both the metabolism and metabolic activation leading to the formation of DNA adducts. However, the mechanism of the promotion stage remains unclear. Among the known biological molecules, lipids are considered to be extremely susceptible to the presence of reactive oxygen species (ROS). In particular, unsaturated fatty acids located in the neuronal membranes are prone to ROS attack, producing lipid peroxides[9]. B (a) P is reported to disturb the antioxidant defense system and responsible to induce oxidative stress and has great potential for causing adverse effects due to redox cycling with their semiquinone radicals generating ROS, increasing oxidative stress and DNA damage. Antioxidants are critical in combating oxidative stress in neurons by scavenging free radicals. Induction of oxidative stress has been proposed previously as a possible mechanism of action for many pathological changes[10].

B (a) P as an environmental neurotoxic compound

As the normal functioning of the brain is essentially dependent on an adequate oxygen supply to maintain energy metabolism, it is also vulnerable to oxidative damage, which can cause alterations in gene expression, impaired cellular signaling, and disruption of membrane integrity, altered neurotransmission and causing neuronal cell death[11]. Due to its high metabolic rate and relatively reduced capacity for cellular regeneration compared with other organs, the brain is believed to be particularly susceptible to the damaging affects of reactive oxygen species (ROS), which are derived from the metabolism of molecular oxygen[12-14]. The ROS decrease the antioxidant capacity or inhibit the antioxidant enzyme activity culminating in toxicant induced oxidative stress[15]. These include its comparatively high oxygen utilization and hence generation

of free radical by-products, its modest antioxidant defences, its lipid–rich constitution that provides ready substrates for oxidation, the reducing potential of certain neurotransmitters, and the presence of redox-catalytic metals such as iron and copper[16]. Additionally, the brain is also susceptible to secondary and self–perpetuating damage from oxidative cellular injury and the activated inflammatory response[17]. In central nervous system (CNS), neurons derive their energy almost completely from oxidative phosphorylation in the respiratory chain of the mitochondria and adenosine triphosphate is generated by the reduction of oxygen to water through the sequential addition of four electrons and four protons. During this process, a leakage of high-energy electrons can potentially cause the formation of superoxide radicals $O_2^{\cdot}$ and through the action of superoxide dismutase (SOD), ultimately produce hydrogen peroxide (H_2O_2)[18]. Under conditions in which mitochondrial superoxide generation increases, or when antioxidant systems are depleted, H_2O_2 may accumulate and react with mitochondrial Fe^{2+}, resulting in formation of reactive hydroxyl radicals ($OH^{\cdot}$ & OH^{-}) via Fenton reaction[19]. Although H_2O_2 is not considered to be a free radical, it can easily form superoxide and hydroxyl radicals.

Normally, in healthy cells, there is a fine-tuned equilibrium between the generation of the reactive oxygen species and different enzymatic and non-enzymatic antioxidant defense systems. ROS normally exists in all aerobic cells in balance with biochemical antioxidants but, these have been shown to be modulated in diseases caused by free radical attack[20]. Oxidative stress occurs when this critical balance is disrupted by excess ROS production or a deficiency in an antioxidant system through B (a) P metabolism[21]. Although the reason for this oxidative stress is not completely understood, it may be caused by the accumulation of toxic metabolites produced by B (a) P metabolism that leads to the excessive production of free radicals. Again an unusual increase in metabolic by-products directly, or indirectly, depletes a cell's antioxidant capacity. When a cell's pro-oxidants exceed its antioxidant capacity, free radicals accumulate and oxidative stress occurs (Fig.1). In brief, these free radicals play integral roles in cellular signaling, physiological immunological responses and mitosis. The resultant cellular damage may range from cellular structural damage and mitotic arrest, to apoptosis and cell necrosis, depending on the level of oxidative stress severity[22-23].

Induction of Cytochrome P450s (CYP) enzymes by B (a) P

The cytochrome P450 (CYP) are a superfamily of ubiquitous enzymes involved in the metabolism of a wide range of either endogenous or exogenous (xenobiotic) compounds. The elimination of PAHs, from organisms is mediated by enzymatic oxidation which the monooxygenase enzyme system with the CYPs as a functional link. CYP1A1 is an isoform that is highly induced by planar aromatic compounds like benzo (a) pyrene and which is able to metabolize a wide range of substrates, in particular PAHs. Benzo (a) pyrene, a model of PAH, undergoes a metabolic activation to form reactive intermediates before it is capable of inducing its mutagenic and carcinogenic effects in biological systems. The first step during B (a) P metabolism is to attach some hand-holds onto these slippery molecules like cytochrome P450. These cytochrome P450 enzymes add oxygen atoms to the rings, making them more water soluble and creating anchors for attachment of larger groups, like sugars or glutathione leading to their elimination. Unfortunately, some of the intermediate forms are highly dangerous and cause damage before they can be removed[24]. However, there is convincing evidence suggesting that high CYP 1A1 activity could lead to toxicity e.g. during B (a) P metabolism. B (a) P when metabolized via Cytochrome P450s sometimes changes into B (a) P-7, 8-oxide, which through hydration by epoxide hydrolase, is metabolized to B (a) P-trans-7, 8-dihydrodiol [B (a) P-7, 8-DHD]. B (a) P-7, 8-DHD may then serves as a substrate for a second CYP-dependent oxidation reaction, generating the ultimate carcinogenic metabolite B (a) P-7, 8-dihydroxy-9, 10-epoxide (BPDE), which could have profound effects on neurological functioning and other health

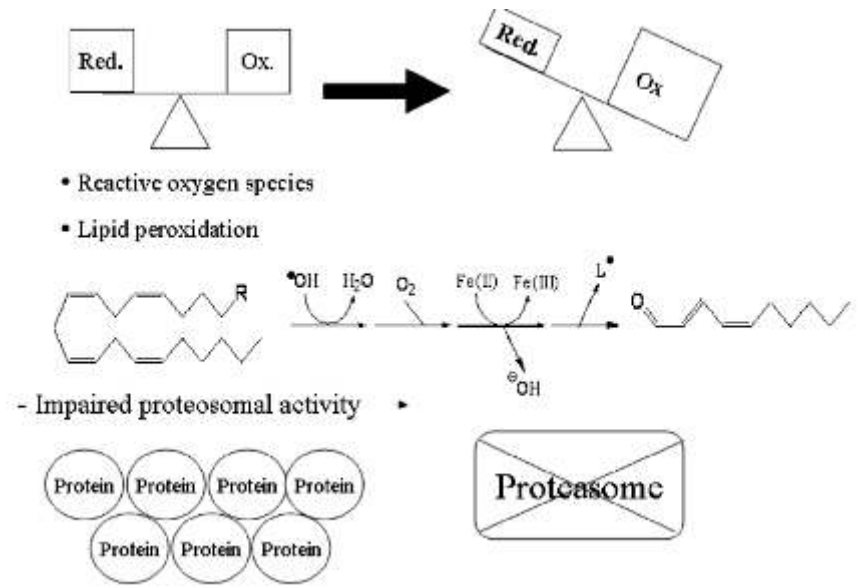

Figure 1. Oxidative stress vs. antioxidant defenses

Reactive oxygen Species ROS normally exists in all aerobic cells in balance with biochemical antioxidants. During B a P metabolism, when the bioactivation exceeds the detoxification it causes in production of biologically reactive metabolites, resulting in the formation of ROS. The increase in lipid peroxidation and decrease in antioxidant defense systems may lead to oxidative stress, sometimes result in insoluble protein an aggregate which fails to be degraded by the existing cellular machinery and accumulates within the cytoplasm.

related issues. In the nucleus, the BPDE may covalently bind to DNA, mainly forming deoxyguanoside-DNA adducts[7], which may result in misreplication and mutagenesis[25]. The ratio between cytochrome P450 enzymes like CYP1A1 and phase II enzyme activities is critical to avoid the accumulation of putatively toxic reactive intermediates of B (a) P metabolism. B (a) P also act as both a substrate and inducer of this cytochrome P450 enzyme activity[26], which converts these substances into more polar, oxygenated products, facilitating their elimination from the cell. Another mechanism is the production of ROS, through which CYP1A1 could lead to toxicity by using B (a) P as a substrate[27]. Hence, the physiological significance of the small amounts of this cytochrome present in brain microsome would depend on several properties like substrate specificity, inducibility, and distribution within the CNS. Thus, high CYP1A1 activity within the cell may be deleterious because of the generation of an intracellular oxidative stress and the subsequent oxidation of biological molecules. When the production of ROS is overwhelming, it will cause necrosis because of the irreversible degradation of cellular macromolecules and can induce apoptosis[28-29]. However, some of the intermediates generated during B (a) P metabolism are chemically reactive, electrophilic derivatives which can be more toxic than the parent compound[30].

In addition to effects of the PAH metabolites, there is another aspect of the action of this class of carcinogens caused by interaction of a nonmetabolised compound with the cytoplasmic Ah-receptor (Aromatic hydrocarbon-receptor). Although, B (a) P itself is not genotoxic, its biological effects are initiated by binding to a ligand-dependent transcription factor termed aromatic hydrocarbon receptor (AhR)[31]. This ligand–bound AhR is translocated to the nucleus and forms a heterodimer with aryl hydrocarbon receptor nuclear translocator (ARNT). After metabolic activation within the cells, some active metabolites of B (a) P trigger to form Ligand-activated AhR-ARNT complexes and can interact with specific promoter elements xenobiotic-response elements (XREs) to initiate the transcriptional activation of many genes, including members of the cytochrome P450 enzymes such as CYP1A1, CYP1A2 and CYP1B1[32]. Mainly, Phase I enzymes are responsible for the production of aryl hydrocarbon hydrolase (AHH), and the oxidative metabolism of AhR ligands. B (a) P is metabolized by AHH to a procarcinogen compound BPDE, (+-)-anti-7β, 8α-dihydroxy-9α, 10a-epoxy-7, 8, 9, 10-tetrahydropyre), which binds to DNA and forms predominantly covalent (+) trans adducts at the N_2 (N2) position of guanine[33]. Phase I enzymes also increase the production of reactive oxygen species (ROS)[34], which have been shown to be associated with lipid peroxidation, oxidative DNA damage and other pathological effects[35]. The biochemical and molecular studies by Nebert *et al*.,[31] also established that AhR plays a key role in cell-cycle regulation and apoptosis. AhR activation by PAHs including B (a) P leads to the induction of AHH, which generate reactive metabolites from the parent compound (by the AhR regulation of AHH enzyme), and which contribute to apoptosis and other cellular damage in biological system.

Studies performed in *in vitro* have demonstrated clearly that CYP1A1 is involved in the metabolic activation of B (a) P into reactive intermediates, rather than the non-metabolised parent compound, and is responsible for B (a) P–mediated mutations, cancer and birth defects[36]. Among the various forms of P450 determined so far, CYP1A1 and CYP1B1 have been shown to be the most important human P450 enzymes in the metabolic activation of PAHs and PAH dihydrodiols[37]. Several cytochrome P450 enzymes are associated with key steps in the oxidation of B (a) P, namely 7, 8-epoxidation of B (a) P and 9; 10-epoxidation of B (a) P-7, 8-diol and CYP1A1 has been demonstrated to be the most active in these oxidations in mammals[38]. Uncoupling of electron transfer and oxygen reduction from monoxygenation by CYP1A1 and CYP1A2 can result in the release of O_2^-, H_2O_2 and OH^-[39]. These reactive oxygen species or oxyradicals react with DNA, proteins and membrane lipids in the intracellular milieu[31,40] thereby contribute to cytotoxic and neurological deficits. Many of the recent reports primarily focus on induction of CYPs[40,42]; however, the mechanism by which the B (a) P acts on CYP450 metabolic activity has not been investigated.

Neurological effects after exposure to B (a) P

There is growing evidence that prenatal exposure to air pollutants from combustion of coal and other fossil fuels have adverse effects on fetal growth and early child neurodevelopment (Fig.2). Recent studies by Saunders *et al*., also demonstrated that oxidative stress in the CNS through the generation of reactive oxygen species and repression of enzymatic antioxidants may be a critical mechanism in the behavioral effects induced by B (a) P[21]. Furthermore, it has been reported that there is close relationship between oxidative stress and locomotor behavior and striatal function, and also between hippocampal oxidative stress and age-induced cognitive decline[43,44]. Molecular and epidemiological research has shown that fetuses and infants are more susceptible than adults to the harmful effects of a variety of environmental contaminants, including PAHs[45]. Experimental animal studies have demonstrated that B (a) P, is a toxicant and produces a variety of neurodevelopmental effects as a result of nervous system damage, including decreased motor activity; neuromuscular, physiologic, and autonomic deficits and decreased responsiveness to sensory stimuli[46-48]. It was also reported that gaiting, loss of coordination, neuromuscular weakness, decrease response to sensory motor stimuli, increased urination and defecation were demonstrated following acute exposure to B (a) P and fluranthene, a closely related PAH compound[21]. They showed inhibition of motor activity in rats exposed to 27μg/m^{3}of B (a) P by inhalation[21]. According to Perera *et al*., DNA-adducts__were associated with reduced DQs in the motor and language areas and also associated with increased odds of developmental delay in the motor area[49].

