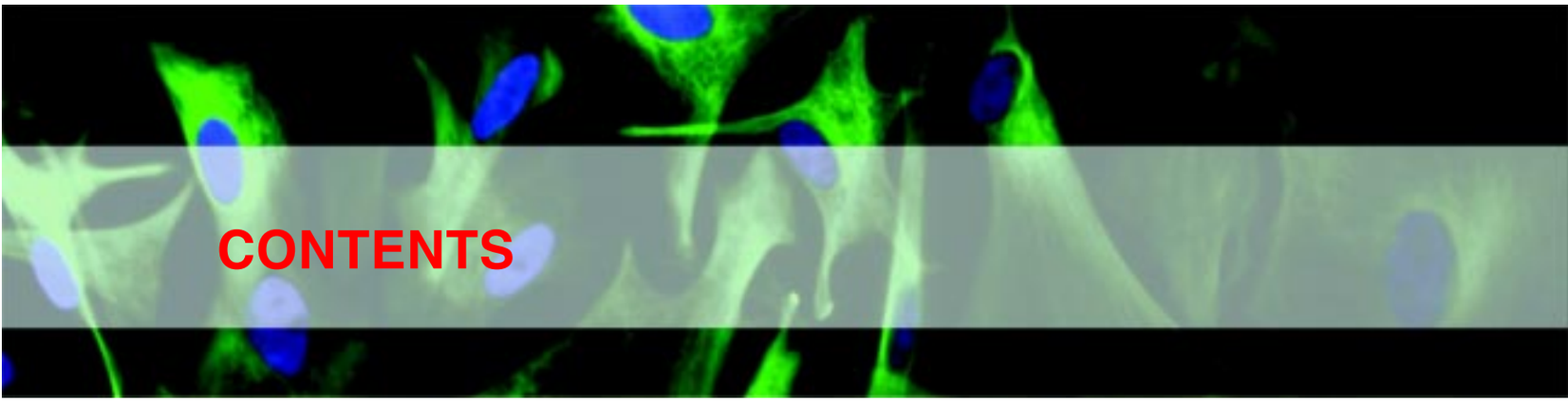
ANNUAL REPORT 2004 - 2005

National Brain Research Centre
NH-8, Manesar (Haryana), India



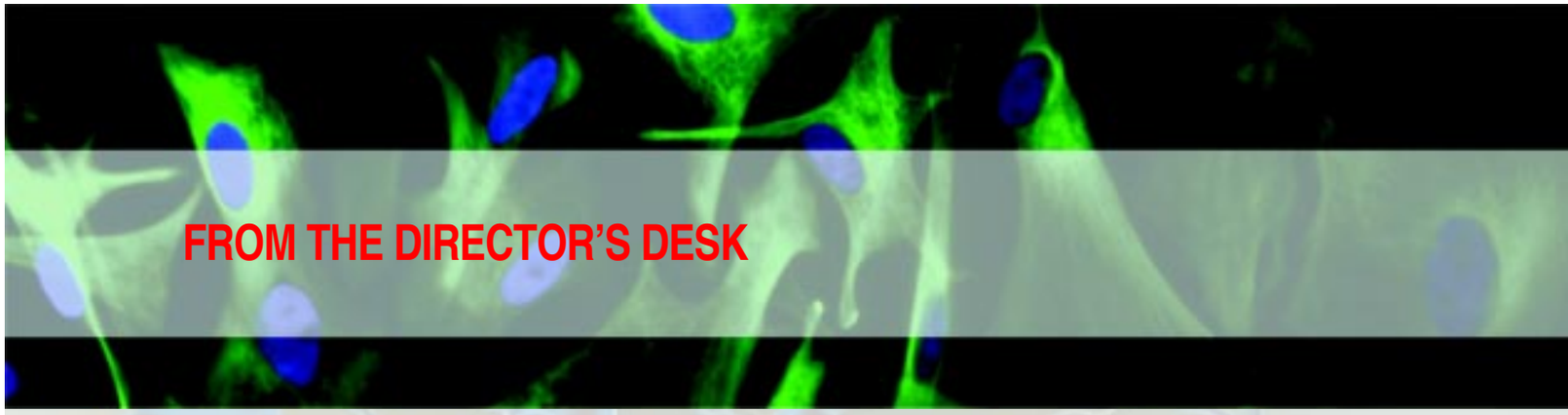
CONTENTS

MANDATE

- Pursuing basic research to understand brain function in health and disease.
- Generating trained human resources with the capability to carry out inter-disciplinary research in neuroscience.
- Promoting neuroscience in India through networking among institutions across the country

OBJECTIVES

- To undertake, aid, promote, guide and coordinate research of a high caliber in basic and clinical neuroscience related to diseases and disorders of the nervous system.
- To develop the centre as the National Apex Centre for neurosciences research and promote neuroscience research at different centres in the country and to provide consulting services to other institutions, agencies and industries.
- To promote, encourage and augment effective linkages, alliances and affiliations between the Centre and National and International Scientific and Research Institutions, bodies, agencies/ laboratories and other organizations working in the field of brain and neurosciences research.
- To establish one or more satellite centers to serve different regions of the country for efficient achievement of the objectives of the center.
- To collect, assimilate, publish and disseminate data and information on aspects relevant to neuroscience to the scientific community.
- To establish, operate and maintain state-of-the-art facilities and database for carrying research and development activities and make such facilities and database available to scientists and researchers from all over the country and abroad;
- To provide for instructions and training in such other branches of learning as the Centre may deem fit.
- To provide facilities for the advancement research and development for advancement of learning and for dissemination of knowledge.
- To undertake extramural studies, extension programmes and field outreach activities to contribute to the development of society.
- To promote, develop, collaborate or otherwise assist in providing services of research, training, consulting or guidance related to neurosciences activities comprising biological, psychological, sociological and clinical aspects; and
- To do all such other acts and things as may be necessary or desirable to further the objectives of the Centre.



FROM THE DIRECTOR'S DESK

Our brains generate numerous thoughts, memories, and emotions, mediate our perception of the world around us. Building upon earlier discoveries provided by a century of work on the anatomical and physiological organization of the brain, it is clear that the phenomenal information processing capability associated with the human brain emerges from an interaction of vast distributed networks that engage 10 billion nerve cells and ten trillion synapses. Understanding how these properties emerge from patterns of electrical activity among interconnected neurons represents a conceptual challenge in modern science. As neuroscientists, we believe that a clear understanding of such complex neural networks will not emerge from existing tools that emphasize the study of molecules or neurons in isolation; rather what is required is a holistic approach where the activity of millions of neurons across different brain areas can be studied simultaneously. It is only from such studies that we can hope to understand complex emergent properties of the brain such as consciousness, perception and cognition.

This complexity is believed to arise as a result of synergistic interactions across multiple levels of organization, with each level of organization emerging from a lower level. For example, in order to understand a neuron, it is necessary to understand the molecular and biochemical machinery that makes up the cell. The interaction of neurons through electrical signals gives rise to local neural networks that are capable of processing simple information. The neural networks interact across different brain areas in order to process more complex information. Integration of information across different networks is necessary for higher order functions such as decision-making and cognition. Thus, a complete understanding of brain function in health and disease entails an understanding that spans across molecular, cellular, systems and cognitive levels of organization.

This is an exciting era for neuroscience. The integrated multi-disciplinary approach to understanding the brain and the emergence of powerful new technologies, such as functional imaging are creating exciting opportunities to explore how decisions are made, how actions are controlled and how errors are made and corrected. These developments have come about because of the vast improvement in tools and techniques that allow us to look into the brain at many different levels of structure and function. This has led to discoveries that have extended the influence of neuroscience far beyond the realm of the laboratories and hospitals. For example, new disciplines of study such as neuroeconomics have emerged, the objective of which is to understand how the embodied brain enables the emergent mind to make economic decisions by combining techniques from cognitive neuroscience and experimental economics. Today, neuroscientists are trying to understand how education can be improved by understanding brain

function. For example, can we tailor education curriculum in schools to match with the development of human brain and the critical period for learning particular skills? Can learning music and art affect other cognitive skills?

While we make progress on understanding the human brain, our utmost concern is to discover better therapies and cures for brain disorders that affect millions of people. This becomes even more urgent as the average age of the population increases over the next decade. As important fundamental discoveries are made, additional research that translates what we know at the level of laboratory into therapies is needed. Translational research, which helps convert basic findings into therapies ready for human testing, has become an important focus of biomedical research. Translational research involves a team – a team that consists of basic scientists working in the laboratory and clinicians who have the obligation to offer and treat their patients with the best care that is available. However an often overlooked but vitally important aspect of translational research requires not only translation of research from the laboratory bench to the bedside of the patient but also the other way around – from the bedside to the bench. For the basic scientist this could mean building better animal models of disease and refinement of research design to understand the disease process. This bi-directional approach to translational research is vital to its success. In a young institute such as the National Brain Research Centre which is set up to pursue excellence in science, the programme for translational research needs to be started now, at its infancy. These could include areas such as injuries of the spinal cord in which basic research is already going on, or in the area of neurodegenerative diseases or in the area of stem cell research, wherein focused teams need to be built to allow for future translation.

Translational research in the field of stem cells is advancing at a rapid pace, mostly in the area of adult stem cells. By some estimates there may be 80 therapies and 300 clinical trials involving stem cells. However, these successes to some extent mask the problems of reducibility, poor therapeutic outcome and lack of understanding of underlying mechanisms that may lead to clinical improvement. Therefore it is important to pursue all sources of stem cells at this point, embryonic as well as adult, since it is not clear which would approach would lead to successful therapy for the different diseases.

In order to realize the potential of human embryonic stem cells in therapy it is imperative that we learn more about the fundamental biology of these cells. We have to understand under what conditions they will not form tumours, under what circumstances they will form the right kind of cells needed, how effectively they will integrate into the host tissue, and which group of patients and at what point during the progression of the disease transplantation would be most effective. To do so one needs an integrative programme that examines different aspects of stem cell behaviour. Only through focused questions, multiple approaches, and cooperative interaction can an institute make a difference in the global world of science.

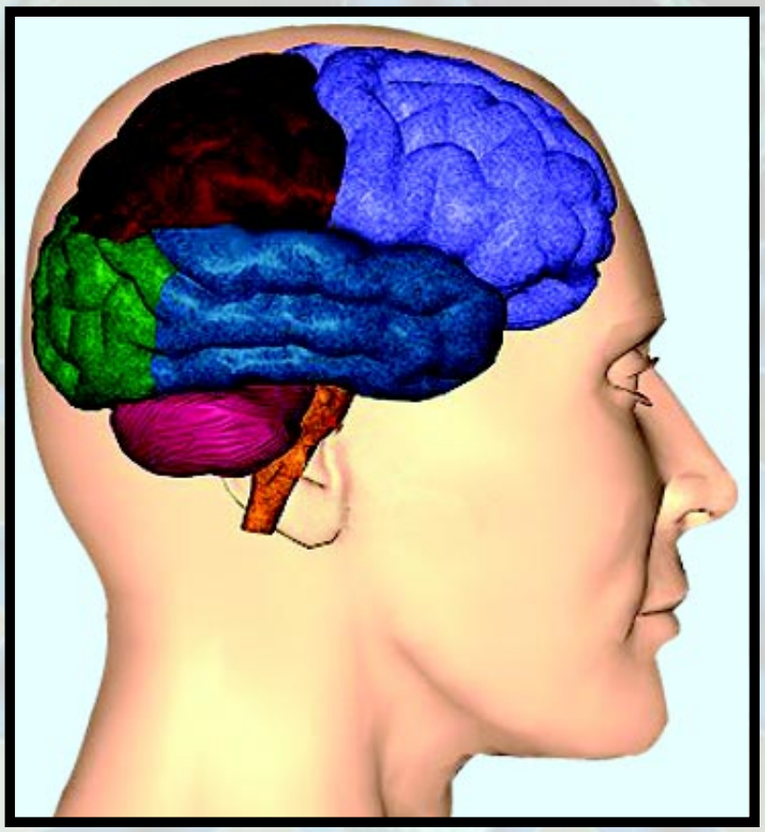
Therefore, capitalizing on the multiple strengths and talents that NBRC possesses, and given the facilities that NBRC has, we have initiated a programme on embryonic stem cell research. We

have brought together researchers who will study the mechanisms that underlie neuronal differentiation, transplant stem cells in animal models of brain and spinal cord injury and neural degeneration and examine their functional integration. In these studies we will measure whether clinical relief is correlated with stem cell functionality in the host environment. We hope that such an approach would be an important step in realizing the potential application of stem cell therapy for treatment of brain diseases.

While we understand the importance of translational research, we realize that basic science for its own sake driven by the interest and passion for excellence of an individual investigator must never be compromised. It is from a wellspring of scientific curiosity and excellence that discoveries occur, from which translational research emerges. Understanding the fundamental mechanisms of how the brain functions, helps not only in the treatment of disease but also helps promote "healthy" brain functioning. The importance of basic research cannot be over-emphasized as we follow promising research pathways for treating disease.

Neuroscience research holds great potential for human progress and health. As we make progress in our understanding of human brain, we are now poised to ask and begin to answer bigger questions. What are the mechanisms and underlying neural circuits that allow us to form memories, pay attention, use language, feel and express our emotions and make decisions? Answering these questions will help maximize human potential. The establishment of the functional imaging facility at NBRC in the coming year will help us address these questions to understand how the human brain functions and what goes wrong when it is diseased or injured.

INTRAMURAL RESEARCH REPORTS



INTRAMURAL RESEARCH REPORTS

MOLECULAR MECHANISM OF THE PATHOGENESIS OF THE CAG REPEAT NEURODEGENERATIVE DISEASES

Principal Investigator : **Nihar Ranjan Jana**

Technical Assistant : **D. Narender**

Research Fellows : **Anand Goswami, Priyanka Dikshit**

Polyglutamine diseases consist of a group of familial neurodegenerative disorders caused by expression of proteins containing an expanded polyglutamine stretch. The group includes Huntington's disease (HD), Dentatorubro Pallidoluysian Atrophy (DRPLA), Spinobulbar Muscular Atrophy (SBMA) and several spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7 and SCA17). These disorders are progressive, dominantly inherited, generally starts in mid-life, and result in severe neuronal dysfunction and neuronal cell death. Increasing length of glutamine repeats in the affected individual strongly correlates with earlier age of onset and disease severity. The repeats show both somatic and germline instability and the successive generation of the affected families experience earlier age of disease onset and rapid disease progression. Interestingly, only a particular group of neurons is affected in each of these diseases, despite the ubiquitous expression of the relevant disease proteins throughout the brain and other tissues. The normal function of most of these proteins remains unknown. A common biochemical feature of most polyglutamine disorders is the occurrence of ubiquitin-positive neuronal intranuclear inclusions. The appearance of ubiquitinated aggregates implies an underlying incapability of the cellular chaperones and proteasome machinery that normally functions to prevent the accumulation of misfolded proteins. But how the formation of these aggregates is mechanistically linked to cellular toxicity is not well understood.

Major aims of this project are (1) to identify and characterize the protein(s) that specifically interact with the expanded polyglutamine tract, (2) to elucidate the mechanism of ubiquitination of the polyQ protein aggregates and modulation of their degradation, (3) to identify the role of mitochondria in polyglutamine disease pathogenesis and (4) screening and identification of small molecules for therapeutic intervention of polyglutamine diseases.

We are studying the mechanism of polyglutamine disease pathogenesis using HD and SCA3 as models. Last year we have shown that CHIP (C-terminus of hsp70 interacting protein) associates with the expanded polyglutamine proteins and promotes their ubiquitination. We have further characterised the functional significance of the interaction and increased ubiquitination of expanded

polyglutamine proteins with CHIP. We have found that transient over-expression of CHIP not only increased ubiquitination but also the rate of degradation of polyglutamine-expanded huntingtin or ataxin-3. Over-expression of CHIP also suppressed the aggregation and cell death mediated by expanded polyglutamine proteins and the suppressive effect was more prominent when CHIP was over expressed along with Hsc70. Now we are investigating the mechanisms through which CHIP protects the expanded polyglutamine protein induced cell death.

This year we have also identified another protein called BAG-1 (BCL2 associated growth regulator-1) that specifically co-immunoprecipitated with the polyglutamine-expanded huntingtin and associated with their aggregates. The association of BAG-1 with the mutant huntingtin was indirect through its interaction with the chaperones Hsc70/Hsp70. We further demonstrated that the over-expression of BAG-1 protects polyglutamine-expanded huntingtin induced cell death. Since BAG-1 is essential for cell survival, its association with huntingtin aggregates might disrupt its normal function and thereby promote polyglutamine-expanded tNhtt-induced cell death.

By using a cellular model of HD, we are also investigating the role of oxidative and endoplasmic reticulum stress on the polyglutamine protein induced cell death to determine whether those stressors modulate cellular proteasomal function. Our preliminary data suggests that the oxidative stress-induced proteasomal malfunction could be linked with expanded polyglutamine protein induced cell death.

Publications:

N. R. Jana and N. Nukina. BAG-1 associates with the polyglutamine-expanded huntingtin aggregates. Neuroscience Letter, 378, 171-175, 2005.

N. R. Jana, P. Dikshit, A. Goswami, S. Kotliarova, S. Murata, K. Tanaka and N. Nukina. Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. ***Journal of Biological Chemistry,*** 280, 11635-11640, 2005.

S. Kotliarova, **N. R. Jana**, N. Sakamoto, M. Kurosawa, H. Miyazaki, M. Nekooki, H. Doi, Y. Machida, H. K. Wong, T. Suzuki, C. Uchikawa, Y. Kotliarov, K. Uchida, Y. Nagao, K. Oyanagi, F. Oyama and N. Nukina. Decreased expression of hypothalamic neuropeptides in Huntington Disease transgenic mice with expanded polyglutamine-EGFP fluorescent aggregates. ***Journal of Neurochemistry,*** 2005.

N. R. Jana and N. Nukina. Misfolding promotes the ubiquitination of polyglutamine-expanded ataxin-3, the defective gene product in SCA3/MJD. ***Neurotoxicity Research,*** 6, 523-533, 2004.

U. Nagaoka, K. Kim, **N. R. Jana**, H. Doi, K. Mitsui, F. Oyama and N. Nukina. Increased expression of p62 in expanded polyglutamine-expressing cells and its association with polyglutamine

inclusions. 2004. ***Journal of Neurochemistry,*** 91, 57-68, 2004.

 # Work done elsewhere.

Presentations:

N. R. Jana. Role of ubiquitin-proteasome system in the pathogenesis of polyglutamine diseases. Annual meeting of the Physiological Society of India, 2004.

N. R Jana, Priyanka Dikshit, Anand Goswami and Nobuyuki Nukina. Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. Japan-China-Korea-India workshop on Neurobiology and Neuroinformatics, 2004.

Funding:

Molecular mechanism of the pathogenesis of the CAG repeats neurodegenerative diseases (DBT).

Molecular mechanism of the pathogenesis of polyglutamine diseases (RIKEN Brain Science Institute, Japan).

Collaborator:

Dr. Nobuyuki Nukina, RIKEN Brain Science Institute, Japan.

