Brain cells - recently unveiled secrets: Their clinical significance

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Twentieth century unraveled the mystery of the atoms and the gene but much to the disappointment of scientists and philosophers alike the hope to achieve a hat-trick by solving the mystery of the mind remained unfulfilled. However, during the latter part of the 20th century the study of the brain moved from a peripheral position within both the biological and psychological sciences to become an inter-disciplinary field called neuroscience that now occupies a central position within each discipline. Those interested in a comprehensive overview of the progress of neuroscience in the 20th century are referred to a concise yet comprehensive review by Albright et al. [1] The declaration of the 1990s as “the Decade of the Brain”, provided a new awareness and impetus to study this most complex, unique and precious possession of human beings. It attracted a large number of scientists to brain research in newly opened departments of neurosciences and establishment of Centers of Excellence and specialized centers all over the world and somewhat belatedly even in India. [3] A brief account of the important gains of the Decade of the Brain has been provided by the author. [4] A summary of Indian contributions in neurosciences during this Decade is published in the Annals of the Indian Academy of Neurology. [5]

Neuroscience research today is a continuum of study from the molecular to the behavioral level. It encompasses the body of research directed towards understanding the molecular, cellular, intercellular processes mediated through electrochemical signals, in the nervous system, integrated to subserve behavior. This has been possible as a result of recent advances in molecular biology, genetics, proteomics and biotechnology along with those in the fields of computer science, microelectronics, and the new imaging techniques (CT, MRI, FMRI, PET, SPECT, two-photon microscopy etc). It has been claimed that 90% of what we know about the human brain has been learnt during the last couple of decades. The new techniques now permit us to study the nervous system - in health and disease - from the gene to the behavioral level - in a seamless intermingling of the Cartesian reductionism to the holistic approaches of cognitive science. Already the knowledge gained from these researches has been translated to provide better diagnostic and therapeutic applications in clinical practice. [6, 7] However, the purpose of this presentation is to highlight some of the path-breaking discoveries in the last decade which have not only exploded longstanding dogmas about the structure and function of the various constituents of the brain but which promise to revolutionize the future of therapy for brain disorders. [8]

Neurons do Multiply Even in Elderly: Neurogenesis in Adults and Neural Stem Cells

Two publications in November 1998 heralded the isolation of the human embryonic stem cells (ESC) which could differentiate into all types of tissues including the neurons and glia. [9, 10] Floyd Bloom hailed it as fulfilling the definition of a breakthrough as a rare discovery “that profoundly changes the practice or interpretation of science or its implications for society”. [11] In 1998 itself, Fred Gage from California and Peter Ericksson and colleagues from Goteborg published the remarkable observations that the mature human brain does spawn neurons routinely in at least the hippocampus, which no doubt would have differentiated from preexisting stem cells. [12, 13] Johansson et al., from Stockholm while confirming that new neurons are continuously added in specific regions of the adult mammalian central nervous system claimed that these have their origin in the ependymal cells. [14] Gould et al., reported neurogenesis in the neocortex of adult primates. [15] As often happens in science, it is remarkable that already in 1965, Altman and Das had demonstrated that new neurons were continuously added in certain regions of the adult mammalian brain. [16] Horner and Gage reiterated that neural stem cells (NSCs) exist not only in the developing mammalian nervous system but also