In epidemiologic studies, prenatal exposure to PAHs has been shown to be associated with reduced birth weight and head circumference[50-53]. In the present cohort, reduction of head circumference was associated with PAH-DNA adducts in

cord blood[52]. Reduction of weight or head circumference at birth has been correlated with lower IQ as well as poorer cognitive functioning and school performance in childhood[51]. In some studies prenatal exposure to inhaled B (a) P was reported to cause deficits in 'learning and memory' as revealed by the fixed-ratio performance of behavioral study and long term potentiation[47,54]. In Wistar rats cognitive deficits have been reported after administration 25mg/kg i.p. dose of 3-methylcholanthrane, another PAH compound[53,54]. According to Cardozo *et al*., unequaled oxidative stress in brain regions has some impact on spontaneous motor activity and cognition[11]. Several studies have associated PAH exposure with decrements in the mental development index on the Bayley Scales of Development at 3 years of age[48]. The deficits in development at 2-3 years of age may be educationally meaningful because compromised function at an early age may have a negative impact on subsequent school performance[55]. Others have also suggested oxidative changes, such as cumulative oxidative DNA damage, to be a common pathophysiological mechanism underlying major depression and medical co-morbidities[56].

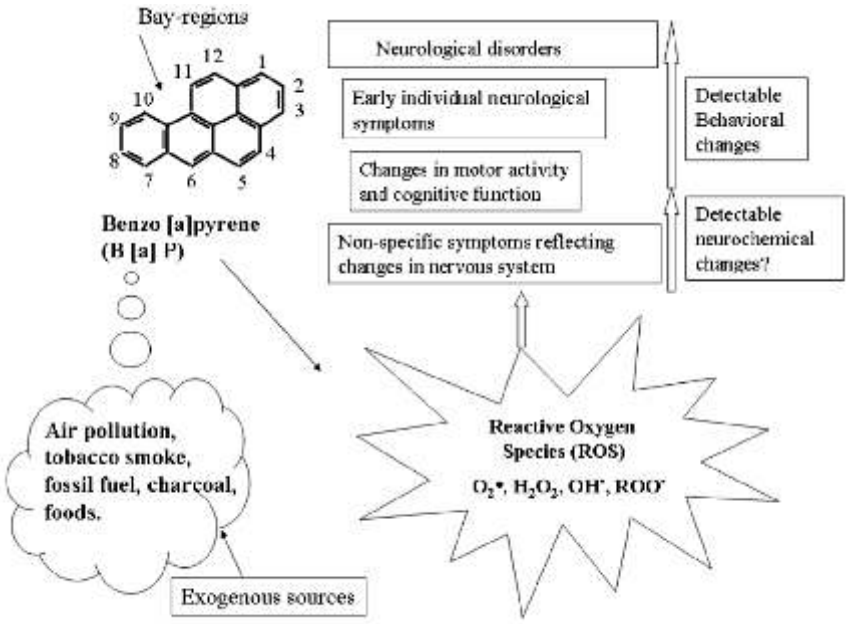

Figure 2. Potential behavioral neurotoxicity of benzo a pyrene

Human exposure to B a P essentially takes place through the ingestion or by inhalation of particulates in the air, cigarette smoking etc. The CNS may be extremely susceptible to attack by ROS reactive oxygen species derived from B (a) P metabolism, which may results repression of enzymatic antioxidants causing in behavioral changes and altering the biochemical's in brain.

Benzo (a) pyrene metabolism and oxidative stress

Oxidative stress is believed to be one of the major causes of many human diseases as it can result in severe cellular dysfunction due to peroxidation of membrane lipids, protein modification, depletion of nicotinamide nucleotides, cytoskeletal disruption and DNA damage[57]. It has been implicated as an important mechanism in the carcinogenicity of PAHs[10]. Oxidative and genotoxic stress induced by PAHs, including B (a) P, activate check point mechanisms for cell cycle control and apoptosis in mammalian cells[31]. It was reported that most of these chemicals induce free radical-mediated lipid peroxidation leading to disruption of biomembranes and dysfunction of cells and tissues[49]. It is a deleterious process that can be an important mediator of damage to cell structure and consequently have an important role in the etiology of various disease states. Some environmental chemicals, like B (a) P when led into oxidative stress, it mainly affect the rates of metabolism, growth and development, higher nervous function, as well as ability to deal with stress due to the antioxidant defence mechanism of a cell or tissue, resulting in some abnormalities. Our brain always operates using a highly intricate chemical communication system and for this to occur properly a receptor must have an affinity for specific chemical ligands or signals, to initiate a response. As brain encounters high levels of oxidative stress as it consumes ~20 % of the inhaled oxygen and possesses low levels of antioxidant enzymes then the metabolism of B (a) P via Cytochrome P-450 generates free radicals, which can disrupt the intracellular oxidant/ antioxidant balance[58]. However, the question whether uncontrolled formation of ROS species is a primary cause of down stream consequence of the pathological process. Because neurodegenerative conditions like Alzheimer's disease, Parkinson's diseases and Huntington's disease have oxidative stress implication in their pathogenesis[28]. In several recent reviews, the role of oxidative stress and oxidative damage to biomolecules has been supported by the pathogenesis of neurodegenerative disease, and specifically e.g. Alzheimer's disease[59].

Generally, three pathways have been proposed by researchers to explain B (a) P metabolism (Fig.3.). The first pathway involves the formation of epoxides, catalyzed by CYP-dependent monoxygenases. Further metabolism includes hydration by microsomal epoxide hydrolase to diols that are oxidized by CYP to produce a diol epoxide, for e.g. the B (a) P-7, 8-dihydrodiol, 10-epoxide (BPDE). The BPDE is the main metabolite that causes toxicity and carcinogenicity by covalently binding to the guanine residue on DNA to form DNA-adducts. The second pathway involves a one electron oxidation of B a P to 6-oxo-B (a) P-radical intermediates that may attack DNA resulting in depurination[8]. The third pathway involves enzymatic dehydrogenation of dihydrodiol metabolites to yield quinone intermediates that may combine directly with DNA or generate reactive oxygen species, capable of attacking DNA[60]. Therefore, quinone formation is

associated with events in the B (a) P metabolism. Of the three pathways, the epoxide pathway is the predominant pathway of B (a) P metabolism (Fig.3), and the vulnerability of neural tissues to B (a) P-induced toxicity depends on the CYT P-450-dependent metabolic capacity of the tissues[36]. Further, dihydrodiol dehydrogenase and peroxidase were reported to be involved in the metabolic conversion of B (a) P to reactive and redox active *o*-quinones, that have been demonstrated in chemical systems to undergo one electron redox cycling with their semiquinone radicals resulting in the formation of ROS and lipid peroxidation[61,62]. The increase in lipid peroxidation and subsequent decrease in antioxidant defence systems may contribute to an increased susceptibility to oxidative stress[63].The increased formation of the dihydrodiols also indicate the tilt of B (a) P metabolism towards toxification and the decreased formation of this metabolite group, points shifting the balance towards detoxification[21]. Therefore, bioactivation of B (a) P to highly reactive metabolites and decreased levels of antioxidants enzymes resulting in oxidative stress might be the cause for neurobehavioral toxicity. Continued research is needed to better understand the mechanisms and specific pathways involved in ROS-induced cell death, and to determine the most rational and effective combination of redox-active agents, resulting in some neurobehavioral changes.

Oxidative stress and neurological disorders

The theory of oxidative stress as a pathophysiological mechanism, at its most basic, can be explained by the concept, 'oxygen paradox' that while oxygen is essential for aerobic life, excessive amounts of its free radical metabolic by-products are toxic[22]. The brain does not have high levels of protective enzymes and the inability of adult neuronal cells to replicate and replace damaged DNA, and owing to lower level of glutathione peroxidase (GSH-Px), catalase (CAT) and having high levels of SOD [25, 64]. Glutathione exists in the reduced (GSH) and the oxidized (GSSG) forms, which can be inter-converted by the enzymes glutathione peroxidase (Gpx) and glutathione reductase (GR). In mammalian cells, the cycling between GSH and GSSG serves to remove reactive oxygen species (ROS), to protect the cells from oxidative stress[65]. A proper balance among these enzymes is required for an effective antioxidant defense because, excess SOD in relation to GSH-Px and CAT, enzymes of peroxide metabolism, contribute to brain pathology[66]. Depletion of GSH is associated with number of human diseases including Parkinson's disease, Alzheimer's disease[67]. The increased SOD activity was associated with manic and depressive episodes, whereas another study found a trend for reduced SOD in bipolar disorder and significantly reduced CAT levels from studies with patient samples that include other psychiatric disorders[68,69]. An increase in the lipid peroxidation product, TBARS, was also reported for both bipolar disorder and schizophrenia[70]. Some of the studies suggested that O_2^- causes changes in intracellular calcium by affecting microsomal and mitochondrial calcium stores and thereby leading to changes in signal transduction and differential gene expression. One possibility is that damage to these organelles can contribute to decreased energy production and increased levels of intracellular free calcium which, in turn can lead to cellular dysfunction and even death. Dysregulation of secondary messenger calcium has been described in bipolar disorder in response to oxidative stress[71].

Sometimes, brain is vulnerable to oxidative damage due to its high oxygen utilization, its high content of oxidisable polysaturated fatty acids (PUFAs), and the presence of redox-active metals copper and iron. The redox state of the cell is largely linked to these redox couple and is maintained within strict physiological limits. Mainly, iron regulation ensures that there is no free intracellular iron; however *in vivo*, under stress conditions an excess of superoxide releases "free iron" from iron containing molecules. The entry and release of iron from iron-storage protein, ferritin, occurs via the "free iron ferrous labile pool", active in Fenton chemistry.

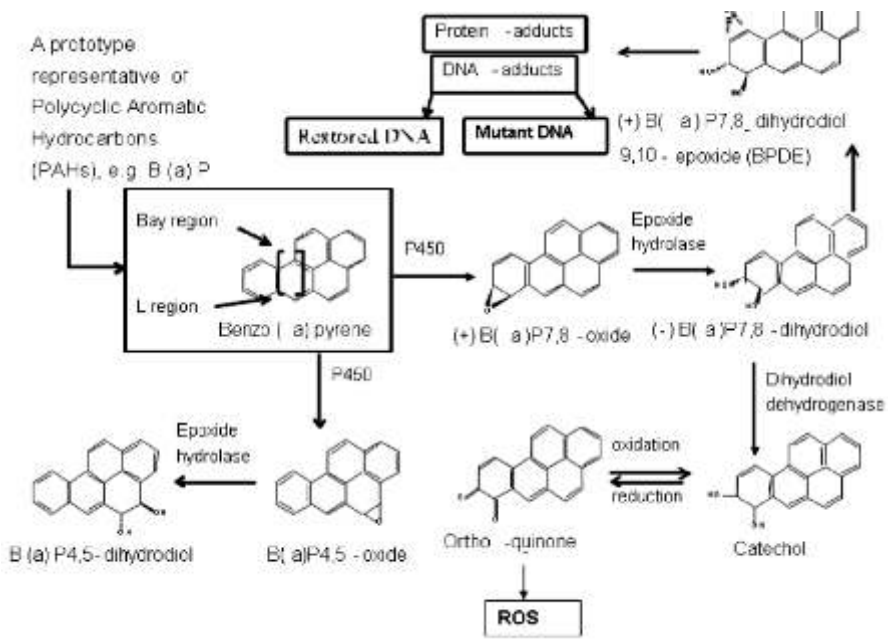

Figure 3. Metabolic pathways of Benzo a pyrene

In the profile of B a P metabolism, major primary and secondary metabolites of B a P are formed as a result of oxidation of the parent compound by the cytochrome P450 enzymes. The epoxides and hydroxyl metabolites, with further oxidation results the formation of quinines, diols, and diol epoxides, are mutagenic. These three predominant quinines can affect the redox cycle between their hydroquinone and semiquinone intermediates to generate reactive oxygen species ROS such as superoxide anion, H_2O_2 and hydroxyl radicals by Fenton chemistry, which can lead to cytotoxicity.