MECHANISMS OF CURCUMIN-INDUCED NEURONAL CELL DEATH AND DIFFERENTIATION

Principal Investigator : **Nihar Ranjan Jana**

Technical Assistant : **D. Narender**

Research fellows : **Priyanka Dikshit**

Project Assistant : **Mou Chatterjee**

Curcumin, a polyphenolic phyto-chemical, is the primary component of the spice turmeric (Curcuma longa). It has been demonstrated to have anti-inflammatory, antioxidant and anti-proliferative activities. The pharmacological safety of curcumin is well documented by the fact that people in certain countries including India have consumed Curcumin as a dietary spice for centuries in amount in excess of 100mg/day without any side effect. Ample evidence exists to support its use in cancer prevention for its anti-proliferative and anti-carcinogenic properties. curcumin, *in vivo*, suppresses carcinogenesis of the skin, stomach, colon, breast and liver in mice and it has been shown to inhibit the growth of a wide variety of tumour cells *in vitro*. Although its precise mode of action remains elusive, studies have shown that chemo-preventive action of curcumin might be due to its ability to induce apoptosis. The pathway through which curcumin induces apoptosis and whether mitochondria play any role in curcumin-induced apoptosis is not fully understood.

The major objectives of this project are (1) to identify the mechanism of the neuronal cell death caused by curcumin and (2) whether and how curcumin promotes neuronal cell differentiation.

We are investigating the cell death mechanisms induced by curcumin using the neuro2a (mouse neuroblastoma) cell line. Last year, we had reported that the curcumin-induced apoptosis is mediated through the impairment of ubiquitin proteasome system (UPS). We are continuing various aspects of this work such as how curcumin inhibits proteasome function and what could be the impact of the curcumin-induced proteasomal malfunction in the cells and how it can be linked with its anti-inflammatory function. We found that the curcumin disrupts the UPS function by directly inhibiting the enzyme activity of the proteasome's 20S core catalytic component. Like other proteasome inhibitors, curcumin exposure induces the stress response as evident from the induction of various cytosolic and endoplasmic reticulum chaperones as well as induction of transcription factor CHOP/GADD153. The direct inhibition of proteasome activity also causes increase in half-life of IκB-α that ultimately leads to the down-

regulation of NF-κB activation. Our result suggests that the curcumin-induced proteasomal malfunction might be linked to both its anti-proliferative and anti-inflammatory activities.

Publication:

N. R. Jana, P. Dikshit, A. Goswami and N. Nukina. Inhibition of proteasomal function by Curcumin induces apoptosis through mitochondrial pathway. ***Journal of Biological Chemistry*****,** 279, 11690-11695, 2004.

Presentation:

P. Dikshit, A. Goswami, A. Mishra and **N. R. Jana**. Curcumin induces apoptosis through the impairment of ubiquitin proteasome pathway. Annual meeting of the Indian Association for Cancer Research, Noida, 2005.

UNDERSTANDING THE PATHOGENIC MECHANISM OF ANGELMAN SYNDROME.

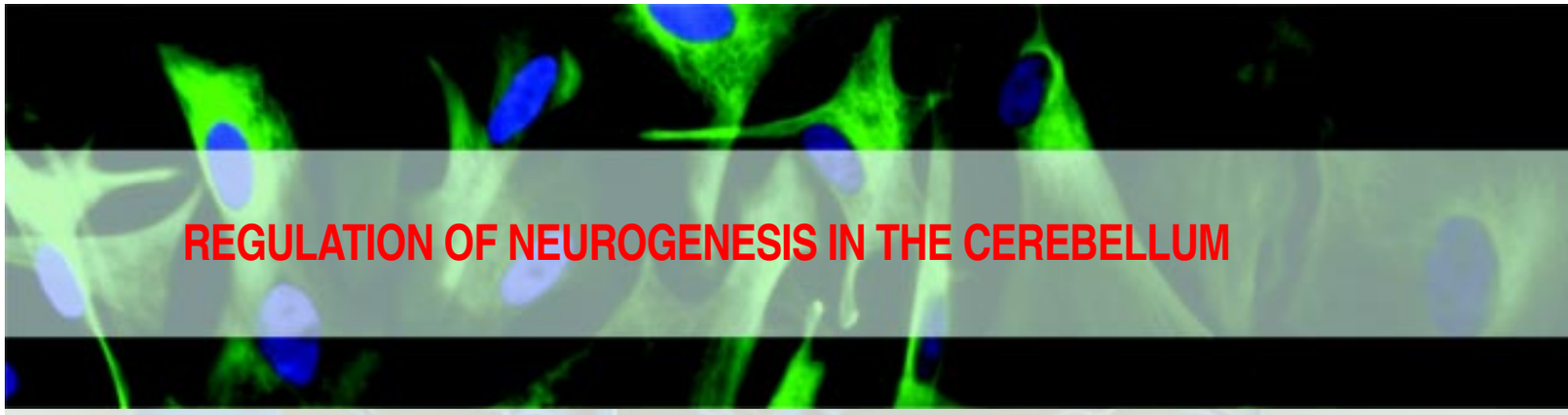
Principal Investigator : **Nihar Ranjan Jana**

Research Fellow : **Amit Mishra.**

The Angelman Syndrome (AS) is characterized by severe mental retardation, microcephaly, a puppet-like ataxic gait with jerky arm movements, hyperactivity, EEG abnormalities and seizures. The frequency of occurrence of AS is 1 in 10,000 to 1 in 20,000 births. Although a mutation of the UBE3A gene (which encodes a protein called E6-AP, a HECT domain ubiquitin ligase) has been reported to cause AS, the detailed mechanism of disease pathogenesis remains largely unknown. Linkage disequilibrium or maternal truncation mutation of UBE3A has also been reported in an autistic family. Though E6-AP is known to be responsible for the ubiquitination of p53 protein with the help of E6 oncoprotein, its other cellular substrate is unknown. Therefore, the identification of the new substrate of E6-AP might help not only in the basic understanding of AS pathogenesis but also the pathogenesis of autism.

The major objective of this project is to determine how the mutation of UBE3A (the candidate gene so far known) causes disease pathogenesis. We are focusing particularly on (1) identification and characterization of the substrate of E6-AP ubiquitin ligase, (2) evaluation of the possible involvement of E6-AP in stress-dependent ubiquitination and protection of stress-dependent cell death and (3) how the mutation of E6-AP affects the ubiquitination of its substrate.

We have cloned full-length and several deletion constructs of E6-AP gene in pcDNA vector and characterized their expression in the mammalian cell culture system. We are now trying to identify the E6-AP interacting protein by co-immunoprecipitation as well as by co-purifying the protein using probond resins. Our effort to clone the E6-AP gene into the yeast vector is underway for yeast two-hybrid screening. We are also making several point mutations (particularly in the ligase domain and reported in AS patients) in the E6-AP gene using site-directed mutagenesis to study the effects of mutating its function. We are also working on the regulation of E6-AP gene expression under different stress conditions using the mammalian cell culture system and are planning to study the developmental regulation of this gene using a mouse model.



REGULATION OF NEUROGENESIS IN THE CEREBELLUM

Principal Investigator : **Shyamala Mani**

Research Fellow : **Rashmi Mishra**

The cerebellum is important for motor coordination and this function depends on the precise synaptic wiring of the different types of cells in the cerebellum. The most abundant cell type in the cerebellum are the glutamatergic granule cells. They control the output of the purkinje cells and thus regulate cerebellar output. Consistent with the fact that the granule cells play a crucial role in cerebellar output, mutations in which there is a loss of granule neurons results in severe ataxia. Much is known about the origins of the granule cells. Unlike other cell types, granule cells are generated in the External Germinal Layer (EGL) where they undergo extensive proliferation during the first two postnatal weeks. Eventually the granule cell precursors exit the cell cycle, extend axons that synapse with the dendrites of the purkinje cells in the molecular layer and migrate past the purkinje cells to form the inner granule cell layer. Several extrinsic cues control each of these events in granule cell neurogenesis. Among these, there is considerable evidence that sonic hedgehog (Shh) expressed by purkinje cells plays a crucial role in controlling cell growth in the cerebellum. In humans, mutations of the patched gene, the receptor for sonic hedgehog is mutated in cases of sporadic medulloblastomas and also in cases of Basal Cell Nevus Syndrome which leads to increased frequencies of tumors. The neurotrophin brain derived neurotrophic factor (BDNF) that is expressed by granule cells during development also plays a role in the survival and migration of granule cell precursors. Several of these signals are present simultaneously throughout the period of cerebellar histogenesis and one of the important questions that remains to be worked out is how these different signals are interpreted by the granule cells such that they progress in a precise fashion through the various stages of neurogenesis producing the required cell numbers for ensuring proper connectivity in the cerebellum. GAP-43 is a nervous system specific protein that is required in order for growing axons to respond to signals that give rise to patterning in the CNS. For example, the GAP-43 (-/-) mice fails to form topographic maps in cortex and cannot form telencephalic commissures. This occurs partly because the GAP-43 (-/-) axons cannot respond to immunoglobulin superfamily (Ig-SF) mediated axon outgrowth and guidance signals. Likewise GAP-43 (-/-) cerebellar granule cell neurons also cannot respond to IgSF and FGF mediated signals and the GAP-43 (-/-) mice exhibit defective cerebellar patterning reflected in abnormal foliation patterns. We have been therefore asking the question of whether GAP-43 is one of the critical molecules involved

in the integration of several extracellular signals and thus plays a role in cerebellar histogenesis. We hope that understanding the molecular mechanisms by which different signals might be integrated will help us understand how the balance between proliferation and differentiation is maintained in the cerebellum which in turn has important implications for therapeutic strategies for treating cerebellar tumors.

Cerebellar cultures from GAP-43 -/- mice were stimulated with bFGF, Shh and BDNF. Cerebellar cultures have been prepared from P4 and P8 mice (n = 3 for each genotype). The number of BrdU cells was quantitated under different conditions and compared to age and litter matched wild type controls. We also quantitated cell death by TUNEL staining and mitosis by PH3 a phosphohistone marker.

The major findings were as follows:

a) There is a two-fold increase in BrdU labeled cells in the knockout cultures as compared to the wild type P4 cerebellar cultures. Current experiments show that this is due to an increase in the length of the S-phase in knockout animals.

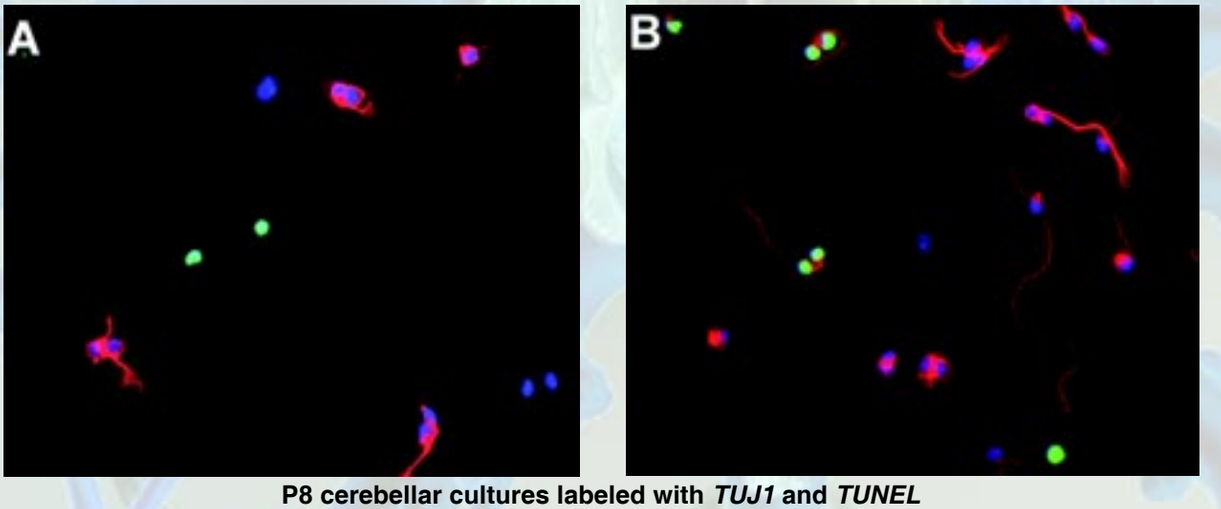
b) Proliferation response to bFGF when added to the defined culture medium is greater in wild type P4 cerebellar cultures than for the knockout cultures.

c) Similarly the proliferation response to Shh, an important factor that regulates granule cell proliferation in the developing cerebellum, is also reduced in the P4 knockout cultures as compared to wild type cultures.

d) Wild type cerebellar cultures respond to BDNF stimulation by an approximately two-fold increase in cell number. Further, addition of BDNF reduces TUNEL staining by about 6 fold. In contrast there is no increase in cell number or a reduction in cell death in response to BDNF in the knockout cultures at P4.

e) Neuronal cell death is five fold more in knockout cerebellar cultures compared to wild type controls at P8.

These results are being corroborated presently with in vivo data.



P8 cerebellar cultures labeled with *TUJ1* and *TUNEL*

Figure 1: Panel A shows cells from wild type cerebellum and panel B shows cells from GAP-43 knockout animals.

Publication:

Y. Shen, **S. Mani**, K.F. Meiri (2004). Failure to express GAP-43 leads to disruption of a multipotent precursor and inhibits astrocyte differentiation. ***Mol. Cell. Neurosci.* 26** :390-405.

Presentation:

Role of GAP-43 in differentiation of cerebellar granule cells, R Mishra, K F Meiri and **S Mani, The National Academy of Sciences 74th Annual Session, Jaipur, Dcember** 2-4 2004.

Funding:

This work is supported by a FIRCA-NIH grant as well as intramural support.

Collaborator:

Karina Meiri, Tufts University

TO INVESTIGATE THE MECHANISMS BY WHICH EMBRYONIC STEM CELLS DIFFERENTIATE INTO DISTINCT NEURONAL SUBTYPES

Principal Investigator : **Shyamala Mani**

Research Fellow : **Manoj Kumar**

Technical Assistant : **Bandita Bagchi**

Intrinsic factors that control the commitment to neuronal lineage and that play a role in neuronal differentiation and cell type specification are largely controlled by transcription factors that contain the basic helix-loop-helix (bHLH) motif. Proneural bHLH factors are involved in the commitment of a multipotent neuroepithelial progenitor cell to the neuronal lineage. These include the neurogenins and Mash. Terminal neuronal differentiation further involves a second class of bHLH factors known as neuronal differentiation factors. This includes NeuroD, NDRF and Nex. Expression of neuronal differentiation factors results in cell cycle arrest and differentiation of neurons in culture. The pattern of expression of neural differentiation genes in vivo is overlapping but not identical. In fact, some of these genes are expressed in specific subsets of neurons, which suggests an additional important function of these factors, that they may be involved in specifying neuronal cell type. Knockout mice have been generated in order to study whether these differentiation factors are involved in the specification of neuronal subtype. However single knockouts of NeuroD, Nex or double knockouts do not show an obvious defect in a subpopulation of neurons missing. Our goal is to elucidate the function of proneural and neural differentiation bHLH genes using ES cells as a model system for studying neuronal differentiation.

This year, the focus has been entirely on human ES (hES) cells. Passaging the hES cells and keeping most of the cells in an undifferentiated state has been a major challenge. This has been standardized for the most part although occasionally the hES cultures tend to exhibit a high spontaneous rate of differentiation. This is mostly because the mouse feeder fibroblast layer is not of a good quality. Occasionally, it has been because one of the key ingredients such as bFGF is not as efficacious. The hES cells have been differentiated under different conditions. We have established a baseline for differentiation of hES cells. This is based on the protocol that is outlined in Zang et al., (2001), which involves the differentiation of hES cells into neurospheres which are then further terminally differentiated into neurons. Following this basic method, we have been able to differentiate hES cells into neurons. We are now in the process of increasing the number of neurons that we obtain as well as characterizing the type of neurons that are seen.

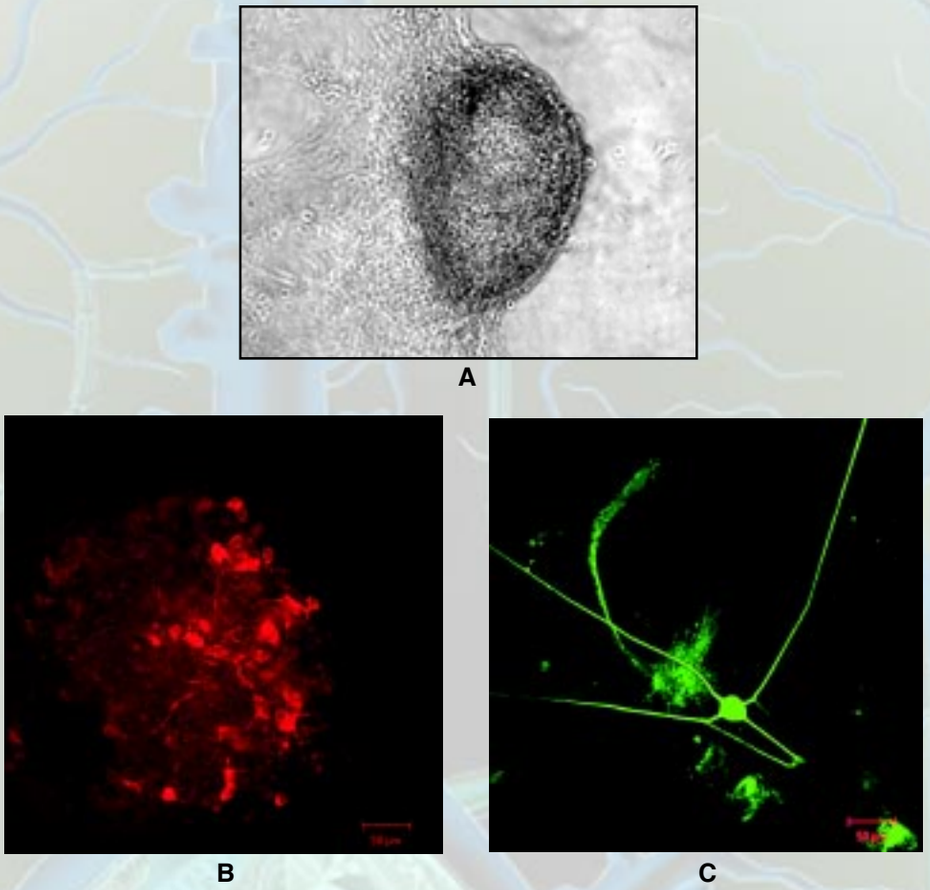


Figure2: (A) An undifferentiated hES cell colony. (B) Differentiated hES cells expressing tyrosine hydroxylase. (C) Differentiated hES cells expressing TUJ1 are able to extend long neurites in culture.

Publication:

The CMV immediate early promoter is regulated by cell-cell interaction and retinoic acid during differentiation of embryonic stem cells. Bandita Bagchi, Manoj Kumar and **Shyamala Mani** (Submitted to ***Cell Biology International***).

Funding:

This work is supported by a grant from DBT as well as intramural support.

CYTOCHROMES P450 DEPENDENT METABOLISM OF DRUGS IN BRAIN

Principal Investigator : **Vijayalakshmi Ravindranath**

Research Fellows : **Reddy Peera, Varsha Agarwal**

Technical Assistant : **Prasanna V.K.**

Cytochrome P450 (P-450), a superfamily of heme proteins is involved in the metabolism of a vast array of carcinogens, drugs and endogenous compounds. The liver is the major organ involved in P450 mediated xenobiotic metabolism, however preferential localization of xenobiotic metabolizing enzymes within specific cell types in extrahepatic organs renders such cells significant capability to metabolize foreign compounds including carcinogens. Thus, minor metabolic pathways of xenobiotic metabolism can produce major effects if they take place at the site of action. Significant differences are seen in the biotransformation of psychoactive drugs mediated by brain and liver P450 enzymes. For example, drugs such as alprazolam are metabolized differently in liver and brain wherein relatively larger amount of the active metabolite is generated in the brain compared to liver. These observations have indicated the existence of unique P450 enzymes in brain that are different from the well-characterized hepatic P450s. Our working hypothesis is that brain cytochromes P-450 plays an important role in the pharmacological modulation of drugs acting on the CNS and it is therefore important to understand major P-450-mediated biotransformation and characterize the enzymes mediating such reactions in the human brain. While many isoforms of P450 are expressed in brain, is there anything unique about their presence there? The long-term objective of the proposed project is to understand the role of *in situ* cerebral drug metabolism in the pharmacological action of psychoactive drugs.