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in the adults including humans. It is now generally recognized that adult neurogenesis occurs at least in two specific brain regions: the subventricular zone and hippocampal subgranular zone. However, proliferating cells isolated from many regions in the adult brain, can give rise to neurons both in vitro and after grafting back into the neurogenic regions in vivo. This would suggest that neural stem cells may be widely distributed in the adult brain and that local environment cues dictate their fate. These observations, since reconfirmed from many laboratories globally, thus established beyond any doubt that generation of new neurons, till recently believed to occur only during embryonic development, occurs throughout life even in humans. In less than a decade since these landmark papers voluminous literature pours in every week from all over the world to translate this knowledge for therapeutic purposes. Hardly a day passes when the lay press, print and electronic media do not bring some aspects of this subject—new discoveries, new techniques, new applications and new controversies—to public awareness. Recognizing its potential for treatment of a large number of hitherto incurable neurological diseases i.e., Parkinson’s, Huntington’s, Alzheimer’s diseases, multiple sclerosis or to replace damaged tissue following spinal cord or brain injury or stroke etc, many governments including India have allocated large sums of money for R and D in this field. This is reflected in the European Commission appointing the European Group of Life Sciences (EGLS) charged with the responsibility of organizing a meeting in Brussels already in December 2001 to discuss stem cells and their possible use in future therapies. The meeting attended by 750 people from 36 countries, among other suggestions concluded that “the potential value of regenerative medicine involving stem cells is enormous” (but neither the full range of possibilities nor the limitations have been clearly identified as yet). It is an extraordinary example of the interest generated at the highest levels of political and administrative leadership within three years of a scientific discovery. Notwithstanding a ban on federal grant for embryonic stem cell research by the President of the US, George W Bush, the State of California approved a $ 3 billion initiative for research in this field and establishment of the new California Institute for Regenerative Medicine. The Department of Biotechnology, Government of India, established a task force to promote this field of research. A number of groups have been established in different institutions in the country for this purpose. The National Brain Research Centre is engaged in a big way in this field.

At the same time there is ongoing debate all over the world about its ethical implications. To overcome the ethical issues attempts are being made to obtain stem cells from sources other than embryos including bone marrow, nasal mucosa, somatic cell nuclear transfer, progenitor cells from cadavers etc. Despite some unresolved technical and ethical issues already individuals have initiated clinical trials. Among them mention may be made of Hongyun Huang from a Beijing Clinic who claims to have treated over 1000 patients with diverse neurological disorders. However, so far no well-controlled, ethically conducted, successful trial has been reported in an indexed journal.

In conclusion it is now well-established that even adults have neural stem cells in at least some parts of the brain, that neurogenesis continues to occur throughout life, that stem cells obtained from diverse sources grown in culture and differentiated into specific cell types can help replace damaged brain cells at least in mammals. It is expected that in the not so distant future their therapeutic potential would be scientifically exploited for human use.

**Astrocytes No Longer Just a Glue**

With their name originating from the Greek word for glue, they were till very recently considered as the brain packing material, holding neurons together. No doubt their role during development as radial glia or to a limited extent in brain development, but their role was still unclear. Recently, it has been shown that astrocytes are required for normal synaptogenesis and synaptic stability through the release of diffusible, extracellular signals, an important one being TNFα. Furthermore it has been demonstrated that in vitro astrocytes regulate synaptic formation and synaptic transmission, thus “reinforcing the view that astrocytes have an active regulatory role - rather than merely supportive role traditionally assigned to them - in the mature central nervous system”. Interestingly enough Noctor et al. and Goldman et al., have produced evidence to suggest that glia, particularly astrocytes, not only regulate neurogenesis but also are the neural progenitor cells.

Astrocytes have been demonstrated to release chemical transmitters, including adenosine triphosphate (ATP), glutamate and D-serine. These chemicals synchronize neural activity and modulate synaptic transmission and

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play an important role during the induction of long-term potential (LTP). On the other hand, astrocytes besides regulating glutamate concentration at the synapses have several transporters on their surface which take up serotonin, dopamine and other monamine transmitters. It is not surprising then that if anything goes wrong with these cells there is going to be dysfunction and disease.

Astrocytes are known to secrete membrane-associated molecules including cytokines, growth factor and neurotransmitters in response to physiological and pathological stimulus.[33,34] It is therefore not surprising that glia may have key roles in central nervous systems disorders, not just being activated following an insult, but in some ways contributing to it. Watkins of the University of Colorado, Boulders, went to the extent of claiming that glia may have a key role in the central nervous system disorders from neuropathic pain and epilepsy to neurodegenerative diseases such as Alzheimer's and may even contribute to schizophrenia, depression and other psychiatric disorders.[35] It is not really not glial cells. However, in the absence of any detailed reports by her, these claims appear somewhat uncalled for. Postmortem examination of patients with schizophrenia, bipolar disorder and depression has revealed glial abnormalities.