Besides superoxide, 6-hydroxydopamine a neurotoxin implicated in Parkinson's diseases can release ferritin iron also. There is also strong experimental evidence showing that oxidative stress and lipid peroxidative products can cause decreases in dopamine and inhibit Na+/K+ ATPase activity as well[72]. In humans, only a handful of relevant studies have been published. These reported elevated lipid peroxidation products and antioxidant changes in obsessive-compulsive panic disorder and social phobia[70,73,74]. A study of anxious women found reduced total antioxidants capacity when compared with non-anxious control group along with other parameter like impaired immune functioning[75]. The notion of oxidative stress mechanism underlying anxiety disorder has been known for some years which might play a major role in setting up a vicious etiological cycle involving free radicals, inflammatory cytokines in post traumatic stress disorder[29]. However oxidation biology research in anxiety disorder is still at its infancy and the bulk of limited literature is insufficient to generate intriguing findings.

Recent studies have also indicated that ROS play a key role in the pathophysiological pathway of wide variety of clinical and experimental diseases[26]. About 100 different types of disorders, like rheumatoid arthritis, hemorrhagic shock, cardiovascular diseases, gastro-intestinal ulcerogenesis and AIDS, have been reported as the ROS mediated disorders[16,17,76]. Some brain related specific examples of ROS-mediated diseases are Alzheimer's disease and Parkinson's disease[15,76]. In neurodegenerative diseases like Parkinson's, Alzheimer's and amyotrophic lateral sclerosis ALS, ROS damage has been reported within the specific brain region that undergo selective neurodeganeration. Protein oxidation has been reported in the hippocampus and neocortex of patients with Alzheimer's diseases, Lewy bodies in Parkinson's disease and within the motor neurons in ALS[77]. Lipid peroxidation has also been identified in the cortex and hippocampus of patients with Alzheimer's disease, substantia nigra of patients with Parkinson's disease and spinal fluid in patients with ALS. It is also known that ROS can cause neuron and astrocyte death through apoptosis and necrosis, but, B (a) P-induced apoptosis have not been fully investigated. Innate deregulation of apoptosis and oxidative processes has been suggested by a recent study, in which the hippocampal expressions of genes encoding DNA repair and antioxidant enzymes were found to be down-regulated in bipolar disorder, while many apoptosis genes were up-regulated[78]. According to Chung *et al*., B (a) P metabolites produced by Cytochrome P450 enzymes can elicits genetic toxicity by forming DNA adducts and results in DNA damage-induced apoptosis[79]. Disorders in DNA repair typically cause additional symptoms such as mental retardation, photosensitivity, immunodeficiency and neoplasia[80].

Oxidative stress is also related to glutamate release and NMDA receptor activation during cerebral ischemia-reperfusion, production of $O_2^{\cdot-}$ in neurons, brain macrophages and glutamate-induced ROS production in astrocytes. Recently, Grova *et al*., demonstrated that chronic exposure to B (a) P in adult mice modulates gene expression of NMDA NR1 subunit in brain areas that are highly involved in cognitive function like the hippocampus[81-83]. Evidences implicating ROS in major degenerative disease is also consistent with their role in brain aging[84]. Thus exposure to various environmental chemicals like B (a) P acting through oxidative stress and the reduced capacity for the homeostatic maintenance of synaptic plasticity mechanisms during brain development may contribute to subsequent behavioral learning deficits. The exact mechanism by which B (a) P administration causes reduction in antioxidant enzymes is not clear[21,85]. Thus, B (a) P–mediated toxicity might be due to its own oxidative properties, its reactive metabolites or both. It is not known whether B (a) P-mediated toxicity is due to its direct toxicity, the toxicity of metabolites or both.

Conclusions and future prospects

Many questions remain unanswered with regard to the role of B (a) P in nervous system dysfunction. Is B (a) P a full carcinogen? What is the major role of ROS and what form of the compound (original or metabolized) is acting? Whether there are changes in functioning of the same regulatory systems as in by nongenotoxic mechanism? Taken together, the study reviewed herein supports the view that an imbalance between oxidants and antioxidants and between proteases and antiproteases may play an important role in the susceptibility of CNS. It becomes clear that reduced antioxidant potential which might result from the binding of free radicals to the active sites of these enzymes, contribute to the increased oxidative stress that is associated with nervous system dysfunction. This ROS production could contribute to repress the expression of oxidative-stress-sensitive genes. The negative regulation of transcription by ROS could affect several CYP isoforms, exhibiting high CYP metabolism, which would rapidly activate the pro-apoptotic machinery leading to a massive cell death following different pathways in order to determine the cell fate. Another potential mechanism for the alterations in enzyme status might be through transcription factors causing changes in antioxidant gene expression. B (a) P is a carcinogen, which induces both the initiation and promotion stages of carcinogenesis. The mechanism of the initiation is well studied while the promotion stage of the carcinogenic action is virtually uninvestigated. We await more experiments on oxidative stress in the CNS through generation of ROSs and the change in enzymatic antioxidants, which may be the critical mechanism in neurological diseases, induced by B (a) P. These chemicals are affecting the normal growth and development at a degree far greater than ever imagined and it needs some of the challenging areas for further research in oxidative stress related diseases in brain. It will be necessary to examine the relationships between metabolite formation of PAHs and oxy-PAHs and DNA adduct formation and metabolic enzyme induction by the processes in detail. Further, more laboratory studies aimed at identifying the underlying mechanisms of B (a) P exposure, particularly on those individuals deemed to be a greatest risk, and are badly needed.

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ANS
MINI REVIEW

Circadian rhythm in patients with spinal cord injuries

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ABSTRACT

Spinal Cord Injury (SCI) is damage to the spinal cord that may result in the loss of mobility. Frequent causes of damage are trauma (motor vehicle accident, falls, etc.) or disease (polio, spina bifida, Friedreich's Ataxia, etc.). However, abnormality in circadian rhythm has been observed in SCI. Melatonin is the primary hormone of the pineal gland and acts to regulate the body's circadian rhythm. Normally, melatonin levels begin to rise in the mid-to-late evening, remains high for most of the night, and then decreases in the early morning hours. Patients with SCI have a lower melatonin secretion during the hours of darkness than in healthy subjects. This may contribute to impaired sleep at night, fatigue during the day and affect pain perception. Disturbed level of melatonin has also been reported in SCI patients that are associated with disturbances in circadian rhythm. Therefore, circadian rhythm can be important in the pathophysiology and treatment of SCI patients and it is important to know the time and the level of melatonin in order to prescribe the appropriate treatment regimen. The expanding science of circadian rhythm biology and a growing interest in human clinical research on circadian rhythm in patients with spinal cord injuries inspired this review. This article reviews the relationship of circadian rhythm in patients with SCI.

KEY WORDS : Spinal Cord Injury ; Circadian Rhythm ; Melatonin

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Introduction

A spinal cord injury usually begins with a sudden, traumatic blow to the spine that fractures or dislocates vertebrae. The damage begins at the moment of injury when displaced bone fragments, disc material, or ligaments bruise or tear into spinal cord tissue. However, injury is more likely to cause fractures and compression of the vertebrae, which then crush and destroy the *axons,* extensions of nerve cells that carry signals across the spinal cord and between the brain and the rest of the body. An injury to the spinal cord can damage a few or almost all of these axons. Some injuries will allow almost complete recovery. Others will result in complete paralysis. A patient with SCI suffers from higher ratings of pain intensity, anxiety and depression and these patients have poor sleep quality due to disturbed melatonin levels.

Circadian rhythm is characterized by the dynamics of 24 hour cycle. Human bodies are in harmony with 24hr light and dark cycles and therefore the secretion of hormones that are sensitive to light and dark orchestrate around the 24 hour cycle and thus ensure optimal functioning. The disturbances in secretion of hormones that are sensitive to dark and light cycle results in abnormal circadian rhythm. Melatonin secretion normally follows a circadian pattern and is highest at night and decreased during the day.

Many of the symptoms associated with SCI, such as difficulty in sleeping, anxiety and depression, are similar to those observed in individuals whose circadian pacemaker is abnormally aligned with their sleep-wake schedule. Many studies have indicated that cervical spine injury results in the complete loss of pineal melatonin production which further affects sleep, causes anxiety and depression[1]. This review will focus on the circadian rhythm in patients with spinal cord injuries. A detailed knowledge of circadian rhythm in patients with spinal cord injuries facilitates designing of novel drugs which combats the disturbed sleep and pain associated with this abnormal circadian rhythm in these patients.

Circadian Rhythm in Spinal Cord

Light plays dominant role in the body, affecting the secretion of melatonin and other hormones thereby affecting the person's wakefulness, blood pressure and body temperature. Serum melatonin levels in patients with spinal lesions at the cervical, thoracic or lumbar region have been formed to be low without diurnal rhythm in patients with cervical lesion (C4-5). However, diurnal rhythms were maintained with high levels in the dark period in the patient with upper thoracic spinal (T2-3) transactions. This study suggests that the cervical region of the spinal cord is part of the neural pathway essential for the diurnal rhythm of pineal melatonin secretion in human beings.[2]

Cervical spinal cord lesions disrupt the circadian rhythm and alter the human melatonin excretion. Levels of serum cortisol, aldosterone, and growth hormone have reflected the rhythmic variations in subjects with SCI. The absence of significant increase in nocturnal melatonin distinguishes quadriplegic subjects from normal males and from subjects presenting with a lesion of the lumbar spinal cord. These differences may be caused by "decentralization" of the pineal organ due to a lesion within the cervical spinal cord interrupting descending sympathetic fibers. The human pineal, like that of other mammals, is regulated, at least in part, by activity within the central nervous system via sympathetic nervous connections.[3]

Circadian blood pressure rhythm was observed in patients with higher and lower spinal cord injury when simultaneous evaluation of autonomic nervous activity and physical activity was done. This study indicated that the central sympatho-excitatory pathway to the upper thoracic cord plays a critical role in the maintenance of normal circadian blood pressure rhythm in humans. Motor nerve functioning and sympathoadrenal secretion are not essential to this regulation.[4] Reduced sleep efficiency in cervical spinal cord injury causes abolished melatonin secretion. Thus, the absence of nocturnal melatonin level in cases of cervical SCI may help in explaining the sleep disturbances which raises the possibility that melatonin replacement therapy could be a plausible alternative to resume normal sleep.[5]

In freely moving rats and monkeys, H-reflex amplitude displays a marked circadian variation without change in background motor neuron tone. In rats, the H-reflex is the largest around noon and the smallest around midnight. This study investigated the effects on circadian rhythm for calibrated contusions of mid-thoracic spinal cord as well as mid-thoracic transection on specific spinal cord pathways. It is also reported that the H-reflex circadian rhythm depends on descending influence from the brain and that, this influence is conveyed by the main corticospinal tract.[6] Thus, circadian rhythm and spinal cord injuries are found to be closely linked and hence an alteration in circadian rhythm in patients with SCI, several symptoms may arise.

Is Melatonin a Circadian Rhythm Hormone Important in Treatment of SCI Patients?

In reality circadian rhythms control the timing, quantity and quality of the hormones and neurotransmitters to be secreted by the body. Hormones and neurotransmitters are the elements that determine how we feel, the sleep patterns, appetite, sex drive and other sleep and mood-related issues. Under normal circumstances our circadian rhythms create circadian balance. When out of balance, the timing and release of hormone and neurotransmitter is disturbed which leads to circadian rhythm disorder (CRD).

A circadian rhythm disorder refers to the condition where the body produces hormones, chemicals and neurotransmitters which are not within the normal limits at the appropriate time of the day. In patients with SCI, melatonin is produced less in the evening thus affecting sleep. Circadian rhythms stimulate the timing and production of several hormones and neurotrasmitters that affect sleep and mood.

Circadian rhythms permeate practically every aspect of our lives because they strongly influence the chemicals that determine our mood and sleep. If we possess prior knowledge about various disturbances in circadian rhythm of melatonin secretion in patients with SCI, it would be helpful in the treatment of SCI patients. SCI is characterized by the diffused pain, disturbed sleep, fatigue, anxiety and depression. Previous reports suggest that in patients with SCI melatonin level remains low,[5] which causes disturbed sleep at night accompanied with diffuse pain in the morning hours.