We had earlier identified that a frame-shift mutation 138delT generates an open reading frame in the pseudogene, cytochrome P4502D7 (CYP2D7) and an alternate spliced functional transcript of CYP2D7 containing partial inclusion of intron 6 is generated in the human brain but not in liver or kidney from the same individual. In liver, the major organ involved in drug metabolism, a minor metabolic pathway mediated by CYP2D6 metabolizes codeine (pro-drug) to morphine (active drug) while nor-codeine is the major metabolite. In contrast, when expressed in Neuro2a cells, the brain variant CYP2D7 metabolized codeine to morphine with greater efficiency compared to the corresponding activity in cells expressing CYP2D6. Morphine binds to μ-opioid receptors in certain regions of the central nervous system, such as periaqueductal gray and produces pain

relief. The brain variant CYP2D7 and μ-opioid receptor co-localize in neurons of periaqueductal gray area in human brain indicating that metabolism of codeine to morphine could occur at the site of opioid action.

CYP1A1, a P450 enzyme plays an important role in the bioactivation of polycyclic aromatic hydrocarbons to the ultimate carcinogen. We identified a splice variant of CYP1A1 in brain by RT-PCR amplification of exons 3-7 (967-1339 bases) of CYP1A1. In all 15 samples examined, a 301 bp fragment was amplified instead of the anticipated 394 bp product, showing the existence of a splice variant for cytochrome P4501A1 in the human brain, which had deletion of exon 6. RT-PCR amplification of the complete open reading frame of CYP1A1 from human brain RNA generated an amplicon of 1593 bp having deletion of 87 bp of exon-6. Using RT-PCR and fluorescence *in situ* hybridization, it was observed that the exon 6 deleted CYP1A1 was present only in the brain but not in other organs, such as the liver and kidney. When expressed in Neuro2A cells, it translated into a functional P450 enzyme as determined by immunoblotting and total P450 content. The exon 6-deleted CYP1A1 metabolized ethoxy resorufin (the classical substrate for CYP1A1) less efficiently compared to CYP1A1 while pentoxy and benzyloxy resorufins (classical substrates for CYP2B) were dealkylated more efficiently.

Cells transfected with exon 6 deleted CYP1A1 did not generate any detectable carcinogenic DNA adducts from arylhydrocarbons while they were formed in significant amount in cells expressing CYP1A1 as determined by ^{32}P post-labelling. Exon 6 deleted CYP1A1 enzyme generated by alternate splicing is functionally different from its hepatic counterpart and does not contribute to bioactivation of arylhydrocarbons to genotoxic ultimate carcinogens that initiate neoplastic transformation through formation of DNA adducts. Structural modeling of the putative protein indicates that there would be alteration in the substrate access channel possibly allowing for less restricted access and altered angle of approach as compared to the normal CYP1A1. A broader substrate access channel would also allow for more free movement of the substrate within the access channel. Size of the active site, character of the substrate access channel and angle of approach appear altered in the exon-6 deleted P4501A1, which may account for the lack of DNA adducts following exposure to arylhydrocarbons.

RT-PCR analysis of 50 samples of human brain revealed that the exon 6-deleted CYP1A1 transcript was present in 49 samples, while only one sample expressed the wild type CYP1A1. PCR amplification of exon 5 to 7 of CYP1A1 using genomic DNA from the human brain samples expressing wild type and exon 6-deleted CYP1A1 revealed no genotypic differences. The nervous system has a propensity for generating alternate spliced forms and splicing diversity may not be related to differences in the genomic sequence but may be regulated by the spliceosomal complex and RNA binding proteins.

Publication:

S.J.Chinta, H.V. Pai and **V. Ravindranath**: Presence of splice variant forms of cytochrome P4502D1 in rat brain but not in liver. ***Molecular Brain Research*** 135:81-92 (2005).

S.J. Chinta, R. P. Kommaddi, H. V. Pai, C. M. Turman, H. W. Strobel and **V. Ravindranath**: Constitutive expression and localization of CYP1A1 in rat and human brain: Presence of a splice variant form in human brain. ***J. Neurochem***. 93:724-36 (2005).

Presentations:

R.P. Kommaddi and **V. Ravindranath**: Identification of a Unique Human Brain Cytochrome P4501A1 Variant Generated by Alternate Splicing at 28 – 30 January, 2005 Indian Academy of Neurosciences, Gwalior

R.P. Kommaddi, H.V. Pai, S.J. Chinta and **V. Ravindranath**: Biotransformation of drugs mediated by brain-specific splice variants of the drug metabolizing enzyme, cytochrome P450 at the Annual Meeting of Society for Neuroscience, October 2004.

V. Ravindranath: Drug metabolism in brain by unique cytochrome P450 enzymes. Symposium on Emerging Concepts in Brain Function at NCBS, Bangalore, March 2004.

Funding:

NIH-RO1 - MH70054

Collaborator:

Prof. H. W. Strobel, Univ. of Texas Medical School, Houston, USA

PROTEIN THIOL HOMEOSTASIS AND MITOCHONDRIAL DYSFUNCTION IN NEURODEGENERATION AND NEURO-PROTECTION BY ESTROGEN

Principal Investigator : **Vijayalakshmi Ravindranath**

Research Fellows : **Smitha Karunakaran, Uzma Saeed, Latha Diwakar,**

Post Doctoral Fellow : **R. C. Kumar**

Project Assistant : **Sujanitha R.**

Idiopathic Parkinson's Disease (PD) as opposed to heritable forms of Parkinson's Disease (PD) accounts for greater than 90% of the Parkinson's disease incidence world over. Among many of the pathological hallmarks, mitochondrial complex-I (NADH ubiquinone 1 oxidoreductase) dysfunction observed in the skeletal muscles, platelets, and lymphocytes of these patients has been shown to be a primary event in PD. Identification of factors involved in maintenance and restoration of complex I function could potentially help develop prophylactic and therapeutic strategies for treatment of this class of disorders. We had earlier shown that perturbations in redox-status of thiols underlies the mitochondrial complex I dysfunction seen in animal models of neurodegenerative disorders and maintenance of redox-status of protein thiols by protein disulphide oxido-reductases, such as glutaredoxin is important for maintaining/restoring mitochondrial function. Down-regulation of glutaredoxin (thioltransferase, a thiol disulfide oxido-reductase) using antisense oligonucleotides results in the loss of mitochondrial complex I activity in mouse brain. Using model neurotoxins such as MPTP and L-BOAA (an excitatory amino acid that is the causative factor of lathyrism, a type of motor neuron disease), we had identified the critical role of glutaredoxin in the restoration of complex I activity. It is our hypothesis that protein thiol modification occurring as a result of oxidative stress results in mitochondrial dysfunction and enzymes such as glutaredoxin play a critical role in the restoration of protein thiol homeostasis and are important mediators of the recovery process. Their differential expression and regulation may help explain some of the gender differences typically seen in both animal models of neurodegenerative disorders and in patient population.

The animal models of MPTP and L-BOAA mimic the gender difference seen in human incidence of the diseases. Treatment with estrogen receptor antagonist sensitizes female mice to MPTP and L-BOAA toxicity. The constitutive expression of glutaredoxin is higher in female brain regions as compared to males and pretreatment estrogen receptor antagonist results in decrease in activity of

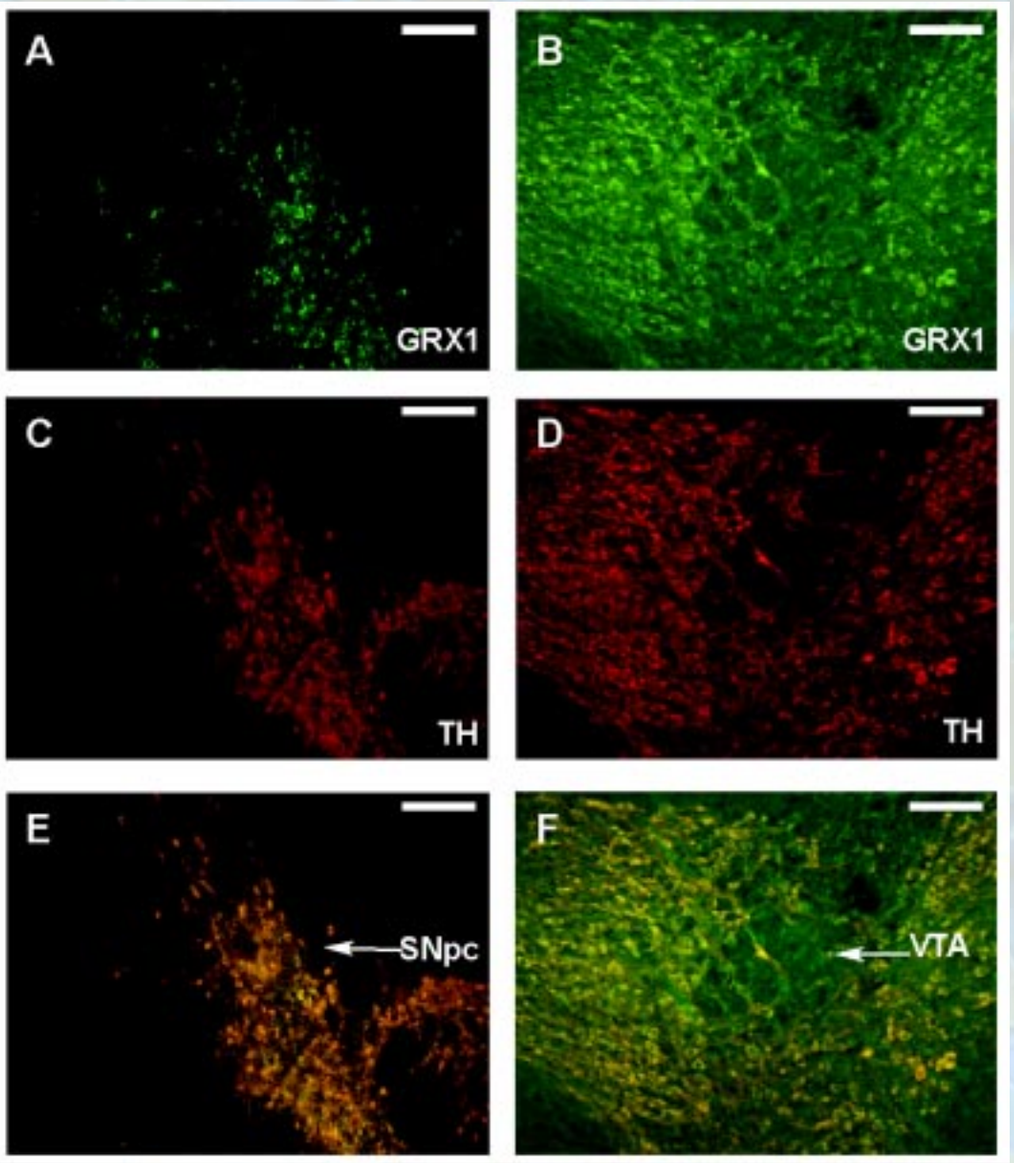
glutaredoxin in brain regions indicating that glutaredoxin is regulated through estrogen receptor(s) in brain.

We examined the effects of ovariectomy on the excitotoxicity mediated by L-BOAA. Administration of L-BOAA triggers glutathione loss and inhibition of complex I activity in motor cortex and lumbosacral cord of male mice but has no such effect in female mice. Ovariectomy sensitizes female mice to L-BOAA toxicity and early events subsequent to L-BOAA administration, such as increased AP1 transcription, upregulation of the mRNA and protein of glutaredoxin were seen only in ovariectomized mouse CNS regions but not in sham-operated females. Constitutive expression of glutaredoxin is significantly higher in female mice brain regions, such as cortex and spinal cord but not in others such as hippocampus and thalamus and in non-CNS tissues such as liver or kidney. Down-regulation of glutaredoxin in female mouse CNS using antisense oligos to Grx1 sensitizes female mice to L-BOAA toxicity indicating that higher constitutive expression of glutaredoxin could potentially contribute to neuroprotection seen in female mouse following exposure to excitotoxins, such as L-BOAA. These results indicate that Grx1 not only plays an important role in the maintenance of mitochondrial complex I function but may also be involved in estrogen mediated neuroprotection. Grx1 does not have an estrogen receptor response element (ERE) element in the upstream promoter region. However, estrogen receptors can interact with transcription factors such as SP1 and EPRE and initiate transcription. Since estrogen regulates Grx1 expression in some brain regions and not others and this is linked to its neuroprotective effects, it would be important to identify the protein interaction networks that govern this phenomenon. In order to address this question, we have now developed an in vitro model in SH-5Y neuroblastoma cell line that expresses estrogen receptors and exposure to estradiol results in increased Grx1 expression. We propose to identify the protein interaction networks that are associated with estrogen-regulated transcription of Grx1. In addition, other redox regulatory proteins, such as thioredoxin and thioredoxin reductase are also being studied in relation to their potential role in neuroprotection.

One of the compelling questions in understanding the pathogenesis of neurodegenerative disorders is the selective vulnerability of specific cell types to neurodegeneration. We are trying to address this question using DNA microarray to examine global gene expression patterns. MPTP model of PD was replicated in C57 mice bred at NBRC using biochemical and histochemical endpoints. We then profiled the early changes in global gene expression patterns following MPTP administration using microarray analyses. Mouse cDNA arrays were hybridized with RNA from midbrain and striatum (the regions affected by MPTP) of control and MPTP treated mice. Genes involved in ubiquitin proteosome, proapototic and MAP kinase pathways were affected. Up-regulation of TNFα, Pak1-BAD, c-jun N-terminal kinase/AP1 (JNK) and p38 mitogen-activated protein (MAP) kinase were observed. The downregulation of pro-survival genes such as Akt and the upregulation of pro-apoptotic pathways were more prominent in midbrain compared to striatum. Our results indicate that MPTP exposure activates cell death related genes at early time points, in a region-specific manner and this may contribute to the

dopaminergic cell death seen in substantia nigra following exposure to MPTP. Identification of factors involved in the regulation of critical checkpoints in these pathways could potentially help develop prophylactic and therapeutic strategies for treatment of this class of disorders.

Colocalisation of glutaredoxin and tyrosine hydroxylase in substantia nigra pars compacta and ventral tegmental area of mouse brain.



Intense fluorescence was seen in the neurons of substantia nigra pars compacta (SNpc; A) and ventral tegmental area (VTA; B) of midbrain when stained with antiserum to glutaredoxin 1. Labeling of dopaminergic neurons in SNpc (C) and VTA (D) when stained with an antibody against tyrosine hydroxylase. Merged images of A and C (E) and B and D (F) indicating the localisation of glutaredoxin in dopaminergic neurons as depicted by yellow color. (Scale bar=50μm)

Ph.D. Thesis (Awarded) – Latha Diwakar

Publications:

Kenchappa, R.S., Diwakar, L., Annepu, J and **Ravindranath, V.:** Estrogen and neuroprotection: Higher constitutive expression of glutaredoxin in female mice offers protection against MPTP mediated neurodegeneration. ***FASEB J.*** **18** 1102-1104 (2004).

Kenchappa, R.S., Diwakar, L., Annepu, J and **Ravindranath, V**.: Estrogen receptor antagonist down regulates glutaredoxin and renders female mice susceptible to excitatory amino acid mediated complex I inhibition in the CNS. ***Antioxidants & Redox Signalling.*** (under revision).

Diwakar, L. and **Ravindranath, V**.: Ovariectomy perturbs protein thiol homeostasis and abolishes neuroprotection against excitatory amino acid in female mice. ***J. Neurosci.*** **(Submitted).**

Presentations:

V. Ravindranath: Global gene expression analysis using microarray to study differential vulnerability to neurodegeneration at FAONS meeting at Tehran, Iran - May, 2004

V. Ravindranath: Molecular Pathogenesis of neurodegenerative disorders at Annual Meeting of IANS, Hyderabad - May, 2004

V. Ravindranath: Oxidative Stress and Neurodegeneration: National Neuroscience Conference, NIMHANS, Bangalore – September, 2004.

R.S. Kenchappa, L. Diwakar and **V. Ravindranath**: Protein thiols, glutathione and complex I dysfunction in Parkinson's Disease. Annual Meeting of Society for Neuroscience, U.S.A. , October 2004.

S. Karunakaran, S. Sampath and **V. Ravindranath:** Constitutive expression, localization and polymorphism of mitochondrial Glutaredoxin-2. Annual Meeting of Society for Neuroscience, USA, October 2004.