It is now known that astrocytes play an important role in the clearance of glutamate. The glutamate-induced neurotoxicity is blamed for pathogenesis of a variety of neurological disorders e.g., epilepsy, trauma, stroke and even neurodegeneration. Activated astrocytes, failing to clear excessive glutamate, may thus be blamed for their involvement in these pathologies.

Yet another function of astrocytes, recently brought to light, is their role in inducing local vasodilation and increased blood flow to the functionally active region of the brain. This is the basis of functional imaging with MRI. Cohen et al., demonstrated astroglial and vascular interactions of noradrenaline terminals in the rat cerebral cortex.[30] Takano et al., have conclusively demonstrated that activation of astrocytes induces this vasodilation.[37] They observed elevated Ca2+ concentration in astrocytic end feet which in turn activated phospholipase A2 (PLA2) and that the resultant arachidonic acid is converted to COX-1 into a vasodilating prostanoid. On the other hand, Mulligan and Mac Vicar (2004) demonstrated vasoconstriction by astrocytes by increased concentration of Ca2+.[38] Increased Ca2+ in astrocytes has also been observed during spreading depression. This is contradictory to the observations of Takano et al. Quoting an earlier study which revealed increase in astrocytes Ca2+ during a stroke, they proposed this to be a novel therapeutic target for treatment of stroke and migraine.[37] It is not the purpose of this write-up to discuss the mechanisms for controlling regional brain circulation but only to highlight the recent observations on the role of astrocytes in this process.

Microglia not just scavengers of damaged or dead neural tissue

Microglia were originally described by del Rio-Hortega in 1932, as a unique cell type differing in morphology from other glia and neurons, comprising approximately 12% of the brain.[39] They are not ectodermal in origin and are believed to be of mesenchymal lineage.[40] Conventionally, they have been recognized as resident macrophages and are responsible for the innate immune mechanism in the brain.[61,43] They kill invading microorganisms, remove debris and facilitate tissue repair after injury.[37] They become readily activated in response to brain injuries or to immunological stimuli. The activated microglia change in their morphology, upregulate expression of a variety of cell surface molecules, produce various pro-inflammatory cytokines. However, till recently it was believed that in healthy mammalian brain microglia are essentially dormant, nonmotile, quiescent cells as if they are silent spectators. Morphologically the resting and activated microglia manifest different characteristics. The former are characteristically elongated cell bodies with spine-like processes that often branch perpendicularly. According to Nimmerjahn et al., the pronounced and ongoing structural changes of resting microglial cells presumably serve an immune surveillance function, in particular microglia can sense subtle changes in their micro-environment through a variety of surface receptors. In response to any kind of brain damage or injury, microglial cells become activated and undergo morphological as well as functional transformation.[44] In contrast in the activated state the cell body increases in size, there is a thickening of proximal processes and decrease in ramification of distal branches. This activation is believed to be preceded by molecular events like changes in their expression of cell adhesion molecules, cytoskeleton reorganization and antigen presentation.[45]

Activated microglia are capable of releasing a variety of soluble factors which are proinflammatory in nature and potentially cytotoxic. Block and Hong have enumerated more than 30 such factors including NO, H2O2, OH, NOO, TGFβ, PGE2 and a variety of interleukins which influence cell survival.[46] With the advent of two-photon microscopy which permits direct imaging of cells in the undisturbed physiological environment of intact organ, Nimmerjahn et al. and Davalos et al., have demonstrated that even in the resting state while soma and main branches remained stable for hours, their processes were remarkably motile, undergoing cycles of formation, extension
and withdrawal on time scale of minutes. These microbial processes and protrusions were also observed to directly contact astrocytes, neuronal cell bodies and blood vessels, suggesting that in healthy brain tissue microglia serve some house-keeping function. Microglia thus are busy and vigilant housekeepers in the adult brain.