Several studies reveal that, melatonin is potentially important as a treatment of acute spinal cord injury. The human circadian system regulates the temporal organization of several endocrine functions, including the production of melatonin (via a neural pathway that includes the spinal cord), TSH, and cortisol. In traumatic spinal cord injury, afferent and efferent circuits that influence the basal production of these hormones may be disrupted. The results of this study indicate that neurological complete cervical spinal injury results in the complete loss of pineal melatonin production and that neither the loss of melatonin nor the loss of spinal afferent information disrupts the rhythmicity of cortisol or TSH secretion.[7] One of the studies demonstrated that the treatment with melatonin reduces the development of inflammation and tissue injury events associated with spinal cord trauma.[8]

In a study on treatment of methylprednisolone (MP), melatonin, and combined treatment of MP and melatonin, there was functional recovery in patients with spinal cord injuries.[9] The effect of melatonin on an experimental spinal cord ischemia was observed and found to be protective with the result that melatonin administration may significantly reduce the incidence of spinal cord injury following temporary aortic occlusion.[10] Melatonin and oxytetracycline are effective in preventing lipid peroxidation in spinal cord injury. Paraoxonase and homocysteine can be used in monitoring the antioxidant defense system as well as superoxide dismutase and plasma glutathione peroxidase, both in injury and medicated groups.[11]

Melatonin has been shown to be very effective in protecting the injured spinal cord from secondary injury.[12] In another study on protective effects of melatonin on experimental spinal cord injury, it was indicated that injection of melatonin reduced thiobarbituric acid reactive substances content and myeloperoxidase activity, facilitating recovery of the damaged spinal cord.[13]

Melatonin may also be potentially helpful for neuropathic pain, activation of the endogenous melatonin system in the spinal cord can reduce the generation, development and maintenance of central sensitization, with a resultant inhibition of capsaicin-induced secondary mechanical allodynia and hyperalgesia.[14] Shift in melatonin production may play a crucial role in pain and abnormal sleep pattern. Thus, the treatment of melatonin in patients with SCI can reduce the pain and also help to overcome the disturbed sleep pattern.

Conclusion

Circadian rhythmicity can be important in the pathophysiology and treatment of SCI patients. The observations of abnormalities in melatonin is highly regulated by circadian pacemaker raising the possibility that there is an abnormality of circadian rhythm in patients with SCI. Controlled studies of circadian rhythm have not been performed in patients with SCI. A clear understanding on circadian pattern of SCI patients will provide useful information to augment the understanding of pathophysiology and help in the treatment of SCI patients. Until the circadian rhythms of SCI are accurately known, appropriate treatment cannot be designed.

Abbreviations

SCI- spinal cord injuries; CRD- circadian rhythm disorder; TSH- thyroid stimulating hormone; MP- Methylprednisolone.

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Brain and the whole new mind*

I am indeed honoured to have this opportunity of participating and interacting with this august gathering. We have people gathered here with expertise covering several aspects as varied as computational brain functions on the one hand to cellular and molecular events on the other, from behavioural levels to basic and applied aspects, from individual to community needs and beyond.

I felt that to address such a wide spectrum of scientists and clinicians is not at all easy. Let me therefore attempt to present a vista with multidimentional moorings on a canvas which should appear coherent and meaningful. I hope I shall be able to make at least a dent in that direction.

No doubt Scientists have long wondered how the brain can do all that it does. Thousands of scientists from many disciplines are on an unprecedented odyssey to explore the most complex matter of the universe i.e. the human brain, and considered to be the blueprint of human destiny. In many aspects it is the highest quest that humans are capable of – the brain attempting to understand brain itself.

The present century with already booming information technology and explosive knowledge expansion, clearly belongs to the era of the brain – to fathom the inner secrets of its working, and the likely implications it may have for the future of the mankind. The revolution in molecular biology and genetics along with the new tools of imaging technology which can discern the chemical traces of thoughts and emotions as they are formed, has paved the way for attempting to attain such heights. Knowing about the potentialities and powers of the brain on the one hand, and knowing how easy it is for things to go wrong with the brain, necessitates that we learn to look into our brain and to 'educate' oneself and one's brain for a purposeful future. What I plan to do is to seek your indulgence in my attempting to discuss the rapidly changing concepts about the functioning of the brain, its power and potentialities in three major dimensions:

i) What are the brain building blocks and their dynamic relevance?

ii) What are the possible damages and aberrations that may be caused in life; and

iii) To cover aspects of remedial measures – repair or renewal, inherent or induced, in the functioning of the brain.

Towards the end, I also propose to briefly cover some of the hitherto unknown aspects of mind which in a way describe a seismic shift now under way and heralding the rising of the Right brain and why Right-Brainers rather than the Left-brainers will rule the future.

Let me begin with the brain growth and development. A series of recent studies no more accept the old concept of a static brain. If anything, both morphology and the functioning of the brain are shown to be the dynamic processes. Genes no doubt configure the framework of the brain, but then the environment takes over – from fetal, neonatal to adulthood and the elderly state, and shape the things linked to these interacting influences.

The brain's real food is its external environment which according to Ronald Kotulak, it gulps "in bits and chunks through its sensory system: vision, hearing, smell, touch and taste. Then the digested world is reassembled in the form of trillions of connections between brain cells that are constantly growing or dying, becoming stronger or weaker, depending on the richness of the banquet". There are spurts in this growth. One of the most effective spurt is during the first three to four years of life by which time much of the fundamental architecture of the brain is completed and foundations laid for attributes such as language, vision, attitudes and aptitudes. The optimization of learning has a second spurt upto about age of 12 years or so. Brain at this period, is highly active as compared to adult brain and is busy in deciding whether to keep or eliminate connections, eagerly seeking information from sense organs and the sensory systems. It does not mean that brain stops learning after that. Learning, however, becomes more and more difficult, but the learning process and consequent brain growth is dynamic, constantly changing its network of trillions of connections between brain cells linked to the stimuli from its environment. Basically, brain cells compete to connect to some part of the body which serves to provide it the requisite food and feedback. If the feedback is not proper, the system fails to grow and function adequately, eg. brain cells connected to visual cortex will die out without proper visual stimulus. Children with inborn cataract are allowed to remain blind, say for two years, have been found to lose the connectivity of the brain cells to visual cortex and the cells will consequently die. If on the other hand these brain cells with connectivity to visual cortex are diverted to speech and hearing areas of the brain and proper auditory stimuli sustained over time these "visual brain cells", potentially take over the functions of the auditory cells. In other words, the functioning of brain cells is just not linked to innate architecture and connectivity, but is profoundly influenced in their functioning by appropriate stimulation particularly during the early phase of life. Part of this problem has been taken care of in nature by providing the number of cells and connectivity which far exceeds actual usage. Trillions of connections that survive the great die-off are dependent on what a child learns during this early phase of life. For instance, connections not reinforced by environmental experiences such as touch, vision, smell or hearing, will accordingly shrink and gradually perish.

Early exposure to violence, bad experiences, poverty with variety of environmental stresses like witnessing murder, stabbing, robbery, rape etc., have a strong effect on the growing brain and may be manifested later in life showing permanent consequences on child development and learning. This happens particularly in those children whose brains are genetically more vulnerable. Recent studies have further shown that stress or drugs of abuse, like cocaine and alcohol, can affect genes, can turn on new genes and set in new connections and receptors

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which may be abnormal, and likely to lead to conditions of permanent, maladaptive behaviour patterns passed on from mother to foetus and the growing child. It is being realized that one can learn bad things like depression and epilepsy and that these are encoded through genes into physical structure of the brain. It seems nature and nurture are both constantly interacting and a disharmony can set up aberrant networks, imprinting how the brain has mislearned and thus increase the risk of having a wide variety of conditions ranging from aggression and depression to epilepsy, asthma, high blood pressure and diabetes. It is thus becoming more and more clear that aberrant behaviour such as aggression or violence is ultimately rooted in early biological reactions of the brain to violence and stressful experiences one undergoes. However, this is not a one-way passage. Given the right opportunities and the right environment, it can bring about dramatic changes in impoverished children when allowed to grow in enriched environment. Sooner this change is brought about in life better are the chances of reversal and the children growing into more useful citizens.

Is there some biochemical basis of abnormal behaviours such as aggression and violence? Can bad experiences of stress or environment bring about changes and / or imbalances in such biochemical parameters? Can changes from impoverished to enriched environment reverse the abnormal balance or profile of these neuro transmitters? The answer to all these questions is yes, but a guarded yes. Brain bio-chemistry has been under close scrutiny during recent decades. Imbalances or abnormal levels of common neurotransmitters such as serotonin, noradrenaline and dopamine, are distinctly related to many of the abnormal behaviours. Low serotonin and high noradrenaline for instance, is related to aggressive behaviour. Bad stressful experiences can tilt the normal balance / values into abnormal ones. However, even a common neurotransmitter like serotonin is known to have more than 5-HT receptor subtypes, each basically concerned with a more specific condition. For example, 5 HT 1 affects aggression, while 5 HT 2 is involved in depression, 5 HT 3 in learning and memory, 5 HT 6 in emotion and cognition, and so on. Combined with this the imaging techniques such as SPECT (Single Photon Emission Computed Tomography) or fMRI and PET, and we get the visual evidence of not only static but functioning brain patterns that co-relate with behaviour like tendencies towards depression, anxiety, distractability, obsessiveness and violence. A clear message, in view of such studies, is that much more attention be paid to children in providing proper environment while the plasticity of the brain is still so flexible. Environment, negative or positive, is likely to influence the child much more as compared to an adult.

The second major domain that I wish to talk about is regarding the regeneration and repair capacities of the brain. Until recently, it was considered that cells of our brain are incapable of regeneration. Recent studies on the brain have revealed a different perspective than was taken for granted for decades. It is now known that neurons can regenerate much the same way as other tissues of the body provided right type of stimulation is provided. This further indicates that given the right stimulus brain does have the capacity to continuously heal itself and replace lost cells. These types of results have led to a tremendous upsurge in research activities to find out if chronic degenerative diseases like Alzheimer, Parkinson's, Huntington and the accompanying memory loss, tremors, dystonia, dementia and other age related signs can be aided by inducing repair of brain cells. It is claimed that our brain associated good chemicals diminish with advancing age. If their levels can be restored like young adults there could be a possibility of managing the disorders. Hormones like estrogen, progesterone, testosterone and growth hormone appear to play a key role in nurturing brain cells just like neurotrophic factors. For instance, in Alzheimer's disease treatments such as estrogen replacement or replacement of diminishing growth factors with additional mental exercises, can postpone disease progression and memory loss. Estrogen also increases sprouting of connections between brain cells, and prevents decline in acetylcholine – the chemical messenger that models new memories. Similarly, neurotrophic factors are known to maintain the normal health of brain cells. Grouping knowledge about the understanding of the role of receptors like NMDA has led to new avenues for research and development.

The discovery of a gene called Neuro-D has potentially opened vistas to learn how neurons regenerate but, more importantly, how new brain cells be made to replace those destroyed by diseases such as Alzheimer's disease. Brain's plasticity is reinforced by the role of brain–derived growth factor and glial-derived growth factor. When neurotrophic factors decline, brain cells eventually die. The discovery of neurotrophic factors has paved the way to repair damaged brain cells, rescue dying ones and generate new cells. There appears to be a growing hope that it may be possible to maintain neurologic functions considered to be diminishing with advancing age. Genetically engineered neurotrophic factors with possibility of crossing blood-brain barrier are in pipeline. This coupled with the nourishing of adult brain cells with epidermal growth factor (EGF) may serve to provide variety of brain cells that may be needed in different parts of the brain.

With ageing, free radicals tend to increase as a result of normal wear and tear of cell chemistry. Anti-oxidants reduce damage to genes and cell structure. A number of drugs acting as super-oxidant are in the market now and show a definite improvement in memory in the elderly. Drugs like PBN or Tacrine, for example, enhance the cross talk between brain cells and facilitate communication and improve memory. Also, the analogy for better mental performance in old age has been drawn from muscles of our body. If a person continues to do physical exercise, the muscles remain healthy and show very little signs, if any, of the weakening or wasting with the advancing age. But, if the muscles are not used, the muscles tend to weaken and eventually waste. It is considered that a similar situation exists for the brain.