V. Ravindranath: Molecular Pathogenesis of Neurodegenerative Disorders. Symposium on molecular basis of disease at IIT, Chennai - January, 2005

V. Ravindranath: Protein thiol homeostasis and mitochondrial dysfunction in neurodegenerative diseases. Indo-French meeting, NBRC - February, 2005

V. Ravindranath: Recent Advances in the Pathogenesis and Treatment of Neurodegenerative Disorders. Sengupta Memorial Lecture, University of Calcutta, Kolkatta - January, 2005

EVALUATION OF THE MOLECULAR BASIS OF THE PHARMACOLOGICAL ACTION OF TRADITIONAL MEDICINAL PREPARATIONS USED IN THE TREATMENT OF DEMENTIA

Principal Investigator : **Vijayalakshmi Ravindranath**

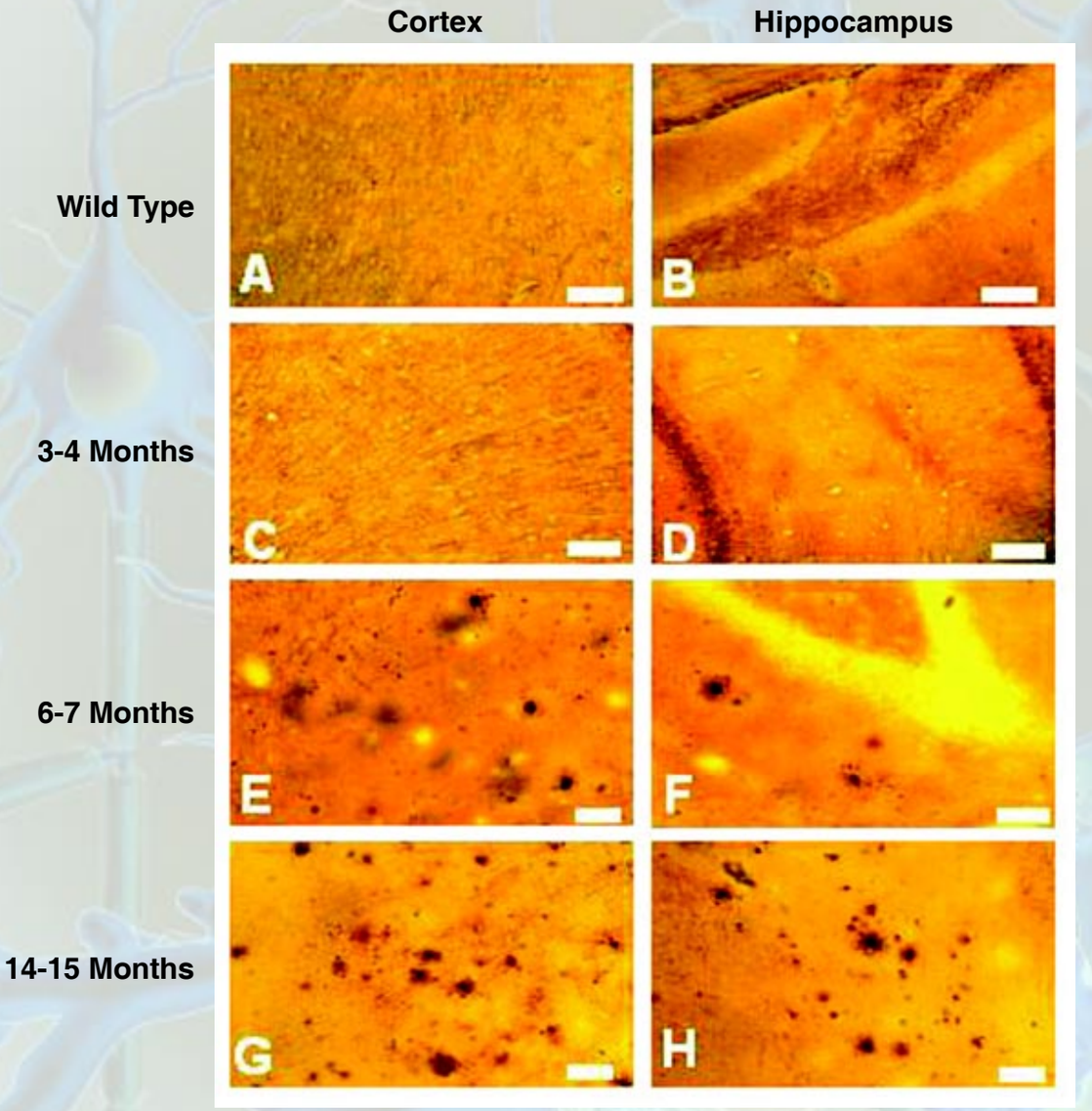
Post Doctorate Fellow : **M Sarath Babu**

Traditional systems of medicine such as Ayurveda offer a knowledge base that can be utilized for development of therapeutic intervention strategies for treatment of neurodegenerative disorders, such as Alzheimer's disease. Neuropharmacological effects of six plants, which are used in the traditional system of medicine for treatment of age-related dementia including Alzheimer's disease are being examined. The plants studied include *Withania somnifera, Centella asiatica, Acorus calamus, Bacopa monnieri, Celastrus paniculatus, Nardostachys jatamansi.*

We had earlier reported the results of in vitro receptor binding assays carried out using membrane preparations from human autopsy brain samples. The plant extracts from *Acorus calamus* and *Withania somnifera* were able to displace the binding of the radioligand to muscarinic receptors indicating their substantial affinity for the receptor. Among the 4 plant extracts tested *Withania somnifera* had the maximum receptor binding activity to muscarinic receptors, followed by *Acorus calamus*. In comparison, *C. asiatica* and *B. monnieri* showed lower activities.

Neuroblastoma cells exposed to amyloid beta peptide were used to assess the potential of the plant extracts to protect the cells. Among the plant extracts screened, *Withania somnifera* exhibited maximal neuroprotective effect in the MTT dye inclusion assay. Simultaneously, the APP/PS1 transgenic mice obtained from Jackson Laboratories were set up for breeding. Using a few middle-aged and old animals, we have observed plaque formation and ubiquitin deposits in the cortex and hippocampus of the APP/Ps1 transgenics, which are absent in age-matched wild type animals. Animals are also being trained on radial maze to identify behavioural deficits. We are now testing the wild type and transgenics for impairment of memory and this will be repeated after chronic administration of *Withania somnifera* to determine if the plant extract is able to reduce/ reverse the memory impairment. For this, we have divided the animals into three groups consisting of wild type, transgenic treated with vehicle and the third group of transgenic mice treated with *Withania somnifera* for 3 months. Our strategy is to test whether the plant extract can prevent/delay the disease progression or if it can reverse the pathological hallmarks of the disease.

Age dependent increase of plaque burden in cortex and hippocampus of transgenic mice carrying mutations in amyloid precursor protein and presenilin on Alzheimer's disease model



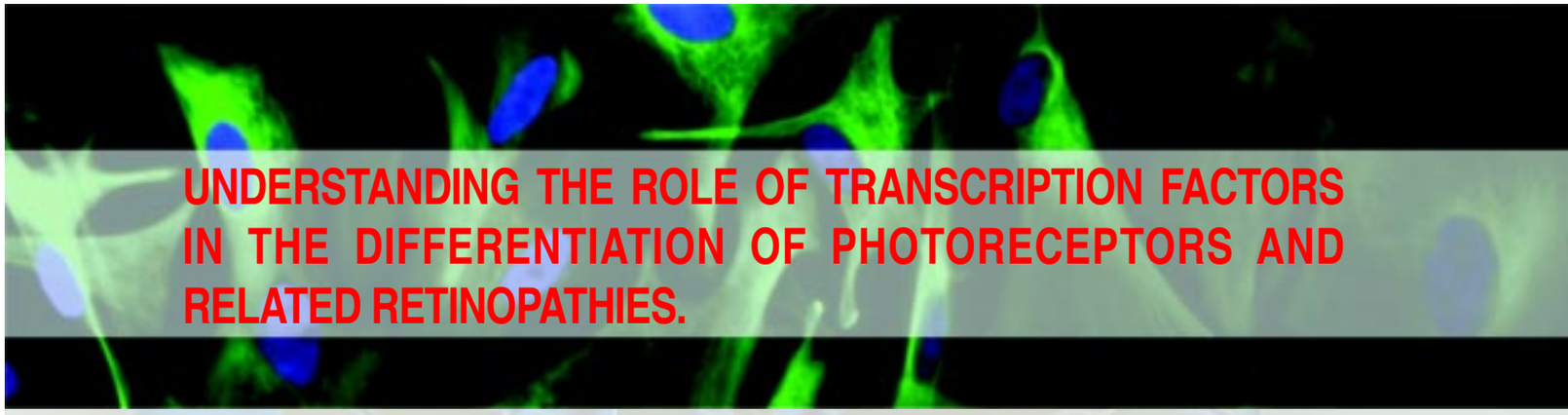
Silver staining was done on sections of cortex and hippocampus in 3-4, 6-7 and 14-15 month old wild type and transgenic mice. Amyloid deposits were seen in the cortex (E) and hippocampus (F) of 6-7 month old animals, which increased in number by 14-15 months of age (G and H). Scale Bar: A and B: 100¼m, C, D, E and F: 50¼m, G and H: 25µm

Funding:

A grant from DBT

Collaborator:

Arya Vaidya Sala, Kerala, Prof. S.C. Jain, Delhi University



UNDERSTANDING THE ROLE OF TRANSCRIPTION FACTORS IN THE DIFFERENTIATION OF PHOTORECEPTORS AND RELATED RETINOPATHIES.

Principal Investigator : **Prabodha Swain**

Research Fellow : **Sandeep Kumar**

Post Doctoral Fellow : **Madhumita Ghosh**

Photoreceptor cells are specialized neurons necessary for photo transduction in the retina. In the mammalian retina, there are two distinct populations of photoreceptor cells known as rod and cones. Each of these photoreceptor cells has a distinct cellular architecture and function in terms of sensitivity to light. Characteristically, rods are sensitive to dim-light vision with low spatial acuity, whereas cones are necessary for colour or bright light vision with high spatial acuity. Retinal cells like any other cell in the body are stringently regulated by transcription factors. Some of the transcription factors including Neural Retina Leucine zipper (NRL) and Photoreceptor specific Nuclear Receptor (PNR) play important roles in photoreceptor differentiation and function in the retina. Several mutations have been detected in both NRL and PNR genes that are associated with different forms of retinal degeneration. It is further substantiated by the phenotypes observed in the retina of humans having NRL/PNR-gene mutations and knockout mice where the expression of the proteins are absent. Interestingly both proteins are conserved in mammals and produce identical functions in the retina irrespective of the species. Recently, these proteins have been localized in the pineal gland of the central nervous system in addition to the usual expression in rod photoreceptors of retina.

Although it has been hypothesized that both factors are involved in the differentiation pathway of photoreceptors (rods), the hierarchical regulation and interaction of NRL and PNR with other retinal proteins are yet to be identified. The identification of such factors is important to understand their role in the disease phenotype. A part of this project submitted to DBT for extramural funding has been sanctioned as a 3-year research proposal entitled "Molecular role of transcription factors in photoreceptor differentiation and associated retinal diseases".

In continuation of the project from last year, we have demonstrated that NRL interacts with TATA-Box Binding Protein (TBP), the general transcription factor necessary to form the pre-initiation complex of any transcription machinery. To determine if such an interaction of TBP and NRL is relevant to its function in retinal photoreceptor, we identified the proximal rhodopsin promoter sequence that was co-immunoprecipitated by both anti-NRL and anti-

TBP specific CHIP assays (Friedman et al. 2004). However, we have also detected a strong interaction between TBP and the carboxyl terminal half of NRL in a GST pull down assay in vitro. We have also observed that phosphorylation of NRL did not significantly affect the in vitro binding of NRL and TBP.

Besides identifying the two interacting proteins TBP and Creb-1 last year, we have identified two new proteins that interact with NRL. Interaction of both cJun and Yb-1 were studied by GST pull down assay. Studies to identify the interacting domains of NRL that binds to these interacting proteins, different deletions of NRL, were expressed as GST-fusion protein and incubated with radiolabelled cJun or Yb-1 protein separately. Both cJun and Yb-1 interacted with the carboxyl terminal half of the NRL without any detectable binding with the amino-terminal half of the NRL protein. The GST protein was used as a negative control and did not show any detectable binding with either of the proteins. We have also identified transcription factor activating factor-4 (TFAP-4) which interacts with NRL. TFAP-4 and cJun are leucine zipper containing proteins. In vitro binding studies suggest that both cJun and TFAP-4 potentially form heterodimers with NRL. Since cJun and Yb-1 are expressed ubiquitously in other tissues including retina, interaction with NRL significantly affects the photoreceptor specific gene regulation of NRL-heterodimer complexes. However, the group of target retinal genes transcribed by the combinatorial NRL complex are yet to be identified. We are using co-expression of NRL and other interacting transcription factors in neuro2a and primary bovine iris cells to study the activated target genes. Differential expression of these target genes will be identified using I-gene arrays.

NRL is a phosphoprotein expressed in mammalian retina. In the affected population, mutations detected in NRL are hypothesized to alter the potential phosphorylation sites in the protein. In-vitro transactivation assays using mutated NRL showed alteration of rhodopsin transcription. To identify the possible kinase(s) and signalling pathways involved in the phosphorylation of NRL, we fractionated bovine retinal proteins using different chromatographic techniques. More than one fraction tested positive for NRL-specific kinases. Interestingly two of these fractions tested immunopositive for the widely expressed mitogen activated protein kinase (MAPK). To confirm if MAPKs directly phosphorylate NRL, we used purified activated MAPK2 and P38 to phosphorylate purified GSTNRL. In-vitro kinase assays showed specific phosporylation of NRL by both MAPK2 and P38. GST was used as a negative control, which did not show any phosphorylation by either of the kinases. For further analysis of the phosphorylation domains of NRL, deletion constructs were designed to express amino terminal 80, 144 and carboxyl terminal 110 amino acids of NRL. Individual proteins were purified and used as substrate to identify the specific phosphorylation domains present in NRL by using purified kinases and kinases present in the retinal fractions. No significant difference was observed between the purified kinases and kinases present in the retinal extract in phosphorylating the amino terminal 80 amino acids of NRL. However, purified MAPK2 did not show any detectable phosphorylation of carboxyl terminal 110 amino acids of NRL suggesting that the specific phosphorylation domain was confined to the amino terminal end

of NRL. A phosphorylation assay using retinal extract as the source of kinases showed weak phosphorylation signal in the carboxyl terminal half of the NRL, suggesting that a kinase other than MAPK2 can phosphorylate carboxyl terminal domain(s) of NRL.

We have also studied the distribution of MAPK2 in developing mouse retina. Immunoblot analysis of retinal extracts using anti-ERK1/2 antibody did not show any change in the developing retinae starting from embryonic day (E) 16.5 through postnatal day (P) 8.5. However, there is an enhanced expression of ERK1/2 and ERK 2 alone in the photoreceptor layer compared to the ganglion cell layer of developing mouse retina. The Erk2 expression was evident throughout the outer segment in the postnatal retinae. The expression was significantly enhanced between P4.5 through P8.5 mice retina examined in the study. Retinae of early postnatal days have Erk2 expression in the area between the photoreceptor and retinal pigment epithelium but it is not detectable in the entire outer segment. Such an expression pattern of ERK suggests that it has an active role in the phosphorylation of NRL with a lag phase of 2-3 days (E18.5 to 2.5) before all phospho-isoforms are detected in the immunoblot analysis of retinal protein.

EFFECT OF NEURAL GENE(S) IN THE DIFFERENTIATION OF RETINAL CELLS.

Principal Investigator : **Prabodha Swain**

Technician : **Sanjay Kumar**

The major objective of this study is to establish an alternate cell culture model that will help study pathways activated by individual retina-specific transcription factors. Neural cell differentiation is regulated by both intrinsic and extrinsic factors. These factors induce programmed differentiation to generate distinct neural cell types in the retina as well as other parts of the brain. Besides transcription factors, different cell cycle molecules also have a major role in the successive steps of differentiation and fate determination of retinal cells. Differentiation of retinal neurons is stage specific. Cell lineage and birth date analysis in rodents suggest that ganglion cells are generated early in the embryonic stage; followed by amacrine, cone and horizontal cells during mid-gestation and rod, bipolar and glia are generated at late embryonic stages. Identification of stage specific factor(s) can potentially serve as markers for one or more retinal cell progenitors. Identification of any such marker of photoreceptor progenitors will be an important tool to characterize and purify such cells from the mixed population of mitotic retinal cells. These cells can be induced by specific extrinsic factors (like FGF, SHH, taurine and retinoic acid) to produce post-mitotic photoreceptors. These progenitors with limited mitotic activity then can be used as potential replacements for photoreceptors in damaged retinae.

Some of the recent findings suggest that iris cells can produce retina specific factors when induced with specific retinal genes. Since iris and retina originate from the same inner layer of the optic cup in embryonic retina, cell culture promises to be an interesting model to study specific pathways that can be triggered by the introduction of retina specific factors.

Earlier, we cultured primary iris cells successfully from cadaver bovine eyes. Inconsistencies in the growth of these primary cells were overcome by standardizing the duration of trypsin treatment and frequency of sub-culturing. Over-trypsinization drastically affects the recovery of cells in primary culture. Though the cultured iris cells grow very slowly, they are mitotically active as was demonstrated by BrdU incorporation. While examining whether NRL had any mitotic effect on these cells, we did not find any significant change in the incorporation of BrdU in NRL transfected iris cells.

We have developed cDNA libraries against Y79 retinoblastoma cells. The Y79 libraries have an insert efficiency in >80% of clones examined. The average insert size varies from

0.6 to 1.5 kbp. The libraries are generated in pCMV Sport6 that can be easily transferred to express protein using LifeTechnologies GateWay technology.

Publication:

Friedman JS, Khanna H, **Swain PK**, Denicola R, Cheng H, Mitton KP, Weber CH, Hicks D, Swaroop A. (2004) The minimal transactivation domain of the basic motif-leucine zipper transcription factor NRL interacts with TATA-binding protein. ***J. Biol. Chem.,*** 2004 Nov 5; 279(45): 47233-41

Presentations:

Invited to speak on the Microarray Tecnology and its Application at Central Goat Research Institute, Mathura on 9th March 2004.

MAPK regulates phosphorylation of neural retina leucine zipper: A key regulator of rod photoreceptor differentiation and function at Neurobiology and Neuro-informatics conference at NBRC, Manesar, Gurgaon.

Funding:

A part of this project is funded by Department of Biotechnology, Government of India

MOLECULAR APPROACHES TO UNDERSTANDING VIRUS INDUCED NEURODEGENERATION

Principal Investigator : **Pankaj Seth**

Project Assistants : **Richa Tewari, Sasidhar Reddy**

Human Immunodeficiency Virus-1 (HIV-1) is the causative agent of Acquired Immunodeficiency Syndrome (AIDS). Without therapeutic intervention, HIV-1 infections in humans often result in a gradual but irreversible immunologic malfunction that eventually leads to the onset of a severe immunodeficiency, the hallmark of AIDS. Despite all efforts in last two decades, AIDS has evolved into a global epidemic affecting more than 40 million people worldwide. Patients infected with HIV-1 invariably experience a wide range of neurological complications associated with AIDS, collectively referred to as human immunodeficiency virus (HIV-) associated dementia (HAD) or AIDS dementia complex (ADC), that often develops in advanced stages of HIV-1 disease. HIV-associated dementia however is not limited to dementia as it is termed. Clinically, HAD involves varying degree of neurological and psychiatric symptoms like cognitive impairment, motor disturbances, hallucinations, delirium and behavioural changes, often leading to progressive deterioration of cognitive and motor skills. The worldwide incidence of HAD is more than 40% of the AIDS population. India is only next to sub-Saharan Africa in the number of suspected HIV infected cases, and has the second largest burden of HIV related pathology. However, the true prevalence of HIV related neuropathology is not available.

HIV-1 has been classified into distinct groups and subtypes that vary based on the genetic make-up of the virus. Remarkably there have been distinct geographic distributions of the HIV-1 strains. Recently, different clades (A-J) of HIV have been reported, of which clade-C has been implicated for around 50% of the AIDS cases worldwide, and more than 90% in India. Most of our current knowledge on HIV-1 induced CNS complications is based on studies with HIV-1B, and limited information is available as to how HIV-1C affects brain functioning in infected individuals. Considering the large number of HIV-1 carriers in India, it is important to carry out research in this emerging area of neurodegenerative disorders. Further, for a precise understanding of the cellular and molecular events of virus-cell interactions during the course of infection, it is important to define the disease and develop potential therapies for it. There is also a need to understand the detailed neurobiology of HIV-1C. Determining the mechanisms by which HIV-1 infection in the CNS causes neurological complications remains a challenging task mainly due to lack of good animal models for HIV-1 and limited access to brain tissue with

different stages of HIV infection or AIDS. To have an improved understanding of neuropathogenesis in patients developing HAD, use of an *in vitro* system is mandatory.

Recently, various cell culture systems derived from human fetal brain cells have been designed to investigate viral-cell interactions in the area of NeuroAIDS research around the world. My laboratory has made significant progress towards the goal of establishing a cell culture system of human CNS progenitor cells in India at the National Brain Research Centre, for their use as a tool to investigate virus-induced neuropathogenesis. This culture system has the ability to be maintained in an undifferentiated state or can be differentiated into highly purified populations of neurons or astrocytes for their use in studying the neuropathogenesis of HIV-1. We have been successful in isolating and maintaining purified cell populations of human fetal brain derived CNS progenitor cells which express nestin, the marker for CNS progenitor cells. These cells have been successfully differentiated into astrocytes and neurons and most of the cells express glial fibrillary acidic protein (GFAP) or Tuj-1, the cell specific markers for the two cell types, respectively.