While the role of microglia in acute insults to the brain was well known, it is only in recent years that their involvement in neuroinflammation associated with neurodegeneration has been brought to light. Using immuno-cytochemical and genetic techniques, chronic inflammation has been observed in Alzheimer’s and Parkinson’s disease, amyotrophic lateral sclerosis, multiple sclerosis etc. These observations have already been translated into trials of non-steroidal anti-inflammatory drugs and minocycline, a second generation tetracycline, for prevention of microglial activation associated with neurodegeneration, in patients of Alzheimer’s diseases or Parkinson’s, HIV-associated dementia, fronto-temporal lobe dementia, multiple sclerosis etc.

It has also been claimed that activated microglia may not only overexcite but also kill neurons and thus be involved in neurodegeneration either directly through release of pro-inflammatory cytokines and other molecular or indirectly by interfering with the glutamate - recycling role of astrocytes. It is interesting to note that activated microglia, as also activated astrocytes, invade the amyloid plaques of Alzheimer’s disease. Glia-mediated inflammatory response is now recognized to contribute to the damage seen in many neurodegenerative disorders. A detailed account of the pathogenetic mechanisms underlying neurodegeneration in these diverse disorders is provided by Block and Hong. In conclusion they summarized, “Microglia are the critical actors of self-propelling mechanisms of neurotoxicity contributing mechanism to multiple degenerative disorders”. And further, “thus, multiple triggers of microglial derived oxidative stress fueling the progressive nature of several independent neurodegenerative diseases”.

Activated microglia have also been shown to play an important role in causing neuropathic pain. Types of injuries that lead to chronic pain trigger microglia in the spinal cord to proliferate and release various signaling molecules which cause sensory neurons’ excitability. More recently scientists from the National Institute of Health Sciences in Tokyo identified a protein that is necessary for neuropathic pain in rats with a severed nerve. A receptor called Toll-like receptor 4 (TLR 4) which is expressed only on microglia in the central nervous system plays a critical role in activating the microglia. The pro-inflammatory cytokines secreted by activated microglia, in turn, are responsible for the increased sensitivity of the neurons.

It is not surprising then that several drugs aimed at inhibiting the inflammatory response of glia are being investigated to provide neuroprotection. These include β-lactam groups of antibiotics which stimulate the astrocytes’ uptake of glutamate for ameliorating amyotrophic lateral sclerosis or memantine for Alzheimer’s disease or Rasagiline which inhibits the monoamine oxidase β enzyme in case of Parkinson’s disease. Recently, attempts have been made to try immunotherapy for Alzheimer’s disease.

Conclusions

Recent researchers on the biology of various brain cells - neural stem/progenitor cells, astrocytes and microglia - have revolutionized our understanding of their role in physiological and pathological states of the nervous system. The existence of neural stem cells and their role in adult neurogenesis has stimulated extensive research to translate this knowledge for therapeutic purposes. Astrocytes, so far believed to be simply as ‘glue’ acting as supportive cells for neurons are now recognized to play an active role in neurogenesis and synaptogenesis, as also maintaining the integrity of synapses. They not only modulate the environment around the neurons by releasing a range of neuronal growth factors, but also actively participate in neurotransmission. They are now known to play a significant role in neuroinflammatory response so intricately linked to the pathogenesis of neurodegenerative disorders. Similarly, it is now established that the microglia, far from being silent spectators in normal brain, awaiting activation in the event of a brain insult and simply acting as a scavenger, play a far more active role as busy and vigilant housekeepers in the adult brain. In addition, their ability to release a variety of pro-inflammatory and potentially cytotoxic factors (more than 30 of them) including NO, H2O2, OH, NOO, TGF-β, PGE2 and a variety of interleukins, permits them to actively participate in neuroinflammation associated with neurodegeneration and other brain insults. These advances in new knowledge have opened up novel potential therapeutic approaches which promise to revolutionize treatment of a large number of hitherto incurable neurological disorders. It is therefore, in the interest of the clinicians - neurologists, neurosurgeons and psychiatrists - to be aware of these developments. This is the motivation for writing this brief review.

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References


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