If mental exercises and activation of the brain is continued over time as age advances, the brain is likely to remain alert and active in persons attaining the old age. Mental exercise for the brain acts in the same way as physical exercise does for the muscles of the body. It is strongly suggested that mental work and mental exercises must be continued to be undertaken. Already, methods to stimulate specific areas of the brain, say of hippocampus, serving as a memory bank for other areas of the brain could help

keep the memory sharp and have been successfully tested in a number of old people. Moreover the functioning of the brain can be substantially improved, provided the brain receives the right type of brain stimulation. This essential stimulation can be supplied by enriched environment and / or by way of various electromechanical devices now available in the market, and have been found effective to in enhancing brain functioning.

Today, more and more scientists are becoming interested in using machines also to help the healthy people in increasing their mental abilities in domains such as: learning, thinking, reasoning, and other cognitive functions. Can a machine actually make you smarter? Enhance your mental functioning? The growing evidence shows that experimental users of various mind-machines responded with dramatic improvements in IQ and EQ, long term memory, attention, reaction time, recall, hemispheric synchronization, and other capacities depending upon the machine in use. For instance, the use of Alpha-stimulator helps develop relaxation and learning in the individual. Use of CAP Scan (Computerized Automated Psychophysiological Scan) allows one to use self-regulatory techniques to eliminate the abnormal brain activity. Mind mirror, Graham potentializer, Mind eye, Tranquilite, Floatation Tank are some of the more common devices used to improve variety of physical and mental performance.

There is another highly challenging aspect of brain activity which needs to be understood. Human brain activity is predominantly focused in one hemisphere at a time, with dominance moving back and forth between the hemispheres depending on the task being performed. In the right handed persons, left hemisphere may show predominance for analytical activity while right hemisphere may show enhanced activity for creative thought. If this asymmetrical hemispheric – EEG pattern can be changed into balanced state of whole-brain integration, the power of the brain may increase several folds, and is reflected in deep relaxation and considerably enhanced learning abilities. While experienced meditators seem capable of achieving 'brain Synchronization at will', these can help one to achieve better cognitive performance.

Let me now turn to our understanding of the brain and the whole new mind. We have progressed from a society of farmers to a society of factory workers to a society of knowledge workers. And now we are progressing yet again – to a society of creators and empathizers, of pattern recognizers and meaning makers. In other words, we are moving from a society built on the logical, linear, computer like capabilities of the present Information Age – the primary attribute of the left-brain, to a new society built on the inventive, empathic amplified capabilities. A whole new mind is for anyone who wants to survive and thrive in this emerging world with legions of emotionally astute and creatively adroit people whose distinctive abilities the Information age has not only often overlooked but not infrequently undervalued.

The so called advanced society of today have been largely dominated by a form of thinking and an approach to life that is narrowly reductive and deeply analytical. Ours has been the age of knowledge-worker, the well-educated manipulator of information and deployer of expertise. But that is changing. We are beginning to witness Right-brain rising. We are all aware that our brains are divided into two hemispheres. The left hemisphere is sequential, logical and analytical and is thus visual and perceptive. The right hemisphere is nonlinear, intuitive and holistic. And, of course we enlist both halves of our brains for even the simplest tasks. But the well-established differences between the two hemispheres of the brain are a key to a powerful base to interpret our present and guide our future. Today, the defining skills of the previous era – the 'left-brain' capabilities that powered the Information Age –are necessary but not sufficient. And the capabilities we once disdained or thought frivolous – the 'right-brain' qualities of inventiveness, empathy, joyfulness and meaning – increasingly will determine who flourishes and who flounders.

The classical 'split-brain' studies undertaken by Roger W.Sperry, led him to write and I quote "The so called subordinate or minor hemisphere, which we had formerly supposed to be illiterate and mentally retarded and thought by some authorities to not even be conscious, was found to be infact the superior cerebral member when it comes to performing certain kinds of mental tasks." He further wrote that "there appear to be two models of thinking." The left hemisphere reasoned sequentially, excelled at analysis, and handled words. The right hemisphere reasoned holistically, recognized patterns and interpreted emotions and nonverbal expressions. Right hemisphere is the key to expanding human thought, surviving trauma, healing autism and more. 'It is the seat of creativity of the soul and even great casserole ideas.'

With my grooming in neurobiology and medicine, I would say that these developments and changing concepts are modifying the emphasis of many medical practices – away from routine, analytical and information-based work and lead towards empathy, narrative medicine and holistic care. We will need to complement our left-directed reasoning by mastering six essential right-directed aptitudes. According to Donald Pink, these comprise of: (a) not just function but also design, (b) not just argument but also the story; (c) not just focus but also symphony; (d) not just logic, but also empathy; (e) not just seriousness but also play, and (f) not just accumulation but also meaning. Left brain directed thinking thus remains necessary but no longer sufficient. We must become proficient in right-brain directed thinking and master aptitudes that are high concept and high touch.

Summary : We possess the necessary tools and technology for not only investigations but also enriching and expanding our mental activities. We can use these methods to influence the brain, its functions and purpose. Let us try to look inwards, balance and dovetail our emotions with intellectual pursuits with emphasis on building right-brain capabilities and tread a path to seek not only individual happiness but also ensuring the continued advancement of civilization.

K.N. Sharma
Emeritus Professor and Executive Director
M.S.Ramaiah Medical Institutions & Hospitals, Bangalore

Primary nocturnal headache – case report

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ABSTRACT

The hypnic headache syndrome or alarm clock headache is a rare entity. It can be diagnosed in a patient presenting with headache occurring during sleep. A rare case of hypnic headache well responsive to indomethacin is being presented here.

KEY WORDS : Trigeminal autonomic cephalagias, Headache, Hypnic.

Introduction

There has been exciting advances in Headache classification, diagnosis, patho-physiology and treatment. The developing field of headache includes the trigeminal autonomic cephalagias. These are rarer syndromes that are likely to be referred to the neurologists who will need to recognize and treat these. The case presented here highlights one of the rare primary nocturnal headaches which is rarely reported in India.

Case Report

A 61-year-old Hindu male presented with 3 month history of nocturnal headache and neck pain at about 3am, occurring daily. It was holocranial in distribution, lasting for about 30-45 minutes, awakening patient from sleep. It was moderate to severe in intensity, bursting in character and associated with dryness of mouth. Pain was not associated with cranial autonomic features, nausea/vomiting, photophobia, phonophobia, auras, weakness of limbs or any exploding noise sensation. There was no family history of such illness, no history of prior similar illness or any seasonal variation. The patient was not having features of raised ICP, sub- occipital or temporal pain, systemic disease or medication over use. His headache was not aggravated by dietary or psychological triggers, physical, environmental or sleep related triggers and was unrelated to head trauma, physical exertion or fatigue. It was relieved by taking one tablet of Dart. The patient was nondiabetic, nonhypertensive, nonsmoker, not addicted to tobacco, alcohol or any substance. There was no history of TIA, Cerebral stroke, anginal pain or M.I, stress, anxiety or depression. General examinations were within normal limits including peripheral pulse and blood pressure. Cardiovascular, respiratory and abdominal systems were within normal limits. On CNS examination, fundus and pupil were normal, and no focal signs were observed. Keeping the above features of new onset of nocturnal headache in mind relevant investigations were requested: ESR -- 20mm/hr, C-reactive protein –normal, CT scan of brain – normal (fig 1). Polysomnographic studies were rather supplemented by sleep history which was consistent with – REM sleep disorder, EEG - normal (fig 2).

The important considerations in differential diagnosis were both primary and secondary headache disorders. Among primary headache disorders, migraine, tension, cluster, hypnic, paroxysmal hemicranias short lasting unilateral neuralgiform headache attacks with conjunctival injections and tearing (SUNCT) and exploding head syndromes were considered. Among secondary headache disorders, Intracranial space occupying lesion (SOL), subdural hematoma (SDH) and epidural hematoma, temporal arteries, cerebro vascular accident (CVA), systemic disease and cervicogenic headaches were considered.

The present case resembles the hypnic headache which is rarely

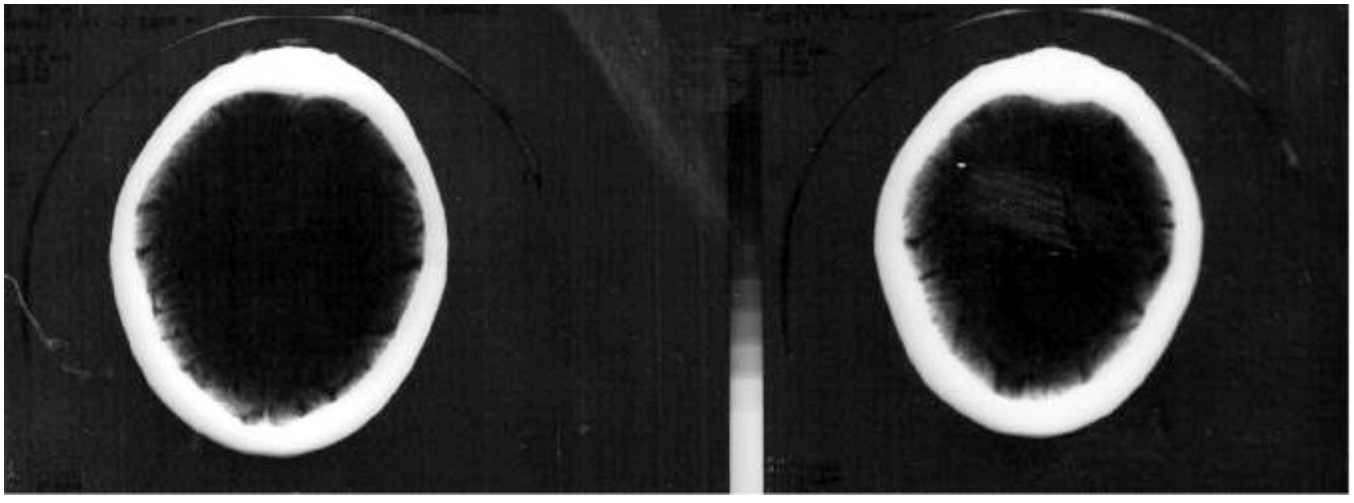

Fig. 1 : CT scan of brain

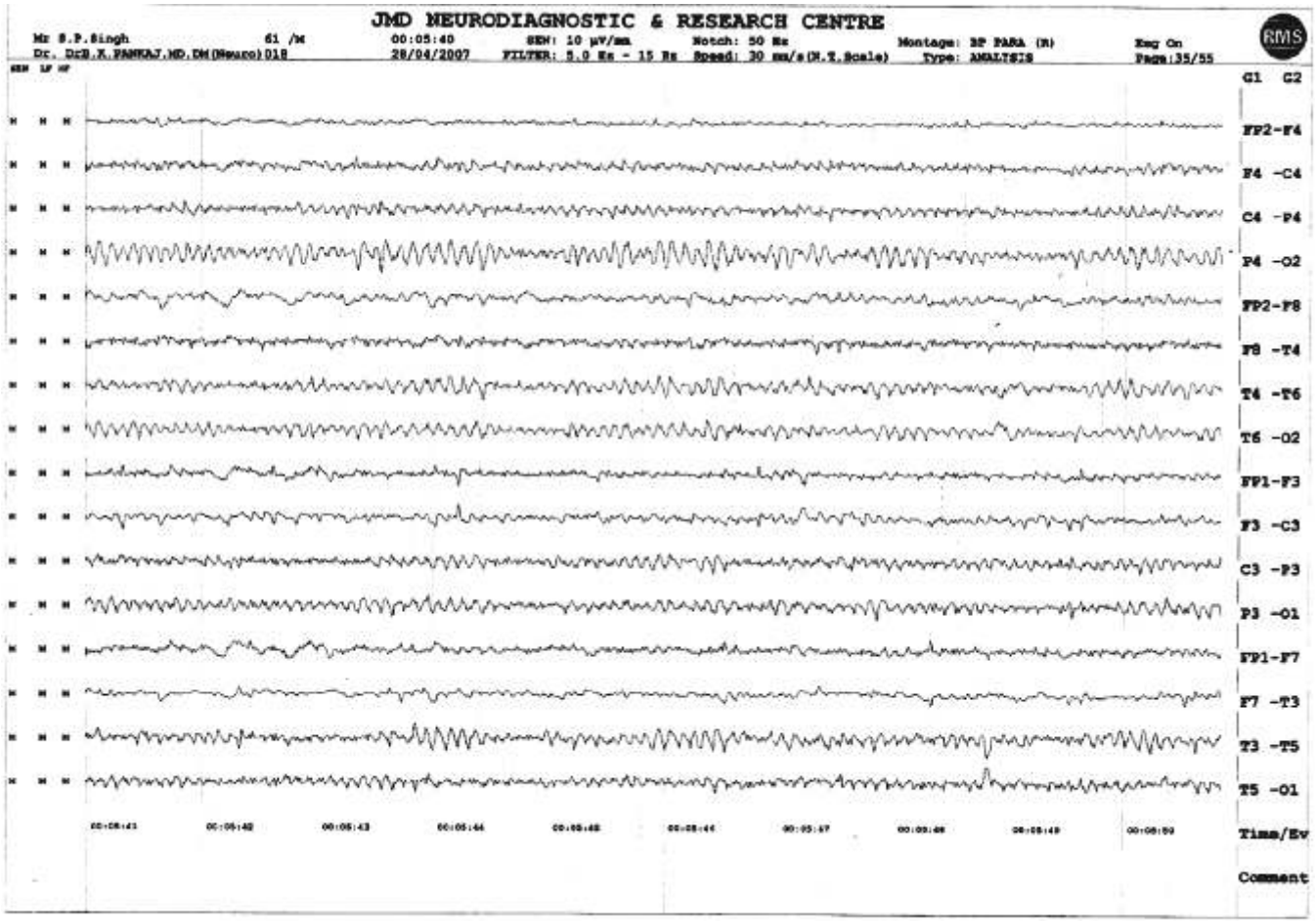

Fig. 2 : Normal EEG

reported[3]. He was given Indomethacin SR 75mg once daily for 1 month. Headache was completely relieved and was symptom free for 2 months. Again he had similar symptoms in 3rd month which was responded well to Licarb-300mg, once daily.