We are actively investigating various aspects of HIV infection in the developing brain and subsequent neuropathogenesis with the help of a human fetal brain culture system. CNS pathology has been induced by the HIV-1 virion, as well as by certain structural and nonstructural viral proteins like gp120 and Tat. Using the HIV-1 transactivating protein Tat from clade B and clade C, we are studying their effect on

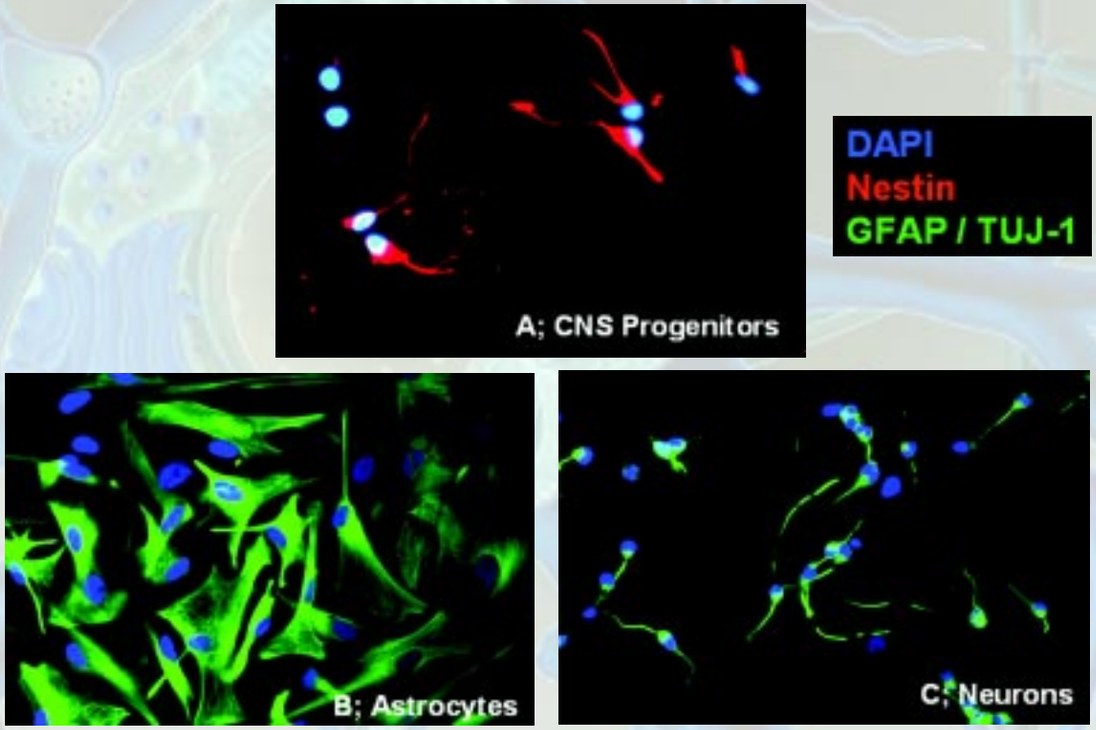


Figure : Human Fetal Brain Derived Cells.
A – Nestin Positive Undifferentiated Human CNS Progenitor Cells.
B – GFAP Positive Human Astrocytes, C – Tuj 1 Positive Human Neurons

human fetal brain derived undifferentiated and differentiated glial and neuronal cells. Our studies and experimental approaches would help understand the viral protein induced inflammatory response in CNS cells and neuropathogenesis of HIV-1.

Publications:

P. Seth, F. Diaz and E.O. Major (2004). JC virus induces a non-apoptotic cell death of human CNS progenitor cell derived astrocytes ***J Virology*** 78: 4884-4891.

D.M.P. Lawrence, L.C. Durham, L. Schwartz, **P. Seth**, D. Maric and E.O. Major (2004). Human immunodeficiency virus type-1 infection of human brain derived progenitor cells. ***J Virology*** 78: 7319-7328.

J. Hou, **P. Seth**, and E.O. Major (2005). JC Virus can infect human immune and nervous system progenitor cells: Implication for Pathogenesis. In: "PolyomaViruses and Human Diseases" (In Press). Editor Nasimul Ahsan, Landes Biosciences Inc, Georgetown Tx, USA.

P. Seth and E.O. Major (2005). Laboratory Models of HIV-1 Infection And Dementia. Neurotoxicity Research (In Press).

Presentations:

P. Seth. HIV-1 associated dementia and progressive multifocal leukoencephalopathy: Viral infections of the CNS. Symposia on Neurodegeneration and Neuroregeneration, Annual meeting of Indian Academy of Neurosciences, Gwalior, India, Jan 2005 (Invited Speaker).

P. Seth. Virus induced neurodegeneration: Implications for AIDS patients. Invited Speaker, Department of Biochemistry, University of Lucknow, Lucknow, India, October 2004 (Guest Lecture).

P. Seth. AIDS associated Dementia: implications for HIV carriers. On World Alzheimer's Day organized by The Alzheimer's Society and Related Disorders Society of India, New Delhi, India, September 2004 (Invited Speaker).

P. Seth. A novel cell culture system for understanding virus induced neurodegeneration. Continuing Education Program at DIPAS, New Delhi, India, September 2004 (Guest Faculty).

P. Seth, F. Diaz and E.O. Major. Understanding Viral Neuropathogenesis By Molecular Approaches. Annual Meeting of Indian Academy of Neuroscience, Hyderabad, May 6-8, 2004 (Invited Speaker).

STUDY OF THE MOLECULAR MECHANISM OF MICROGLIA/MACROPHAGES MEDIATED NEURO INFLAMMATION IN JAPANESE ENCEPHALITIS

Principal Investigator : **Anirban Basu**

Project Assistant : **Khaleel Saheb**

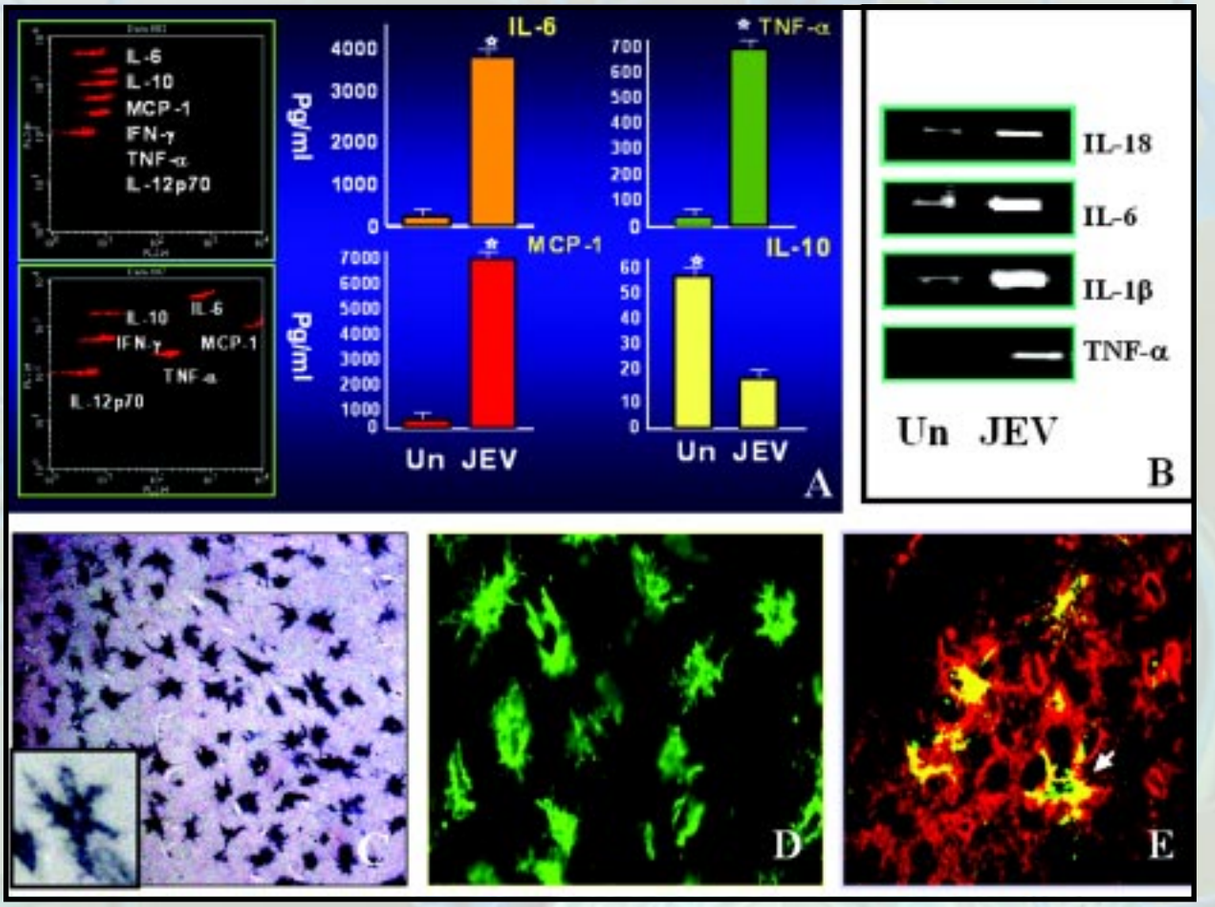
Japanese encephalitis (JE) is an acute viral infection of the central nervous system occurring throughout Asia. After entry into the host, JEV generates a rapid inflammatory response including peripheral cell infiltration in CNS. The inflammation results in an increased level of cytokines such as macrophage derived chemotactic factor (MDF), TNF-α and IL-8 in the serum and cerebrospinal fluid (CSF).

Studies have shown that microglia, the major immuno-competent cell type in the CNS, becomes activated following infection or stress. However, very little is known regarding the direct involvement of microglia and infiltrating macrophages in JEV mediated inflammation. In this study, we plan to evaluate the role of microglia and infiltrating macrophages in an experimental model of JE virus infection.

The major objectives of this project are to determine (1) the magnitude and the nature of inflammation in the brain of mice after JE virus infection (2) the role of microglia/macrophages in the inflammation process and identify (3) signal transduction molecules and transcription factors that are involved in the inflammatory response following JE infection.

Seven days old BALB/c mice were infected intra-cerebrally with 100 p.f.u of JE virus strain JaOArS982. Mice were sacrificed on the fourth day of infection and tissue was further processed for immuno-staining, RNA and protein isolation. Our preliminary results indicate an induction of pro-inflammatory cytokines (IL-6, TNF-α) and chemokine (MCP-1) after JE virus infection. We have also observed (1) enhanced number of activated microglia and (2) cellular colocalization of Iba-1 (microglia specific marker) and Hx-2 (flavivirus cross reactive antibody) following virus infection.

Microglial activation and the induction of proinflammatory mediators in Japanese Encephalitis.



The following results were obtained from JE virus infected BALB/c mice pups:
(A) Cytokine Bead Array analysis shows the induction of multiple pro inflammatory mediators (B) Induction of proinflammatory cytokine transcripts (C) Tomato lectin staining showing activated microglia. The inset depicts a higher power magnification of an activated microglia (D) Iba-1 immunoreactivity depicts highly activated microglia. (E) High power view of a microglia co-expressing Iba-1 and Hx-2 (Flavi virus cross-reactive antibody).

Publications:

A Basu, J K Krady and S W Levison (2004) Interleukin 1: A master regulator of neuro-inflammation. ***J Neurosci Res*** **15; 78(2):** 151-156

 # A Basu, J Lazovic, J K Krady, D T Maguer, R P Rothstein, M B Smith and S W Levison (2005) Interleukin-1 and the Interleukin-1 Type 1 receptor are essential for the progressive neurodegeneration that ensues subsequent to a mild stroke. ***Journ of Cereb Blood Flow and Metab*** **25:17-29 [Nature Publishing Group (**cover photo**)].**

J K Krady, **A Basu,** C M Allen, Y Xu, K F LaNoue, T W Gardner and S W Levison (2005) Minocycline Reduces Pro-Inflammatory Cytokine Expression, Microglial Activation And Caspase-3 Activation In A Rodent Model Of Diabetic Retinopathy. ***Diabetes*** 54:1559-65.

 # Work done elsewhere

Presentations:

Indo-French Workshop in Neuroscience, 14th-15th February, 2005, NBRC, Manesar
Abrogated Interleukin-1 response confers lasting protection to mice from ischemic brain damage.

Poster, **35th Annual meeting of American society for Neurochemsitry (ASN), New York City, 14-18th August, 2004**.
Ciliary Neurotrophic Factors diminishes microglial reactivity and stimulates their production of neurotrophic factors. C M Liberto, J K Krady, **A Basu** and SW Levison.

Poster, **13th Annual meeting of International Society for Magnetic Resonance in Medicine (ISMRM), Miami beach, Florida, 9-13th May, 2005**
Neuro-protection following hypoxia-ischemia in IL-1 type 1receptor deficient mice: suppression of inflammation and inducible nitric oxide synthase (iNOS) production. J Lazovic, **A Basu**, J K Krady, R P Rothstein, M B Smith and S W Levison

Collaborators:

We are indebted to Dr Sudhanshu Vrati and his associates from National Institute of Immunology, New Delhi not only for his continuous help in setting up a virology laboratory at NBRC, but also for providing us JE infected mouse brain samples to perform the initial experiments to test our hypothesis.

PROBING THE CONTROL OF ACTION USING SACCADIC EYE MOVEMENTS

Principal Investigator : **Aditya Murthy**

Research Fellow : **Supriya Ray**

Our visual sensitivity is not uniform but rapidly declines centrifugally from the centre of gaze as a result of which objects in the periphery cannot be identified clearly. To counter this problem our brain has evolved a mechanism whereby the visual scene is explored in discrete steps, each of them corresponding to an eye movement called a saccade, followed by a fixation. By carefully observing the pattern of fixations, a number of behavioral studies have shown that saccades are not random but direct gaze to objects of interest. Therefore, before each gaze shift, perceptual processing must identify potential targets for the eye movement, and motor processing must prepare and execute the motor command. The role of cognition also provides an added level of complexity since behavior is not strictly dictated by perceptual processes: internal goals are important. The challenge therefore is to understand the representations of the image that guides orienting responses and the computations that subserve and link visual and cognitive processing, and eye movement programming. The long-term goals of the proposed project are to understand how vision and cognition guide action. These questions will be approached through investigations of visually guided saccades in novel paradigms designed to probe oculomotor control in normal human subjects.

Probing the sensory consequences of eye movements

As a consequence of eye movements, images on the retina are displaced resulting in blurring of the retinal image. However, despite frequent occurrence of eye movements our perception of the world appears stationary. The inability to perceive such induced motion despite the occurrence of saccadic eye movements is due to a phenomenon called saccadic suppression during which visual sensitivity is reduced perceptually. However, several studies have shown dissociation between perception and action during voluntary movements and that perceptual judgments based on relative locations of visual stimuli is different from that of goal directed movements. In view of this dichotomy between visual representations dedicated to perception versus action, it remains unknown whether the goal directed movements that rely on visual information are also subject to the effects of saccade suppression as measured perceptually.

To test the perisaccadic sensitivity of the visual representation that guide saccades, we used a version of double-step saccade task in which single targets when displaced to successive locations commonly resulted in the

generation of a sequence of two saccades. By manipulating the stimulus (low contrast and high temporal flicker) we attempted to selectively test the perisaccadic sensitivity of the magnocellular (motion) pathway. In previous studies using two-alternative forced choice paradigms, it has been shown that saccadic suppression of the magnocellular pathway occurs predictively (e.g. Ross et al Nature, 1998), i.e. prior to the movement of the eyes.

To access whether such predictive suppression occurred in our double-step task, reaction times to the second target as well as the intersaccadic interval were used as metrics to determine the extent and time-course of suppression of visual representations that guide saccades. Using a framework previously used by us (Ray et al Vision Research, 2004), we have shown that unlike the visual representations responsible for perception of motion, the magnocellular pathway that drives saccades is only suppressed during, but not before the saccadic movement. These results raise the possibility that the sensitivity of visual representations to the effects of saccade suppression may differ depending on whether the representations are used to subserve motion processing or whether they are used for guiding saccades.

Publications:

S. Ray, J.D. Schall and **A. Murthy**. Programming of double step saccade sequences: Modulation by cognitive control. ***Vision Research*** 44: 2707-2718 (2004).

A. **Murthy,** Ray S., Thompson, K.G., Jacobi, S.S. & J.D. Schall. Predictive error correction in frontal eye field. (Submitted to *Neuron*)

Presentations:

A. Murthy. Computational Vision. Lecture for Computational Neuroscience workshop, given at University of Hyderabad (2004).

S. Ray (2004) Neural control during saccade sequences. 4th Indo-Japan-Korea-China workshop in Neurobiology & Neuroinformatics, India.

Funding:

A grant from DST, India

BRAIN MECHANISMS OF ACTION CONTROL IN HUMANS

Principal Investigator : **Aditya Murthy**

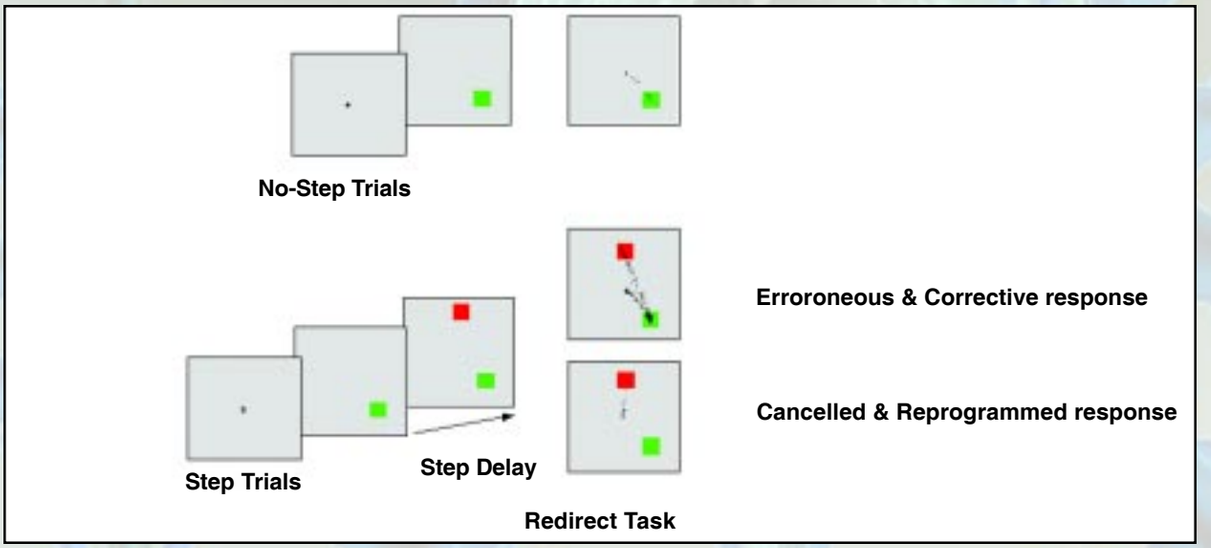
Research Fellow : **Supriya Ray**

Project Assistants : **Snehal Chokandre, Jyoti Prakash**

Understanding the neural basis of voluntary control is a central problem in cognitive neuroscience. Goal directed movements involve the participation of a number of different brain areas. Two subcortical areas that have been implicated in motor control are the basal ganglia and the cerebellum that are reciprocally connected to motor cortical areas. However, the specific nature of control exerted by these two areas remains unclear. Understanding how the brain controls actions is necessary to understand the causes underlying various psychopathologies and motor abnormalities where there is a failure of control. These series of experiments are being carried out in collaboration with Dr. Sharat Chandra and Dr. Madhuri Behari of CNS, A.I.I.M.S. where a facility to measure eye movements in real time under computer control has been set up.