Discussion

Primary nocturnal headaches are chronobiological disorders, usually associated with REM sleep[1]. Headaches can be thought to be either intrinsically related to sleep by anatomy and physiology e.g.--migraine, cluster headache, paroxysmal hemicranias and hypnic headache[2-6]; cause of sleep disturbance, such as CTTH or chronic migraine with or without analgesic overuse; or as a result of a disrupted nocturnal process of the underlying process that disrupts sleep. Sleep in the adult consists of four to six sequential cycles, each lasting approximately 90 minutes during which a relatively longer period of NREM sleep is followed by a relatively shorter period of REM sleep. NREM sleep is divided into four stages of increasing depth (stages I-IV). Slow wave sleep (Stage- III and IV) predominates early in the night, and REM sleep predominates towards morning. From adolescence, the percentage of slow wave sleep tends to decrease until it is absent in individuals over 60 years old.

REM sleep is thought to be initiated by cholinergic neurons in the laterodorsal tegmental nucleus and pedunculo-pontine nucleus[7], and REM 'off' cells have been found in the noradrenergic locus coeruleus and serotonergic dorsal raphe nucleus (DRN). Cell recordings in the DRN have shown a slow and regular firing rate during wakefulness, a decreased firing rate during NREM sleep, and near cessation during REM sleep[8].

Hypnic headache (also known as 'alarm – clock headache') is a rare syndrome first described by Raskin[9] in 1988. Over 70 cases have been reported in the literature[3]. Its prevalence has been estimated at 0.07%[10].

It is a benign syndrome characterized by a dull headache, occuring only during sleep, and wakes the patient at a consistent time, usually between 01.00 and 03.00 hours[10,11]. It lasts approximately an hour, and occurs once per week to six per night[3], usually at least 4 times a week[10]. The diagnostic criteria given by International Classification of Headache Disorders for hypnic headache are as following:

A. Dull headache fulfilling criteria B-D.
B. Develops only during sleep, and awakeness patient.
C. At least two of the following characteristics:
    1. occurs > 15 times per month
    2. lasts ≥ 15 minutes after walking
    3. first occurs after age of 50 years

D. No autonomic symptoms and no more than one of nausea, photophobia or phbonophobia.

E. Not attributed to another disorder.

This case report highlights the importance of taking a detailed history in the case of headache supplemented with general examination and necessary investi-gations. It also emphasizes the need for recording accurate history specifically keeping in mind the salient points of the International Classification of Headache Disorders criteria for the correct diagnosis and treatment of headache patients.

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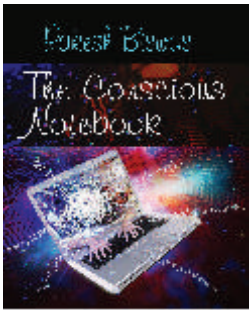
A metanarrative of academic life



The Conscious Notebook : Narrative Human Ontology

Rakesh Biswas

Nova Publishers, 2009
Hard Cover, $ 79
ISBN : 978-1-60692-735-9

Reviewed by Carmel M Martin and Pranab Chatterjee

'The Conscious Notebook: A Narrative Human Ontology' by Rakesh Biswas, a professor of medicine from Bhopal is a book on medical fiction (labeled, the science and fantasy of medicine) recently published by Nova Science Publishers, New York to predominantly cater to the rapidly evolving specialty of Medical Humanities in US and Europe. The author creates a fictional conceptual model of a human that is visualized in physical form analogous to a notebook computer that hides an infinite backend process of cognition representing the human mind driven by its consciousness. This particular backend entity is labeled 'con' (shortened from a consciousness that is universal to all humans and other sentient life also sometimes represented simply as being) and is fictionally endowed with powers that enable it to run on multiple notebook computers (analogous or metaphorically morphologically indistinguishable from humans). The biggest problem with this book is its length and the usage of concepts such as ontology that acts as a barrier for an average medical reader. However this is also a strength, as it prompts the medical reader to reflect on the ontologies that impact on everyday practice. The refusal to accept a dualistic mind body perspective requires the reader to make an effort to comprehend the non linear and even sometimes chaotic intrusions about mind and body in the narrative sequences in the book. Of course this is a major purpose of the book, to simulate the realism, the almost magical realism of everyday medical care and medical lives, if we open our minds and our hearts. Once you open up your consciousness to the earthy poetic narratives, you become involved and entranced. There is even a graphic chapter which is highly recommended as a first read even before you read the first chapter.

'The Conscious Notebook' which weaves around the lives of two medical students representing two generations promises to touch our medical souls and bodies. On a first reading it seems to have kept its promise (although a few more readings maybe necessary to grasp the entire significance of its portrayal of mind body duality and breaching that duality). It asks the reader to comprehend the multiple layers of reality and consciousness that interweave in our complex medical worlds, that influence our practice. This book should be read alongside medical journals in journal clubs and by medical students, as well as reflective practitioners. It is unfortunate that the present price tag of 70$ might confine it to US libraries but perhaps the publisher should consider a lower priced Indian edition.

Carmel M Martin, MBBS, MSc, PhD, MRCGP, FRACGP
Associate Professor,
Northern Ontario School of Medicine,
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This book by Dr. Rakesh Biswas, is an experience in reading. The opening parts smashed any preconceived notions that I had conjured up in my mind after glancing through the title. In a unique literary endeavor, on a theme that can be best described by me to be something like a Patch Adams-meets-Hindu philosophy, the author takes on the philosophy of the practice of medicine as a whole. Although discussing a holistic approach, it is written up in a manner which is reminiscent of the medical school training we receive, where often we end up not viewing patients as individuals with very discrete and individual problems, which have entirely different connotations, complications and consequences to them despite the congruency of their clinical or etiological diagnoses, but as machines carrying an "issue" which can be solved by predetermined and pretested methods. The author and the characters in his rather uncanny work end up implicitly reminding us that despite our best efforts, we end up treating diseases, never patients!

The characters like Dr. Joatmon (acronym for: Jack of all trades, master of none) takes a head-on challenge to the concept of super-specialization that is so rampant in medicine today. Through this character, the author comes up with thought provoking, and often, controversial statement, like: "In medicine one tries to look at the infinite like… looking at the whole forest and not just concentrating on its trees" and then broaches the topic of External-Internal Medicine. The eccentric Dr. Joatmon appears to be one from the Patch Adams school of thought where he endeavors to treat the patients, as individual human beings rather than a diagnosis. An approach which makes the protagonist, June, end up commenting on the irony of the trade of being a physician: "Well, don't you think it's ironic that a profession so very devoted to the care of individuals should care so little about individuality."

Adding to this heavy dose of philosophy, there is a dash of romance as well, as the attractive, yet weirdly unworldly June, ends up moving from one relation onto the next, providing some much needed succor and oxygen to the story line which might have otherwise stifled and become asphyxiating for the average medical reader, who is more habituated to read straight shooting words, where spades are called spades. In my view, it adds a human touch to the narrative which, otherwise, ran a high risk of becoming a didactic instrument on the philosophy of the practice of medicine.

Written in a lucid manner, with a cogent flow of thought stringing the whole together, the book appears to move from one phase to another with much poise. It is a page turner and makes a good reading. In effect, this is more of a biography, with a lot of off-shoots into medicine, philosophy, philanthropy and the vanishing art of medicine. As the author states in the Epilogue: "It was the story of our nerd Con(sciousness) and his notebook body as his sole source of existential sustenance on an Earthy plane". A must read for every medical student moving into medical practice, I am sure, they will identify some parts of this whole narrative (which at times, in my opinion, becomes beguilingly difficult to interpret) with their own lives and professions.

Pranab Chatterjee, MBBS
Intern, Medical College, Kolkata

Molecular Shots

Parkin Immunostaining as seen under 40X

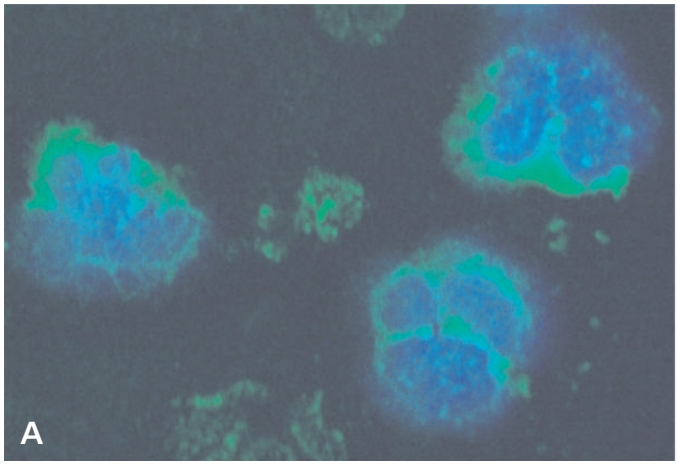
A) Co staining showing Nuclear expression by DAPI and cytoplasmic expression of protein in PBMCs by FITC antibody

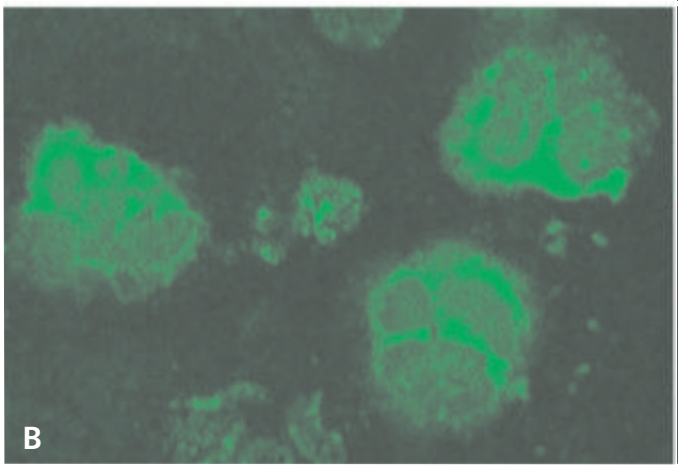
B) FITC conjugated rabbit anti parkin antibody reveals cytoplasmic expression of parkin protein

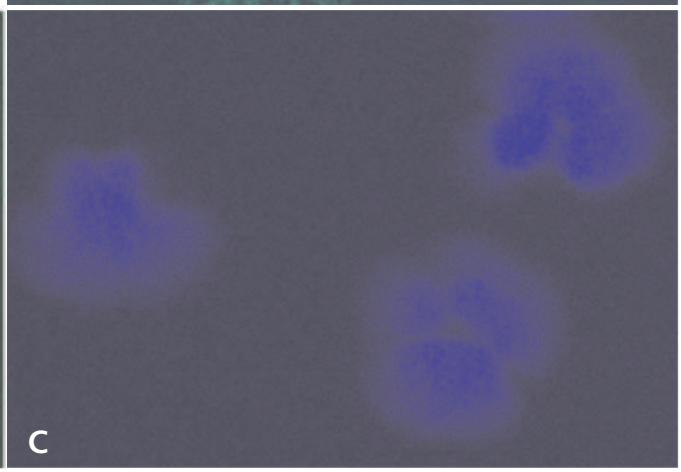
C) DAPI Immunolabeled PBMCs showing nuclear staining

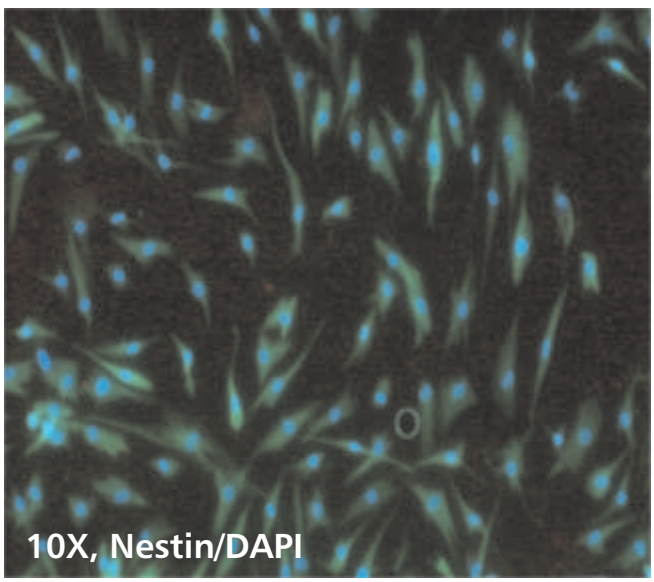
Monika Vinish, Sudesh Prabhakar, Madhu Khullar et al.