Inhibitory control of action

A hallmark of the voluntary control of action is the ability to inhibit a planned



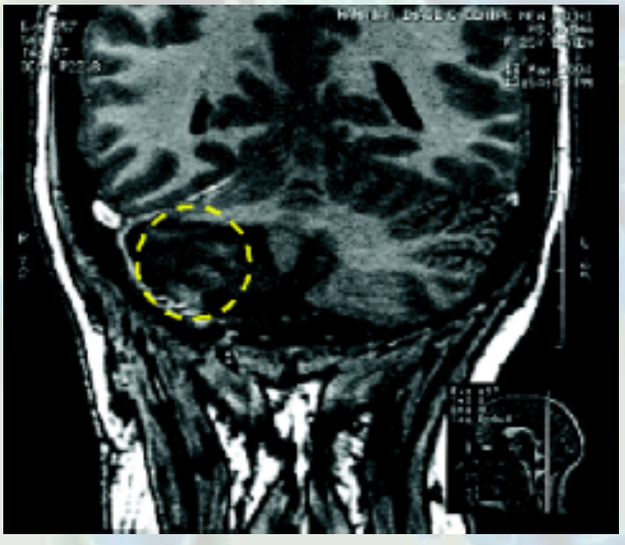
During the REDIRECT task, patients were instructed to redirect their gaze to the second (red) target in step trials. In Random No-Step Trials (not shown), the red target did not appear and patients were instructed to direct their gaze to the green target

movement when confronted with situations that render current goals inappropriate. This ability to inhibit inappropriate actions is of considerable interest because it involves an internal act of control, which redirects overt movement. Inhibitory control can be probed in the REDIRECT task that entails inhibiting a preprogrammed eye movement for successful performance. Using a simple theoretical construct, which will be described in detail during the presentation, we have used the REDIRECT double-step task to estimate the duration it takes to inhibit a partially prepared movement in normal subjects and patients with lesions in the cerebellum and Parkinson's patients having compromised basal ganglia function. The aim of this line of work is to understand how inhibitory control is implemented in the basal ganglia and cere bellum.

Using the REDIRECT task we have recorded data of 8 patients with Parkinson's disease. We have also recorded data of 4 patients with Cerebellar lesions and are in the process of determining whether these patients have deficits in inhibitory control.

Brain mechanisms of error correction.

In the REDIRECT task when inhibitory control is successfully implemented, it results in the cancellation of the preprogrammed eye movement. However, as the delay between the appearance of the target and the stop signal increases, subjects increasingly fail to inhibit their responses leading to errors. When subjects make such errors they frequently make quick corrective movements. Such behavior implies that the brain has the capacity to evaluate the consequences of actions and provide a means to correct erroneous behavior. Thus, response to errors in the cancel task can provide novel insights into the basis of supervisory control of actions. The aim of this line of work is to use a neuropsychological approach to delineate the neural circuits that instantiate supervisory control. Data from the same subjects used to investigate inhibitory control will be used to evaluate whether these patients had a compromised ability to correct errors.



MRI Scan of a Cerebellar lesioned patient who performed the REDIRECT task

Presentations:

Chokandre S., S. Ray, Ahmad F.U., Sarat Chandra P. and **A. Murthy** (2005). Hierarchical control of action during error correction in a patient with saccadic dysmetria. Indian Academy of Neuroscience. Gwalior, India.

A. Murthy (2004). Oops, I did it again: insights into the cognitive control of action during errors. International Conference on Cognitive Science, Allahabad, India.

A. Murthy (2004). Executive control of action. 1st National Frontiers of Science meeting, INSA, New Delhi, India.

A. Murthy (2005). Executive control of action. 1st Indo-US Frontiers of Science meeting, Indo-US SAT forum, Bangalore, India.

A. Murthy (2005). Cognitive neuroscience of errors. Indo-French Workshop, NBRC, India.

A. Murthy (2005) Cognitive neuroscience of errors. Indo-German Symposium, Bangalore, India

Funding:

A grant from DST provides support for the project.

Collaborators:

Dr. Faiz-Udin Ahmed: Resident, Dept. of Neurosurgery, CN Centre, AIIMS.

Dr. Sarat Chandra: Assoc. Prof., Dept. of Neurosurgery, CN Centre, AIIMS.

Prof. M. Behari: Dept of Neurology, CN Centre, AIIMS

NEURAL CONTROL OF ACTION BY BASAL GANGLIA NETWORKS

Principal Investigator : **Aditya Murthy**

Research Fellow : **Supriya Ray**

While much progress has been made in understanding the function of sensory and motor networks, the nature of neural networks mediating their interactions remain obscure. As a consequence, we have a poor understanding of how sensory information is transformed into a movement. One of the key structures thought to play an important role in the transformation of sensory signals into motor commands is the basal ganglia network, which receive no direct sensory input and sends little direct output to the spinal cord. Rather, their primary input is from the cerebral cortex and their output is sent back to the cortex via the thalamus.

Within this general scheme, the basal ganglia-thalamocortical circuit implements a number of functionally distinct loops involving different modalities in which information from somatomotor, oculomotor, cognitive and limbic systems are processed in parallel. Although the anatomical significance of such loops between cortex and basal ganglia have been appreciated, their functional significance remains largely unspecified. Here we use the non-human primate model to study the function of one such loop, namely the oculomotor loop, in which information from the frontal eye fields (FEF) is relayed to the basal ganglia, processed and sent to the mediodorsal nucleus of the thalamus and sent back to the FEF. Here we propose to study the sensorimotor transformations in this loop in context of how basal ganglia-thalamocortical circuitry initiates actions, how actions maybe cancelled and reprogrammed by this circuit, and how this circuit may help in the correction of erroneous actions.

To address these issues, we are training non-human primates in a modified double-step task in which they need to cancel a preprogrammed saccade following the appearance of a new target. As a control, subjects will also perform double step tasks in which they will follow the sequence of target steps with successive saccades. The two tasks will be designed such that the identical pattern of saccades yielding correct performance in the follow task is incorrect in the cancel task. This comparison would enable us to examine how the FEF/ basal ganglia neurons help inhibit actions and corrects errors induced by sudden changes in instructions that render current goals inappropriate.

Funding:

A grant from DBT.

NEURONAL PROCESSING IN NORMAL AND IMPAIRED BRAINS

Principle Investigator : **V. Rema**

Post Doctoral Fellow : **Renuka Ramachandra**

Research Fellows : **Zia Ud Din, Manisha Chugh**

One of the apparent consequences of cortical injury is degeneration of neurons at the site of injury, which in turn could lead to disruption and reorganization of synaptic contacts formed by these neurons. As a result there would be loss of functional synaptic connectivity and loss of integration of synaptic responses. The ensuing disruption in flow of excitation in the cortical network can be postulated to be the primary reason for disabilities of both neuronal and behavioral functions.

The focus of our studies is to understand (1) the synaptic changes that occur following brain injury; and (2) determining the behavioral consequences of brain injury and the extent of recovery. Using single unit electrophysiological recording we are measuring initial changes in the activity of neurons in the region adjacent to a unilateral focal lesion in the somatosensory cortex in adult rats. Spontaneous activity, evoked activity and receptive field were recorded from whisker barrel cortex of control and lesioned adult rats. As we move from the edge of lesion and beyond into the penumbra, we see differential neuronal activity ranging from no activity at the edge to higher than normal response and larger receptive fields about 1mm away from the edge within four days of recovery. These alterations in the neuronal activity could be explained by the differential expression of molecules involved in excitatory and inhibitory neurotransmission we had seen using immunohistochemical localization.

Behavioral tests using the whisker dependent task of gap crossing showed long-lasting deficiencies in whisker usage after lesions of the whisker barrel cortex. Since limited improvements are seen in neuronal function in lesioned animals after exposure to a complex environment, we are examining whether the prior exposure of animals to different environments will influence the outcome of recovery. We see that animals reared in an enriched environment post weaning or hand-handled from the day of birth onwards show a better performance on several behavioral tests compared to control animals. After unilateral somatic sensory cortical lesions, the performance of these animals considerably reduced even after long recovery times suggesting that additional interventions are

needed for better recovery. In this respect, the effects of a pharmacological agent CDP-choline on functional recovery is being examined. In the initial experiments, we tested this drug on primary cortical neurons and our results indicate that CDP-choline is neuroprotective. It also increases neuronal survival and arborization. In later experiments, this drug will be used in vivo in lesioned animals in combination with exposure to an enriched environment with the objective of improving recovery.

Gap- Cross Task

Figure: A rat is performing the Gap cross task which depends on sensation from its whiskers. This behavioural task is useful in assaying deficits in the sensory pathway since the whisker to cortex pathway is the main somatosensory pathway in rats.

Presentations:

V Rema, R Ramachandra, Z.U.D. Dharokhan, N. Jain (2004) Unilateral lesions of the somatosensory cortex reduces expression of NMDA receptor subunits in the contralateral homotopic region. 34th Annual Society for Neuroscience. San Diego, CA.

Manisha Chugh, Zia Ud Din Dharokan, **V. Rema** (2005) Stimulating environment reduces anxiety and improves exploratory behaviour. 22nd Annual Conference of Neuroscience "From Basic to Clinic".

Funding:

International Senior Research Fellowship from The Wellcome Trust, UK.

BRAIN REORGANIZATION FOLLOWING SPINAL CORD INJURIES

Principal Investigator : **Neeraj Jain**

Research fellow : **Shashank Tandon**

Project Assistant : **K B Ramakrishnan**

The somatosensory system processes inputs from receptors in the skin, joints and muscles in a number of different areas in the lower brain stem, thalamus and cortex. Motor areas of the brain receive feedback from the somatosensory system which enables finely controlled movements such as during palpation and grasp. My research program aims at understanding how the sensorimotor system processes sensory information to enable tactile perception and motor control, and how spinal cord injuries affect functional organization of the system in adult animals and during early development. We perform unilateral lesions of the dorsal columns of the spinal cord leaving spinothalamic and other ascending and descending pathways intact. Using multiunit mapping and intracortical microstimulation techniques, we are determining the effects of these injuries on the somatosensory and motor areas of the brain. These plastic changes in brain organization are then related to the behavioural effects of the injuries in order to understand the mechanisms of recovery of behaviour following such spinal injuries and to develop interventions for better recoveries.

In addition to ongoing work on primates, experiments have been initiated on rats to determine the effects of spinal cord injuries. As a first step, normal organization of the movement map in M1 or the primary motor cortex is being determined since considerable uncertainty exists about this map. Our data supports the presence of a medial eyelid movement area and a lateral hand/arm movement zone. The hindlimb area is caudal to the forelimb area. The region evoking movements of the lower jaw and tongue is present rostro-lateral to the forelimb area.

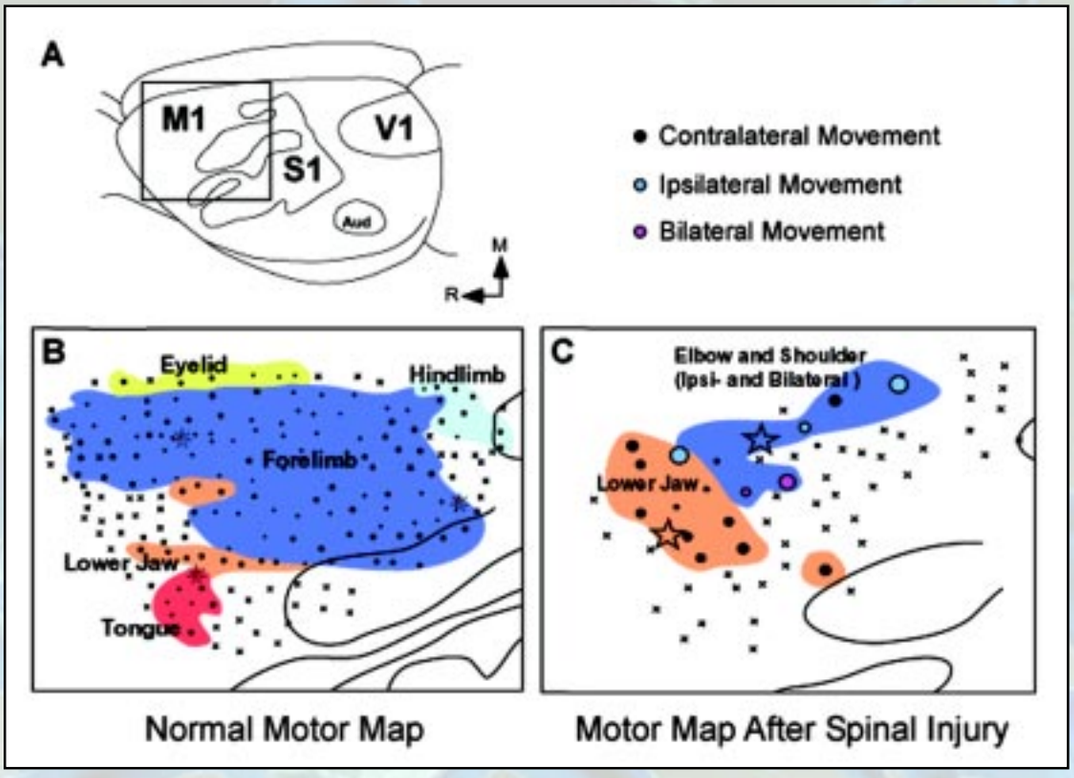
We mapped the somatosensory and motor areas of rats with unilateral lesions of the dorsal columns at upper cervical levels 5- 8 months after the lesion. The results show that after such injuries, neurons in the somatosensory cortex do not respond to the stimulation of the deafferented regions of the body. Instead, the neurons are either non-responsive to the stimulation on any part of the body or show responses to the stimulation to the part of the body with intact inputs.

Intracortical microstimulation of the motor cortex in rats with lesions of the dorsal columns

at upper cervical levels showed that stimulation at many sites that were expected to evoke movement of the forearm, no movement of any body part was evoked. However, at some of these sites, movements of the ipsilateral elbow and wrist were evoked or there were bilateral movements. In normal animals, bilateral movements are elicited only at a few points and that too only for the proximal shoulder. The mechanisms of emergence of distal ipsilateral movements following lesions of the dorsal columns are currently under investigation.

We are also continuing our efforts to determine how injuries early in development affect organization of sensorimotor areas. The goal of these experiments is to determine if in animals with lesions early in development there is a more 'normal-like' organization of the sensorimotor areas because the system is still immature and capable of compensating for the partial lack of inputs. We have initiated these experiments with a unilateral lesion of the dorsal columns in one newborn monkey. We will map somatosensory areas 1, 2 and motor areas in order to extend our results reported during the previous year.

Effects of unilateral lesion of the dorsal columns on the organization of the motor cortex of a rat.



(A) Dorsolateral view of the rat brain showing locations of the primary motor (M1) and the primary somatosensory (S1) areas. Locations of the primary visual cortex (V1) and the auditory cortex (Aud) are shown for reference. The boxed region demarcates the area shown in detail in 'B' and 'C'. (B) Normal motor map showing locations of the regions where movements of the forelimb, hindlimb, eyelid, lower jaw and the tongue are represented. (C) Organization of the motor cortex in a rat with unilateral lesion of the dorsal columns following a recovery period of eight and a half months. Note that at most of the sites (marked by 'x'), no movement is evoked (c.f. 'B'). At the sites that do evoke forelimb movement, either ipsilateral or bilateral movement was observed. The lower jaw representation remains normal.

Presentations:

Shashank Tandon, K B Ramakrishnan, Leslee Lazar and **N. Jain** (2005). Effects of injuries to the dorsal spinal cord on the organization of the sensorimotor cortex. Abstract in the 22nd Annual Meeting, Indian Academy of Neurosciences, Jan 28 – 30, Gwalior, MP, India.

"Brain reorganization following sensory loss early in development". Development, Epigenetics and Plasticity', a symposium organized by Indian Society of Developmental Biologists, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore. Dec 21-23, 2004.

"(1) Principles of organization of mammalian brains, and (2) Plasticity of the brain following injuries". Short Course on Computational Neuroscience. University of Hyderabad, Hyderabad. October 11-21, 2004.

"fMRI for systems and cognitive neuroscience: filling the gaps and taking it beyond. June 20, 2004". 'National School on NMR: molecules to human behaviour' organized by Sophisticated Instruments Facility, Indian Institute of Science, Bangalore, and held at Institute of Himalayan Biology and Technology (IHBT), Palampur, June 15-25, 2004.

Funding:

International Senior Research Fellowship from the Wellcome Trust, UK.

EMERGENCE OF PRIMARY AND NON-PRIMARY AUDITORY CORTICAL AREAS DURING LATE FOETAL AND EARLY POSTNATAL AGES IN HUMANS

Principle Investigator : **Soumya Iyengar**

Technical Assistants : **OP Sharma, Arvind Singh Pundir**

Project Assistant : **Radhika Rajan**

This study focuses on the development of primary and surrounding non-primary (association) auditory cortical areas in humans. While the primary auditory cortex is mainly important for perceiving pure tones, the non-primary auditory areas appear to perceive speech related sounds in adulthood. However, the exact developmental time-line of how and when these areas developed is not known. Our immediate goal therefore is to identify these areas using histochemical and immunohistochemical markers in post mortem brain tissue at different stages of development.

Our initial results have corroborated earlier studies demonstrating that the non-primary auditory areas (TA, TB and TD) can be distinguished from each other and from the primary auditory area (TC) in adults based on differences in cytoarchitecture revealed by staining for Nissl, acetylcholinesterase (AChE), and three calcium binding proteins Calbindin, Parvalbumin and Calretinin. Nissl staining in the left hemisphere of one adult and one adolescent (14 years of age) has shown that the primary auditory cortex (area TC) has a prominent Layer IV (granular layer) and very narrow radial columns whereas non-primary areas TA and TB have a smaller granular but wider supragranular layers and wider radial columns compared to TC. Area TB can be distinguished from TA since it has prominent pyramidal neurons in Layer III. Area TD can also be easily differentiated from TC, TA and TB since the six layers and radial columns are not well-organized in this part of the auditory cortex. Staining for AChE has shown that area TC predominantly stains for fibres whereas both fibres and neurons are labeled in TB. Especially prominent are large pyramidal neurons in Layer III of TB. Both TA (see Fig 1) and TD demonstrate mainly neuronal staining.

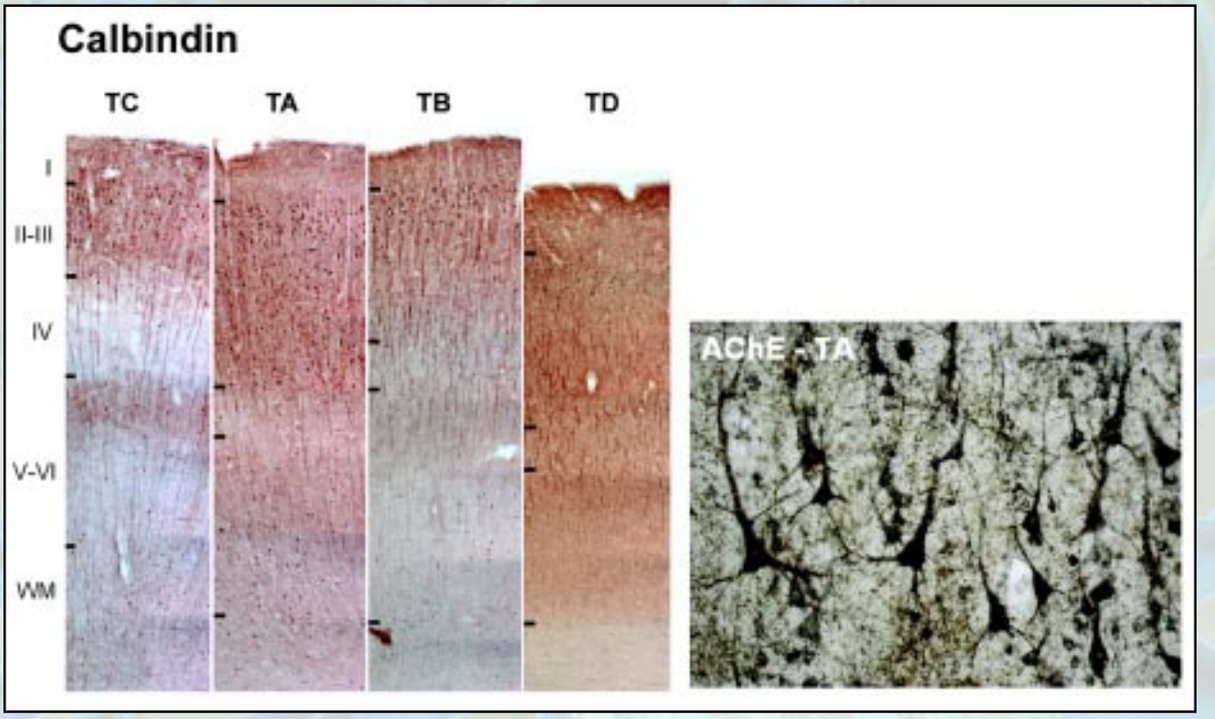
Staining for calcium binding proteins revealed that calretinin is only present in bipolar neurons in all components of the auditory cortex. A number of calretinin-positive bipolar neurons are present in the supragranular layers of TC and layers II and IV of TB whereas a small number are present in supragranular layers of areas TA and TD. TD can also be delineated from other areas since neuropil in layer I of area TD is highly positive for calretinin.

A large number of parvalbumin-positive neurons are present throughout the auditory cortex. Neuropil is positive for parvalbumin in

all layers of TC, although Layer IV is more darkly labeled compared to the other layers (earlier reports have shown that neuropil is highly positive for parvalbumin only in layers III and IV). Parvalbumin-positive neuropil is present in Layer IV of TA as well as in TB.

We also found that neuropil was highly positive for calbindin in layers I-III and V of TC as shown in the figure. Whereas neuropil in layer IV was negative for this protein for calbindin, vertically aligned bundles of calbindin-positive fibres were present throughout this layer. Calbindin-positive pyramidal and stellate neurons were present in layers II and III in all four auditory cortical areas. Neuropil was also positive for calbindin throughout TA and vertically arranged fibres were present in the three non-primary auditory cortical areas in addition to TC, although they were not as thick. Area TD had very few neurons positive for calbindin compared to other auditory areas.

Expression of the calcium binding protein calbindin and the enzyme Acetylcholinesterase in different subdivisions of the adult human auditory cortex



(A) **(B)**

Fig. 1: (A) Coronal sections of the auditory cortex stained for the calcium binding protein calbindin which can be used to differentiate between the primary and non-primary areas. Neuropil was highly positive for this protein in layers I-III and V whereas layer IV was negative for this protein in area TC (primary auditory area). This was also true for areas TA and TB although the distinction between different layers was not as sharp as in TC. Vertically aligned bundles of calbindin positive fibres were present in all auditory cortical areas although they were most distinct and appeared to be the shortest in length in TC. Calbindin-positive pyramidal and stellate neurons were present in layers II and III in all auditory cortical areas, the greatest in density in TB and the least in TD. (B) Large pyramidal neurons that were positive for AChE in layer III of area TA.

Our preliminary results demonstrate that we can differentiate between the primary and non-primary auditory cortical areas in adult and postnatal human brains based on cytoarchitecture and the expression of different markers. Since the organization of different auditory areas was similar at the two ages studied, it appears that the adult pattern of organization of the auditory cortex emerges by about 14 yrs of age, although a larger sample is needed to validate this result.

We are currently processing samples from fetal and pediatric age groups for comparison with data obtained from adults. We have also begun reconstructing the volumes of the auditory cortical areas using image analysis and specialized software for comparison across different age groups.

Funding:

A DBT grant awarded for project entitled "Emergence of primary and non-primary auditory cortical areas during late fetal and early postnatal ages in humans" in March, 2005.

Collaborator:

Dr. PC Dikshit, Maulana Azad Medical College, New Delhi

NEUROGENESIS IN THE SONG CONTROL SYSTEM OF ZEBRA FINCHES

Principle Investigator : **Soumya Iyengar**

Research Fellow : **Nazia Khurshid**

Technical Assistant : **Arvind Singh Pundir**

Project Assistant : **Radhika Rajan**

New neurons are continuously produced by the ventricular zone of the brain throughout the life of songbirds such as zebra finches. These new neurons are incorporated into functional circuits within the song control system of these birds as well as to other regions not directly associated with song. Zebra finches learn their songs during a sensitive period during early development (25-60 days) and attain adulthood at 90 days by which time their song is stable. Since their songs remain stereotyped throughout their adult life, it is still not known why neurons have to be replaced in the song control regions. The goal of this project is to study whether changing levels of adult neurogenesis would change the pattern of a specific behaviour, that is, song. An additional outcome of this project would be to find whether the levels of neurogenesis in adulthood are regulated and maintained at a particular level. The specific aim of this project is to use opioid receptors as a tool to change the levels of neurogenesis in the zebra finch brain since it has been shown that blocking these receptors increases the level of neurogenesis in the ventricular zone of developing mice (Reznikov et al., 1999).

Western blots of brain tissue obtained from male and female zebra finches have shown that δ- and μ- opioid receptors are present in appreciable amounts in male and female zebra finch brains. We have also demonstrated the presence of these receptors on the somata of neurons within song control regions lMAN (lateral magnocellular nucleus of the anterior neopallium), Area X (a striatal nucleus), HVC (higher vocal center) and RA (robust nucleus of the arcopallium) in adult male zebra finches as well as in the ventricular zone using immunohistochemistry.

We will now study the pattern of expression of these receptors in female zebra finches and male and female juvenile birds. We will also study whether they co-localize with neurons that have recently divided by injecting birds with BrdU (bromodeoxyuridine).

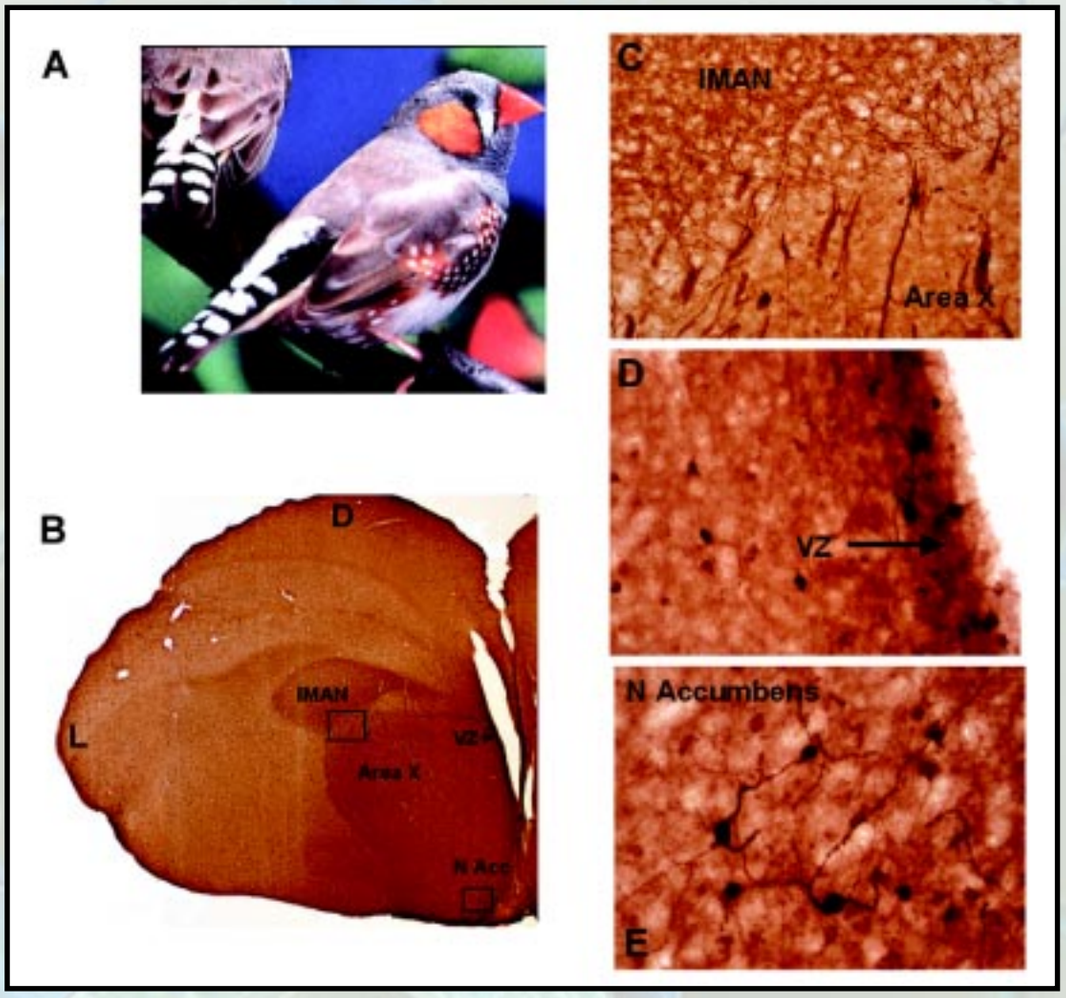


Fig.1. Patterns of expression of μ opioid receptors in zebra finches (A). A cross section of the anterior forebrain of an adult male zebra finch (B) showing that neuropil in IMAN and Area X is highly positive for the μ opioid receptor, also photographed at high power in (C), D, dorsal, L, Lateral. Neurons adjacent to the ventricular zone extend their dendrites into this region (D) and neurons in the nucleus accumbens in the ventral striatum also express the μ opioid receptor (B and E).

DEVELOPMENT OF NOVEL COMPUTATIONAL TOOLS TO INVESTIGATE SPEECH

Principle Investigator : **Nandini Chatterjee Singh**

Research Fellow : **Latika Singh**

Project Assistant : **Anindita Bir**

Clinically, speech is one of the earliest markers for development or acquired pediatric neurological disorders. Speech is the expression of ideas by the voice; sounds, expressive of thought, articulated by the vocal organs. It is the spoken form of language. Studies over the last 50 years have revealed unexpected learning strategies for language. These strategies demonstrate statistical and computational skills coupled with pattern perception and provide grounds for the unique ability of infants to learn new sounds by simply being exposed to them. Developmental studies suggest that early in life, perceptual representations of speech are stored in memory. Subsequently, these representations guide the development of motor speech. Young children across all cultures learn their mother tongue rapidly and effortlessly, from babbling at 6 months of age to full sentences by the age of 3 years. In the light of these new developments in language acquisition, it is important that we obtain an accurate picture of the development of speech in normal children. We also realize that this could have useful implications in our understanding of disorders that disrupt speech in pediatric populations.

Our primary aim is to investigate the development of speech in normally developing children and children with communication disorders using various tools of signal analysis.

From a signal processing approach, human speech signals have a time-varying frequency structure and are joint representations of time and frequency. We employ various techniques like spectrographic analysis, fast Fourier transforms and modulation spectra to study these vocal productions. We have also set up new quantifiers to characterize various spectral and temporal features of speech.

Computational analysis of speech development in normal children

We are currently involved in studying the development of speech in normally developing children in the age group 4-8 years. We obtained utterances for various words, vowels and picture naming tasks from males and females in the age group mentioned above. The speech material consists of various phrases and words in English. All speech samples were collected using a laptop computer with a high quality microphone. We have obtained data

from nearly 100 children from different schools in Gurgaon. We are in the process of setting up a database of speech samples from normal children. On the basis of age, we have divided the children into three groups and are carrying out different kinds of acoustic analysis namely:

1. Spectrographic analysis of speech.

2. Modulation spectrum analysis (2-D Fourier decomposition of speech)

Sound spectrograph - A spectrogram, like a musical score, is a visual representation of sound. The horizontal dimension corresponds to time (reading from left to right), and the vertical dimension corresponds to frequency (or pitch), with higher sounds shown higher on the display. Frequency is measured in Hertz (Hz), or cycles per second; and in kilohertz (kHz) or thousands of cycles per second. The relative intensity of the sound at any particular time and frequency is indicated by the darkness of the spectrogram at that point. The most obvious feature of a speech spectrogram is the energy modulation, both in time in any given frequency channel, and along the spectral axis at any instant, due to formant peaks and their transitions, spectral edges and rapid amplitude modulations at onsets and offsets. Figure B below shows the spectrogram for the word "biscuits" as uttered by an child aged 4 years and figure A shows the spectrogram for the same word "biscuits" as uttered by an adult (in this case a female).

A visual inspection of the spectrograms shows that there is a fair amount of difference between the production of vocal utterance by a child and that of an adult. This difference is partly due to differences in the vocal tract and partly due to differences in the brain. We are presently involved in characterizing development of human speech through various acoustic parameters using different techniques of signal processing.

Modulation Spectrum

We also use a 2-D Fourier decomposition of speech called the modulation spectrum in order to obtain insights into the processing of speech by the brain. The modulation spectrum presents a representation

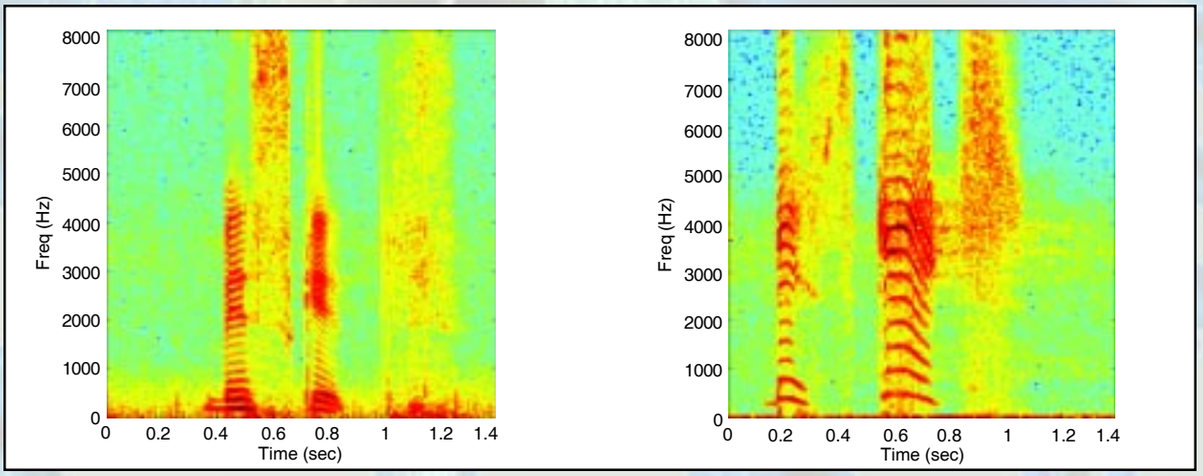
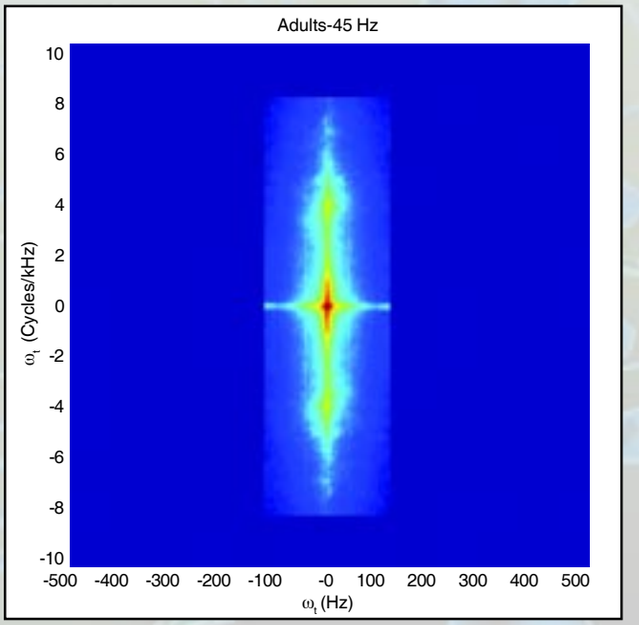


Fig A. Spectrogram of an adult female uttering the word "biscuits"

Fig B. Spectrogram of a 4 yr old child uttering the word "biscuits"



where any sound can be broken into a series of temporal and spectral modulations. Thus we have a joint representation of both temporal and spectral components of sound. The modulation spectrum is obtained by taking the 2-D Fourier transform of the auto-correlation matrix of sound in its spectrographic representation. The ω_t which are plotted on the x-axis are the temporal modulations and ω_x are the spectral modulations. The colour in the figure is a measure of the power and the dark colour indicates that most of the energy is found for low spectral and temporal modulations and shows that most of the high frequency spectral modulation power is found at the very lowest temporal modulation and vice versa. In other words there is a scarcity of sounds with both high spectral and high temporal modulations. The figure shows a modulation spectrum of sentences in English produced by adults.

Our analysis so far shows that variability in the speech of children is high as compared to adults. This variability decreases with age but does not follow a set pattern. We are presently quantifying these parameters to characterize this variability. Once this has been standardized we hope to set up a database of speech samples of children, which could be used to compare with children with communication disorders.

SPEECH DEVELOPMENT IN CHILDREN WITH COMMUNICATION DISORDERS

Principle Investigator : **Nandini Chatterjee Singh**

Research Fellow : **Latika Singh**

Project Assistant : **P. Shantisudha**

A second project aims at characterizing the differences in speech production in normally developing children and children with communication disorders. Research by Tallal and colleagues (Tallal et al.,1996) had earlier demonstrated that Language Learning Impaired (LLI) children require hundreds of milliseconds (msec) between acoustic events to discriminate between them, while children of the same age and intelligence level only need tens of milliseconds. They also showed that this basic sensory integration deficit interferes with the ability of LLI children to discriminate the critical brief acoustic cues within syllables and words that distinguish one phoneme from another. Their studies also highlighted the fact that temporal integration thresholds are highly correlated with the degree of receptive language impairment in younger LLI children, and later with these children's difficulty learning phonological decoding skills for reading. Using spectrographic and modulation spectrum techniques, we are investigating speech in children with autism and specific language impairment. Pilot data shows that differences in the speech of normal children and high functioning autistic children do exist, and we are currently in the process of quantifying these differences.

Since the modulation spectrum is a representation of sound in terms of temporal and spectral modulations, one would expect that a modulation spectrum of the speech sounds of children with SLI would reflect the modulations missing in their speech versus the speech of normal children. Such an approach could also test the hypothesis that children with LLI (Language Learning Impairment) often require longer time periods between acoustic events to discriminate them as compared to normal children. Our study and analysis will address some of these issues and make an attempt to obtain easier and earlier clues of this impairment. Based on our results, attempts will also be made to design therapies to overcome language learning impairments.