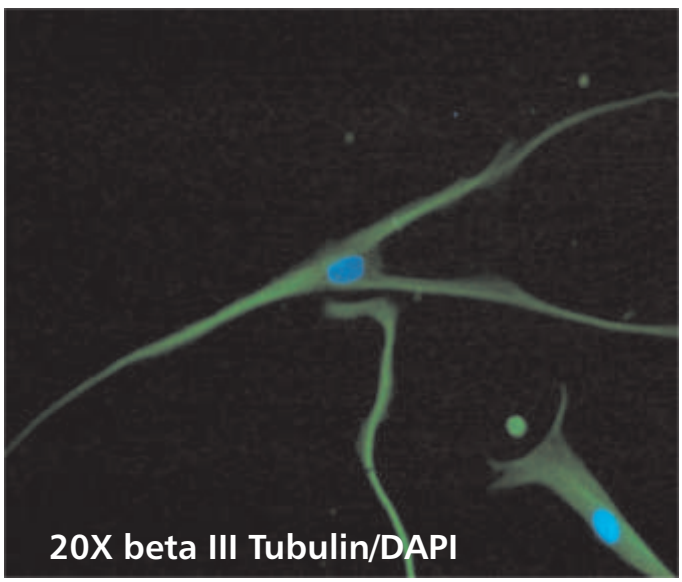
PGIMER, Chandigarh



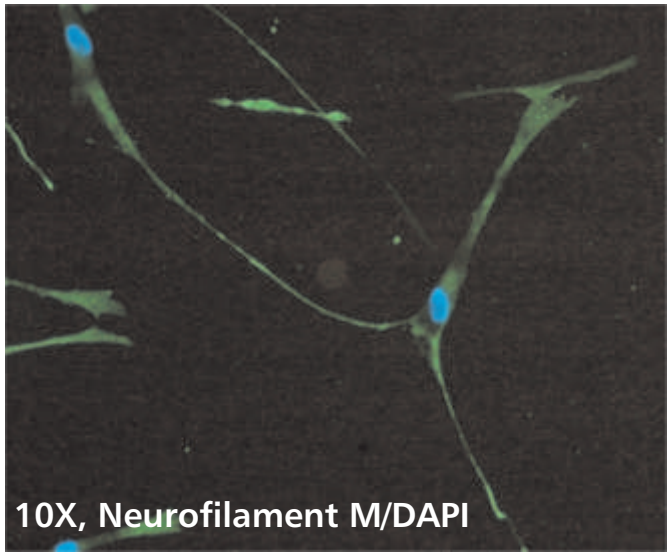


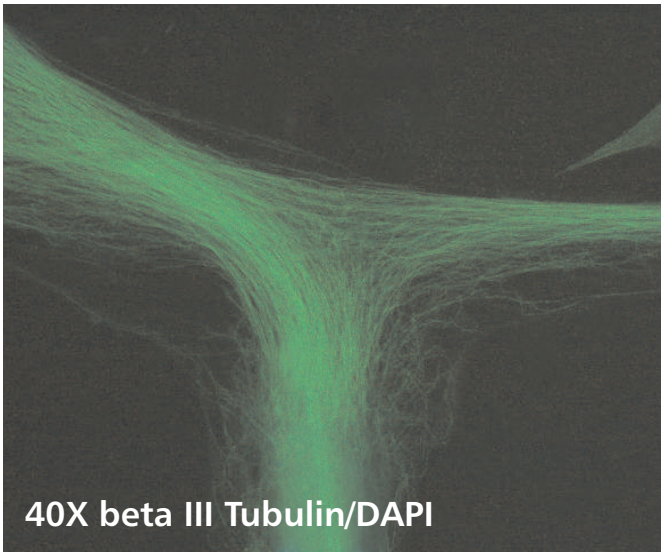






Sushmita Bose and Sujata Mohanty
AIIMS, New Delhi





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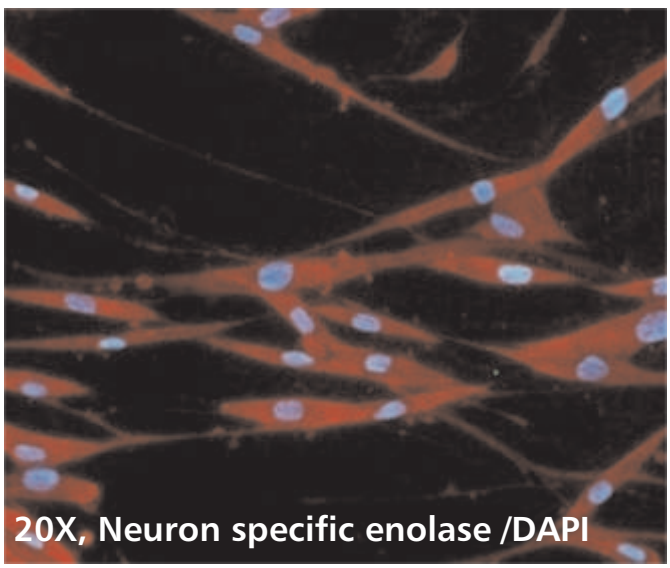

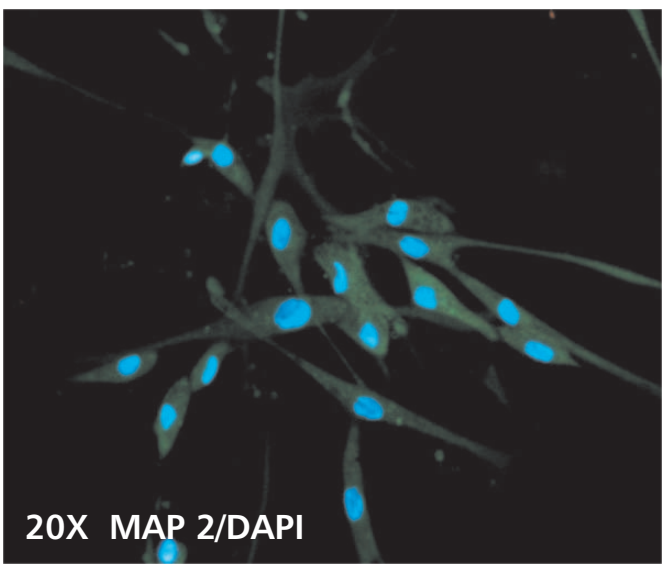

Sushmita Bose and Sujata Mohanty
AIIMS, New Delhi

Letters to Editor

Dear Editor :

I have always wanted scientific journals in India to be internationally competitive: however, this can happen only when our "leading" scientists believe in publishing in Indian journals. This belief has often been missing and consequently, most of the papers published in journals published from India carry papers which either have been returned from the so-called "international journals" or were thought by the authors themselves to be not meeting the required "international standards"! Given this, the quality of most journals have remained poor. Poor quality of submissions is compounded by rather poor peer-review that most peers in the country are willing to provide. The undue love for "impact-factor" in science-establishments in our country is also impacting Indian journals because with poor submissions and poor peer-review, the quality of papers remains sub-standard and these obviously cannot attract good citations [1,2].

Given this rather disappointing experience, I would hope that under your leadership the Annals of Neuroscience would really strive to attract good manuscripts and have good peer-review system alongwith facilities for online submission and online reviewing. I also hope that this journal is quickly listed by indexing services like the Pubmed and ISI Web-of-knowledge, Scopus etc

I will try to contribute, whatever limited that I can, to help your efforts.

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Dear Editor :

I congratulate you on taking over as the Editor and for your very forward looking plans. As I have been a journal editor or associate editor for 15 years and on several editorial boards, I would be happy to help your journal and the other members of the Advisory Board to draft new editorial guidelines, etc that can make it competitive. Let me provide you with a little input with regard to a print journal and a publisher which should be engaged in order to defer your publication costs and become competitive. From the publishing company point of view, if the publishers can sell the journal at about the cost and can make most of their money from advertising, they are looking at a threshold of about 2000 sold-copies to be able to make a profit. In my experience, if you can have the journal subscription to be part of the Academy membership dues, then you have a "captured readership" and the publishers will take on the print version with alacrity. I do not have experience of a pure eJournal but feel that most of the website programs like AOL, Google etc make their money out of the advertisements that they add. That is how they provide content free. You may have thought of this, but perhaps the pharmaceutical, instrument and biologic manufacturers may be willing to provide support based on the inclusion of their advertisements. Alternatively they might like to buy advert space, based on the number of hits. It is also a good idea to convene a meeting of the Editorial Advisory Board once a year.

Good luck with your endeavor.

Sincerely,

Walter G. Bradley, DM, FRCP
Professor and Chairman Emeritus
Department of Neurology
Miller School of Medicine
University of Miami

Dear Editor :

I am providing you with my insights about how the *Annals of Neurosciences* can be improved with the help of new team you are building. Annals could provide a means to encourage critical reading and writing skills in the graduate student and postdoc population in India, or elsewhere within its readership. I suggest that an entire issue each year be devoted to "Journal club" articles along the lines of those carried by the Journal of Neuroscience http://www.jneurosci.org/misc/ifa_features.shtml with the difference being that we could feature contributions written about ANY neuroscience paper written in the previous year. Students and postdocs could be motivated to participate in this – to identify a paper that interests them and write a Journal club about it- by awarding a prize to the best written such contribution. The issue should carry the best 20 or so articles, and perhaps one or more awards. The prize(s) should be substantial, such as $1000 toward travel to an International meeting such as IBRO or SFN. All contributions would be reviewed rigorously for the quality of their analysis of the original article. Making this an annual feature would serve to stimulate the student/postdoc community to develop critical reading and writing skills, and to participate in this as a community- we may see multiple entries from a single Institution or lab. Such writing may even become a part of graduate coursework, all of which would be very good for Neuroscience research in India.

Prof. Shubha Tole, Ph.D.
Department of Biological Sciences
Tata Institute of Fundamental Research
Mumbai, India

Dear Editor :

I have been associated with the journal as its editor for over last five years and found it an interesting and a challenging job. I am confident of your capabilities that you will keep the quality of the journal high so that it will touch new heights in the coming years under your dynamic leadership. I will however provide certain suggestions that will help. You may reconstitute the Editorial Board keeping an equilibrium of basic and clinical experts so that it may cover all areas of neurosciences and also keep regional balance. You may also keep a co editor, if that helps you, to share your work from time to time. You might want to redesign the cover if you feel so and raise funds from pharmaceutical firms as

sponsors. With this I am also forwarding some of the manuscripts which may be included in the first issue of 2009. The subsequent issues are due in April. July and October. In addition to this, the abstract issue is published in December in coordination with Secretary of the Annual meeting.

Rakesh Shukla, DM
President, Indian Academy of Neurosciences
Professor, Department of Neurology
Chattrapati Shahuji Mahraj Medical University
Lucknow

How to make a medical journal more citable?

Dear Editor :

It is heartening to note that the new editorial board of Annals of Neurosciences (ANS) is trying to enhance its quality, readership and create a new model for communicating research results, commentaries and reviews. ***Open Access*** journals[1] are likely to be cited more and therefore having a proper Open Access Policy for any journal is desirable.

Citation of a journal article is measured variously by Impact Factor, Weighted Impact Factor, PageRank, Eigenfactor, h-index, and other criteria[2-6]. Bollen *et al*.[7], have done a comparative study of the scientific measures of impact of a paper. However, concerns still remain on how to judge a journal or an individual publication. Therefore, it should be prudent for the editors to carefully deal with certain contentious issues like ***Conflicts of Interest***. The recent case between JAMA[8] and Jonathan Leo[9] has raised a considerable hue and cry among the academic researchers and the general public. Such conflicts of interest can be very important while interpreting any research publication.

Further, the editors can ensure that the papers published in the journal are clear, concise, complete and correct[10].

I sincerely hope that the editors will succeed in making ANS the fountainhead of new knowledge and expression in the field of neurosciences.

Suptendra Nath Sarbadhikari
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10. Gastel B (April 12, 2009), Editorial Help: More Than Correcting Grammar, http://www.authoraid.info/news/editorial-help-more-than-correcting-grammar

Dear Editor :

I appreciate your view towards improvement of the scientific quality of of the journal. Since inception of the journal we are facing the problem of getting sufficient number of papers to get proper review and incorporate valuable suggestions. On this aspect, I will suggest you to approach individual members through email requesting them to send you interesting observations/ possible review on their research subject of study. This may act as a great facilitator for your efforts. Your request could also include soliciting valuable suggestions towards improvement of scientific content of the journal. Your continuous writing to established members will generate good response. I am confident you will get some good manuscripts for publication. Once you have enough papers automatically the quality will start moving up. You will get many more suggestions and then you can discuss among your editorial board and give it final shape.

Best wishes

A.K. Agrawal
Developmental Toxicology Division
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Dear Editor :

I was wondering Neuroscience could introduce two features
1) Specifically adding a section on Cognitive and Computational Neuroscience
2) Increase student interaction by solicit reviews of interesting papers published in Neuroscience journals.

Nandini Singh
NBRC, Manesar

Dear Editor :

We need to increase the submissions from research labs and from clinical researchers. Looks like a lot of work for you to get the Indian neuro community on board.

Colin L Masters, MD
Executive Director and Laureate Professor
The Mental Health Research Institute
National Neuroscience Facility
University of Melbourne
Parkville Vic 3010 AUSTRALIA

ANS Mission and Scope of Journal

The Journal is a free-access, multi-disciplinary publication of the Indian Academy of Neurosciences that aims to cover new advances in Neurosciences. It provides a platform for papers that range from computational and experimental work in the neurosciences to those that fit the interface between experiments and clinic. The Journal accepts research papers as research articles, brief communications, reviews, commentaries, book reviews, molecular images, student's perspectives on published reports in the form of journal clubs and people and views. It also includes editorials on Policy which may include Intellectual property, Technology commercialization and inter-disciplinary issues. Journal is committed to the rapid publication of original findings that increase our understanding of the molecular structure, genetics, function, behavior, physiology, toxicology and development of the nervous system. Experimental papers should have implications for the neurological disorders and may report results using any of a variety of approaches including anatomy, theory, biophysics, *invitro* systems, imaging, and molecular biology. Papers investigating the physiological mechanisms underlying pathologies of the nervous system, or papers that report novel technologies of interest to researchers in neurosciences are also welcome. The criteria for acceptance of manuscripts will be scientific excellence, originality, defiance of dogma based on sound evidence and relevance to the field of neuroscience based on peer review of manuscripts. Although independent peer reviewers are requested by Editor, the authors may suggest a list of three names with expertise to comment on the paper and upto two people who need to be excluded from review. The editorial board reserves the right to refuse review process if the paper does not meet the scope of the journal or is not scientifically appealing. We encourage authors to define the established principles and policies that defy the norms and to argue against accepted dogma. The Editor in Chief will make a final decision on the acceptance of the manuscript and would be under no obligation to seek further opinions. Our intent is that this publication becomes a gateway through which a wide array of information can be accessed in a single periodical. The goal is to provide peer-reviewed and rapid communications that will foster clearer understanding of the Neurosciences, generation of better diagnostic tools, encourage journalism among young scientists and development of effective cures for Neurological disorders. All experiments described in the ANS that involve the use of animal or human subjects must have been approved by the appropriate institutional review committee and conform to accepted ethical standards.

Guide to Authors

General

The format of the manuscript should be as follows: Title page, Abstract, Introduction, Methods, Results, Discussion, Acknowledgements, Abbreviations, References, Tables, Figure Legends and Figures. Review articles are not required to follow this outline. First mentions of figures and tables in the text should be in numerical order. Headings and sub-headings should not end with a full stop. ***The manuscript should be formatted in double spacing and the lines should not be numbered. The text of your paper should be saved as a .doc file. with the corresponding author's last name and sent to the editor by e-mail.*** Manuscripts are expected to document the origin and specificity of reagents used, particularly antibodies and document institutional authorization for conducting research in humans and animals, to conduct adequate statistical analyses and comprehensively report statistical results, and to be written in English.

Articles and Reviews could also be accompanied with represen-tative colour photograph or illustrations which summarizes the work. This will be displayed in the content.

Title Page

*The Title page **must** include*:

- A clear and concise title
- The authors name(s) and surnames
- The address(es) from which the work originated
- The name, address, fax number and e-mail address of the person who will deal with correspondence, including proofs.
- The total number of pages, figures, tables and equations
- The total number of words in: (i) the whole manuscript; (ii) the Abstract; and (iii) the Introduction.
- A list of four or five keywords not appearing in the title, preceded by "Keywords:"

Abstract

Research Articles and *Reviews* should have an Abstract, which appears before the main body of the text. The Abstract should be written in complete sentences without headings and should provide a summary *not exceeding 250 words* suitable for publication without the full article text. Thus, if references must be cited in the Abstract they must include the author(s), journal title, volume number, page range, and year. It should begin by discussing background, methods, results and conclusions but without these headers. The use of abbreviations in the abstract should be avoided. Statistical results need not be described in the Abstract.

Introduction

The body of the *Research Articles* should start with a brief Introduction, *not exceeding 450 words*, which outlines the historical origins of the study and stating the aim of the study and/or hypothesis to be tested. It should not have information from Abstract or discuss the results.

Methods

The methods section should be written such that another researcher is able to reproduce your work. Important methodological aspects of your work, should be described, even if such descriptions can also be found in prior publications. Companies from which materials were obtained should be listed with their country.

Discussion

The Discussion could contain the summary of the major findings, while avoiding repetition of the statements in Abstract or the Results. Citing literature supporting or defying the findings should be provided. The Discussion should be able to project a major advancement of scientific concept, method or effect.

References

All references must be listed, and all listed references must be cited at least once in the main text

Preparation of the manuscript

Research Article

The research article should be typed in Calibri font size 12, double space word format, readable in Word 2003-07 format. The length of the article should not exceed 5000 words and all the illustrations should be in Power point edition 2007 or more. The supplementary information, if any, should be provided with Tables in word format while all figures must be included in power point. From a research article the references should be written as shown below.

From a Research Article

Jaime C. Montoya, Grace O., et al. Diagnosis of Tuberculous meningitis using Enzyme-Linked Immunosorbent Assay utilizing a 30,000-Dalton native antigen of Mycobacterium tuberculosis. Phi J Microbiol Infect Dis 2000; 29 (4): 156-161.

From a Chapter in a book

e.g. Fullplus BW. Characterisation, isolation and purification of cholinergic receptors. In : Theselff S Ed., Motor innervation of muscle, 2nd ed. Longdon: Academic Press 1976; 11-26.

Review Articles

Review Articles in ANS are full length articles on topics of particular current interest. Proposals for *Review Articles* are invited by the Editors-in-Chief, Executive Editor and the Associate Editors. Journal also encourages independent submission of innovative and comprehensive reviews that provide complete framework of the topic including the historical aspects, progress in the field including controversies and promises. The review should not simply provide an outline of every point published by a paper but rather be structured to address a point in science covered the Journal.

Case Report

Only one case report of novel and extra ordinary importance will be published. Unstructured abstracts also required.

Journal Club

Graduate students or post doctoral fellow can write a commentary or a published paper from any reputed neuroscience journal and describe the fundings under background study design, criticism and implication.

Molecular Shots

JPEG Images from experiments such as immunocyto chemistry can be submitted with legends and authorship.

Competing interests

If there is likely to be economic gain (patents, financial investments, consulting agreements) resulting from having utilized experimental approaches, instruments, methods or drugs, or of having discussed such items in the manuscript, represents a competing interest. Competing interests should be clearly disclosed and described in detail in the Acknowledgements. It is in the interest of the author to disclose such interests, and ANS requires such disclosure.

Funding

All sources of funding should be declared in the Acknowledgements. If a private/commercial sponsor supported the research, authors are advised to describe the role of the study sponsor(s), if any, in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. If the funding source had no such involvement, this should be stated.

Evaluation of manuscripts

Submitted manuscripts are assigned to an Associate Editor and/or and Editorial Board member who is responsible for its evaluation. He may get it evaluated from another expert in the field. The Editor-in-Chief's decision regarding publication is based on the recommendation of the reports of reviewers, which will, at the Editors' discretion, be transmitted to the authors.

Ethical standards

All studies using human or animal subjects should include carry a declaration identifying the Institution or Review Committee which approved the study. Editors reserve the right to reject papers if there is doubt whether appropriate procedures were followed or competing financial interests have not been disclosed.

(I) Studies with human subjects

When human subjects are used, manuscripts must be accompanied by a statement that the experiments were undertaken with the written consent of each subject, and that the study conforms with institute ethical committee guidelines.

(ii) Studies involving experimental animals

The methods section must briefly state measures which were taken to minimize pain or discomfort and the ethical committee should be identified.

Experimental animals

The ***species, strain, sex, age, supplier and numbers of animals*** used should be specified. If transgenic or knockout animals are used the established nomenclatures must be used

Reagents

The control experiments that were conducted to ensure the specificity of the method should be described, along with key references to previous work with this reagent. For antibodies, this documentation includes a precise description of the antigen, the nature of the antibody (species, purification), the supplier, catalogue number, and specificity tests performed

Statistical methods

A complete description of statistical methods is required. The main statistical results should be described in the Results section. The description of statistical results in the figure legends should be limited to important analysis. The description of the statistical results should include the degrees of freedom.

Acknowledgements

The financial support should be acknowledged alongwith those from collaborators or if the author has worked on a fellowship, the sponsoring agency should be mentioned. If the manuscript has been previewed by someone, the individual's name should be acknowledged.

Cover illustrations

Colour illustrations suitable for front cover, can be submitted to the Editor for consideration.

Online submission

ANS would be shortly launching the online submission of manuscripts at the new web site.

Announcing

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for Graduate students and Post doctoral fellows

Submit your commentaries to ANS Journal

ANS is introducing an exclusive section dedicated to nurturing Scientific expression among graduate students and post doctoral fellows. ANS will welcome structured commentaries on leading articles already published in the field of neurosciences.

Editor-in-Chief
Akshay Anand, PhD

Such commentaries may refer to articles which have an impact on neurosciences, research methods or policies and may be extracted from ANS or other journals. All such submissions can be made to Editor directly who will secure peer review of these submissions. Please refer to guide to authors for details.

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Plan To Attend

IAN CONFERENCE 2009

International Conference on Advances in Neuroscience & XXVII Annual Meeting of Indian Academy of Neurosciences

December 18-20, 2009

Venue
NIMS UNIVERSITY
Shobha Nagar, Jaipur-Delhi Highway,
N.H. No. 8, Jaipur – 303001 (Rajasthan)

Last Date for Registration
10th September 2009

Last Date for Applying for Travel Awards
10th September 2009

Last Date for Applying for Young Scientist Awards
10th September 2009

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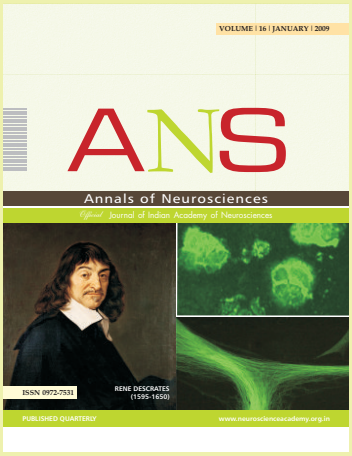

Cover Illustration of Rene Descrates (LEFT), Parkin immunostaining of human lymphocytes (from Vinish et al, TOP RIGHT) and Neurons stained with β III Tubulin (from Bose et al, BOTTOM RIGHT, See Molecular Shots inside)