Publication:

Variability in speech production - an acoustic analysis of data from bilingual children. Latika Singh, P. Shantisudha and **N.C. Singh** (submitted to ***Journal of Applied Accoustics***)

Presentations:

N.C. Singh A new computational tool to study speech impairments in children - Frontiers of Science, Indo-US SAT forum, New Delhi and Bangalore, India, January 2005.

N.C. Singh Techniques in computational neuroscience lectures delivered at the IBRO School of Neuroscience at Bangkok in December 2004.

APPLICATION OF STOCHASTIC RESONANCE AND STABILITY ANALYSIS FOR BRAIN IMAGING AND THERAPY

Principal Investigator : **Prasun K. Roy**

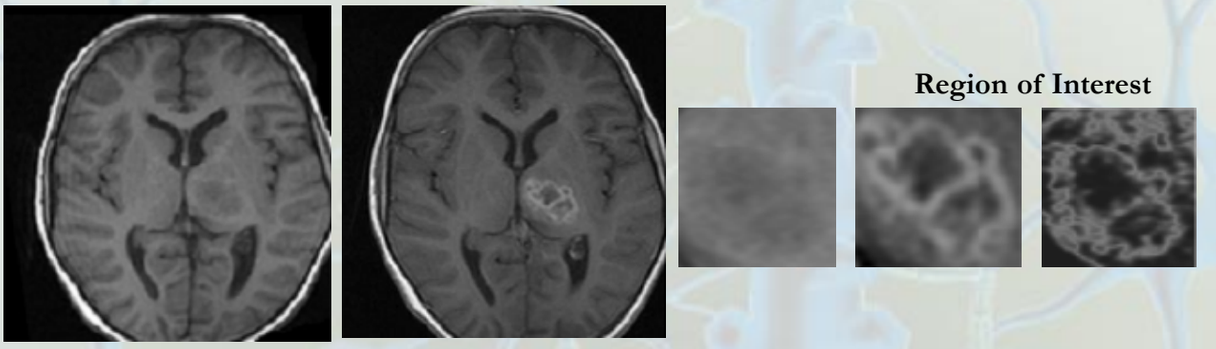
Post Doctoral Fellow : **Vani K. Kashyap**

Trainees : **T. Ray and A. Banerjee**

A promising prospect to enhance the efficiency of neuroradiological processes, whether diagnostic or therapeutic, is offered by the Stochastic Resonance (SR) effect, an emerging research field in computational neuroscience and bioengineering. SR is a general principle of nonlinear dynamics applicable to various systems, whether physical, chemical, biological, computational or quantum. In other words, noise is added to decrease the noisiness of a system, that is, noise is used to counter noise. Though this may seem counterintuitive, SR has been used to enhance various processes relevant to neurobiologists, such as (i) x-ray/γ-ray/raman spectra, electron paramagnetic resonance of organic chemicals (ii) peptide-induced neuromodulation, or electrostimulation, for neuronal signal transduction, and treatment in cerebral stroke, neural injury or sleep apnoea. However the practical application of SR effect as a novel technique in neuroimaging or therapy has not been systematically pursued, and the applicability is the goal of our study.

Perturbative Activation: Our aim is to use SR to enhance (i) MR, CT and fMRI signal for diagnosis, and (ii) radiotherapeutic signal for treatment of radiosensitive lesions. One of our questions in neuroimaging is whether stochastic resonance enhancement of image contrast and image processing can be used in MRI as a surrogate for high-priced injectable Gadolinium-based contrast scan in India. Gadolinium deglumine is a costly organometallic chelate, has side-effects and sometimes appreciable toxicity. We probe the possibility of using the approach of stochastic resonance imaging (SRI) to enhance MR images so that the enhanced image could approximate gadolinium-enhanced images. Gadolinium (Gd) enhancement of MR proton signal is basically effected by a stochastic activation induced by Gd atoms. Proton relaxation processes occur, actuated by microscopic effects as probabilistic fluctuation of local dipolar field, due to the stochastic kinetic motions of the gadolinium nuclei. We perform a stochastic resonance enhancement of the voxellated MR signal and administer a programmed stochastic perturbation on conventional (non-gadolinium) T_1 image of different brain lesions.

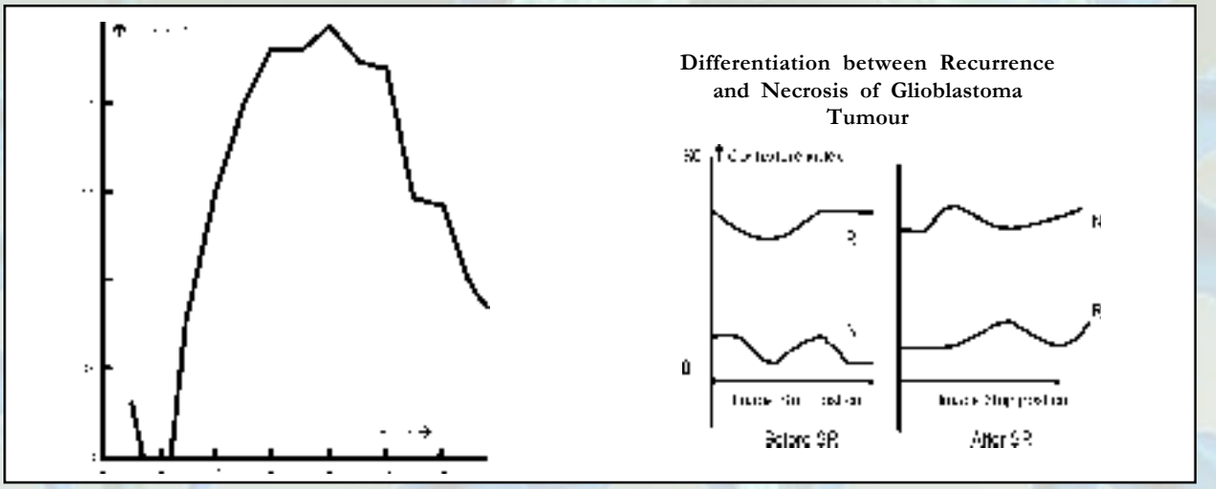
Applications: There is appreciable enhancement of the images, which compare with the gadolinium-enhanced images of the lesion. An example is shown in fig. 1. The noise



(a) Conventional Image, tuberculoma enhanced (b) Gadolinium enhanced image (c) Conventional (d) Gadolinium (e) SR-enhanced

Fig. 1. Stochastic Resonance Imaging: (a): Conventional T_1 image of tuberculoma in MRI, no selective contrast available. (b): Gadolinium-contrast enhanced image of tuberculoma in MRI: note enhancement of tuberculoma lesion. (c): Region of interest [ROI] of tuberculoma, obtained from image (a) on left; resolution is poor. (d): ROI of Gd-enhanced lesion from image (b); note the increased patterned structuration or trabeculation; (e): stochastic resonant enhancement of conventional ROI, produces the necessary enhancement without gadolinium. The degree of contrast of the trabeculation in the SR-enhanced image (e) compares with the contrast of the trabeculation in the Gadolinium enhanced image (d).

correllogram between (a) the gadoliniated image and (b) the stochastic resonant image shows an inverted U-shaped graph, the characteristic signature of stochastic resonance, that indicates that maximum enhancement and correlation occurs at a particular optimum stochastic input level of the SR image (fig. 2a). We apply the technique to investigate MR image enhancement of lesions, which are of considerable importance to neurology and neurosurgery in the Indian scenario, such as neoplastic and infective/infestive lesions of the brain (e.g. cysticercosis, tuberculoma, tumour necrosis or recurrence). We are probing the utility of SR as a useful clinical tool; for instance, in the radiological differential diagnosis between



(a) (b)

Fig.2.(a). Correllogram between SR restored image and original image shows maximal correlation at an optimal noise level, indicating the signature of stochastic resonance (b) Contexture parameter of image (y-axis) against position (x-axis). Left: Original image (before SR enhancement). Right: Treated image (after SR Enhancement). R - tumour recurrence; N - necrosis. Note reversal of the order of curves.

recurrence or necrosis of brain tumours, which is an important contemporary problem in clinical neuroscience. We have also shown the validity of the contexture index to quantify as the performance indicator of SR enhancement [fig. 2b].

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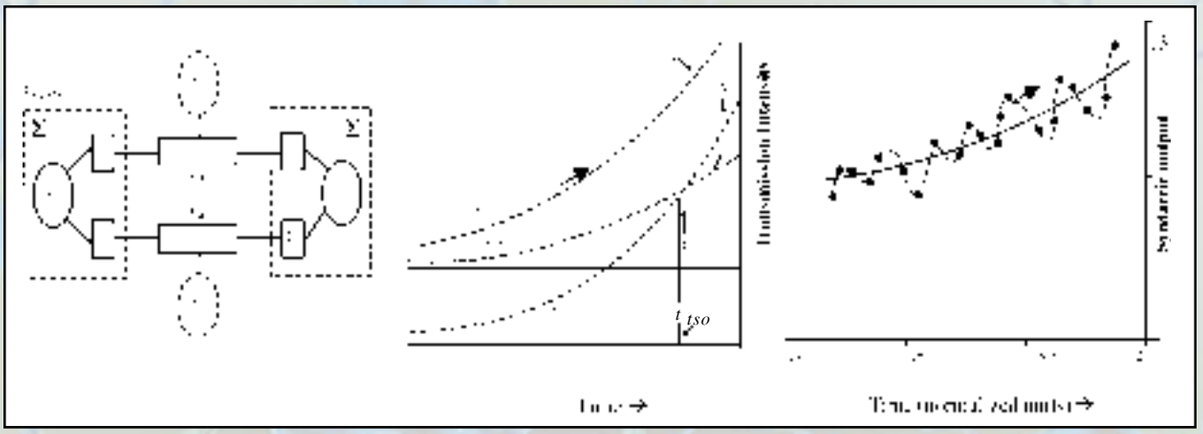
NON-EQUILIBRIUM INFORMATION THEORY AND SPATIO-TEMPORAL PROCESSING IN NEURONAL SYSTEMS: A NEUROCOMPUTATIONAL APPROACH

Principal Investigator : **Prasun K. Roy**

Trainee : **Vineet Punnoose**

A fundamental challenge in neuroscience is understanding how the brain thinks or computes, namely how the neural system communicates information under influence of stimulus and neuromodulators. The main difficulty arises because the alphabets of neural information transmission, that is, spikes, are identical. On the other hand, in two other information transmission systems in biology, namely in genetic and immunological coding, there is a well-organized transformational grammar and the alphabets are different and specific (e.g. different aminoacids or different immunoglobins). It is now known that the same spike can transmit different types of information depending on inter-spike arrangement and its statistical characteristic (as Fischer information metric tensor), which can undergo alteration, by the neuromodulator environment. There is considerable empirical neuroimaging data showing that cortical neural information transmission correlates with excitatory neuromodulation. One needs a quantitative mathematical computation approach that can account for information transmission, and its neurometabolite modulation, at various neurobiological levels, from neurons, to cortical ensembles, to the complete neurocognitive system.

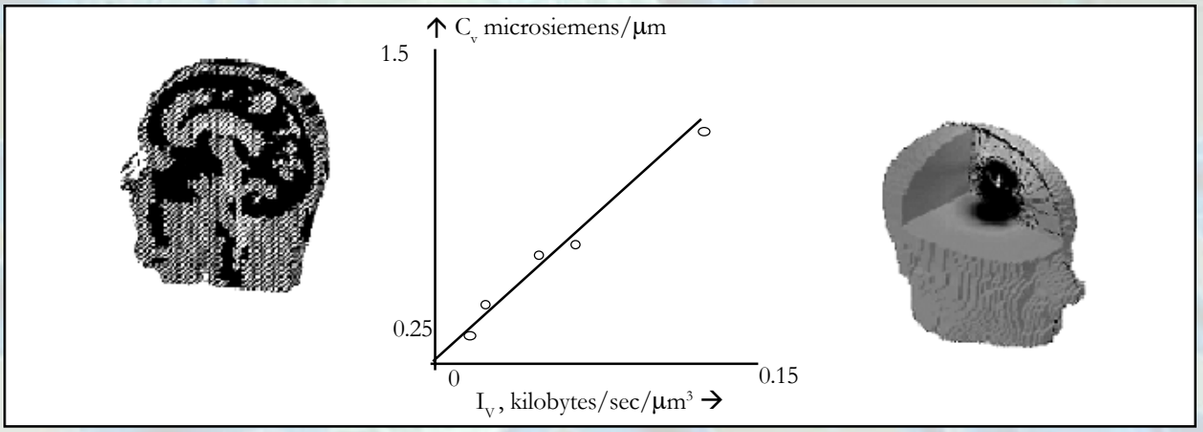
Multiplexed Information processing in neural systems: Using irreversible thermodynamic approach, we have found out that information transmission occurs via two modes, a first-order mode Y' and a second-order Y" mode. We found that at an optimal level of fluctuations, there is a transition point where first and second order modes equalize (the iso-activation point P). For equipoised transmission, there is an optimal level of system fluctuations, thus indicating a process of stochastic activation and resonance. Applying this approach to the neurocognitive system, we have found that in language communication, the two modes are characterized respectively by the syntagmatic word output (primary process output, using syntactic linkage) and the paradigmatic word output (secondary process output, using semantic linkage). We have shown that (i) the primary and secondary process output alters systematically as activation increases, either by neuromodulators or cognitive arousal, and (ii) the mathematical model correctly describes experimental data [fig. 1].



(a) (b) (c)

Fig. 1. (a) Information processing model of communication between neural systems: language communication channels between experimenter E_1 (box A) and subject E_2 (box B). Items C, D & N denote encoder, decoder and system noise for the two systems. (b) Thermodynamic model of information transmission showing the rise in the two component modes of multiplexed information transmission of a neural system as activation increases (Y' = dynamic mode; Y" = stationary mode; Y curve is total information flux, i.e. Y' + Y"). P is iso-activation point. (c) Experimental data for language production under increasing activation, confirming the computational model.

Information flux mapping: For cortical information transmission, we probe how the transmission can be characterized by a transmittivity tensor. Themodynamically generalizing the concept of diffusion transport tensor in brain tissue (a well known procedure in neuroimaging), we construct the concept of generalized transport tensor, using thermodynamic cross-property relationship and Neumann Principle in a two-phase fluidized



(a) (b) (c)

Fig. 2. (a) Finite-element model of brain, to simulate information flux and conductivity dynamics. (b) Plot of mean eigenvalues C_v of conductivity tensor (in microsiemens/μm) and mean eigenvalues I_v of information flux tensor (in kilobytes/sec/μm³) in different regions; points from left to right: subcortical white matter, anterior internal capsule, parasagittal sulcus, average of cortex, cerebellar white matter. Note the linearity of the graph ($p < 0.01$) corresponds to the thermodynamic cross-property relationship in brain tissue. (c) Schema of mapping a tensor field, as information flux connectivity, across the brain during seizure disorder.

porous medium consisting of extracellular and intracellular pools. We are exploring a computational simulation of the tensorial transport process in a 3-D finite element model of the brain with the different tissue elements: grey or white matter, cerebrospinal fluid, vascular spaces and subcortical nuclei [fig. 2a]. Utilizing the two-phase media model, we obtained a linearized relationship between the eigenvectors of the information transmission tensor and the eigenvectors of the conductivity tensor [fig 2b]. Thereby we are constructing the schema of the informational flux mapping across the brain tissue, which could be applied to track ectopic informational connectivity in seizure disorders or movement disorders, as well as neurotransmitter correlates [fig. 2c]. A potentially important application of our informational connectivity approach would be to determine functional connectivity between deeper brain regions, via connections or tracks that *actually* transmit information. This functional approach is complimentary to the usual indirect oxyhaemoglobin approach, and deals directly with the basic currency of the functional linkage in brain, namely information transmission.

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Funding:

Intramural funds of NBRC.

Collaborators:

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Dr. T R Seshadri, Delhi University, Delhi.

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R.S. Kenchappa, L. Diwakar and V. Ravindranath: Protein thiols, glutathione and complex I dysfunction in Parkinson's Disease. Annual Meeting of Society for Neuroscience, October 2004.

S. Ray (2004) Neural control during saccade sequences. 4th Indo-Japan-Korea-China workshop in Neurobiology & Neuroinformatics, India**.**

V. Ravindranath: Drug metabolism in brain by unique cytochrome P450 enzymes. Symposium on Emerging Concepts in Brain Function at NCBS, Bangalore, March 2004.

V. Ravindranath: "Molecular Pathogenesis of neurodegenerative disorders" on May 6-8, 2004 at Hyderabad.

V. Ravindranath: "Global gene expression analysis using microarray to study differential vulnerability to neuro-degeneration" on 17-19 May, 2004 at FAONS meeting at Tehran, Iran.

V. Ravindranath: "Genetics of Autism" at Ranbaxy Science Foundation's 14th Round Table Conference on 6th August, 2004 at New Delhi - Brain Disorders Across Life Span: Hopes and Challenges

V. Ravindranath: Oxidative Stress and Neuro-degeneration: National Neuroscience Conference, NIMHANS, Bangalore – September, 2004.

S. Karunakaran, S. Sampath and V. Ravindranath: Constitutive expression, localization and polymorphism of mitochondrial Glutaredoxin-2. Annual Meeting of Society for Neuroscience, October 2004.

V. Ravindranath: "The Plasticity of Human Brain" on 2-4 December, 2004 at National Academy of Sciences, Jaipur.

V. Ravindranath: "Molecular Pathogenesis of Neurodegenerative Disorders" on 8 January, 2005 at IIT, Chennai

V. Ravindranath: "Recent Advances in the Pathogenesis and Treatment of Neurodegenerative Disorders" on 12 January, 2005 at Dipti Sengupta Memorial Lecture, Kolkatta

Reddy P. Kommaddi and V. Ravindranath: "Identification of a Unique Human Brain

Cytochrome P4501A1 Variant Generated by Alternate Splicing" at 28 – 30 January, 2005 Indian Academy of Neurosciences, Gwalior.

V. Ravindranath : "Emerging Concepts in Brain Function" held on 4th and 5th March, 2005 at National Centre for Biological Sciences, Bangalore.

V. Ravindranath: "Protein thiol homeostasis and mitochondrial dysfunction in neurodegenerative diseases" on 14 –15 February, 2005 at Indo-French meeting, NBRC, Haryana

V Rema, R Ramachandra, Z.U.D. Dharokhan, N. Jain (2004) Unilateral lesions of the somatosensory cortex reduces expression of NMDA receptor subunits in the contralateral homotopic region. 34th Annual Society for Neuroscience. San Diego, CA.

Manisha Chugh, Zia Ud Din Dharokan, **V. Rema** (2005) Stimulating environment reduces anxiety and improves exploratory behaviour. 22nd Annual Conference of Neuroscience "from Basic to Clinic.

DISTINCTIONS, HONOURS AND AWARDS

Vijayalakshmi Ravindranath:

Scientific and Academic Recognitions:

1. Fellow, Indian National Science Academy,
2. Member, Working Group on Neuro-informatics of Global Science Forum.
3. Member, Governing Council of International Brain Research Organization.
4. Secretary, Federation of Asian and Oceanian Neuroscience Societies.
5. Member, Asia Pacific Regional Council, IBRO.
6. Foreign Secretary, National Academy of Sciences, India.
7. Member, Task Force on Medical Biotechnology, Immunodiagnostics and Vaccines, DBT.
8. Member, Medical Sciences Research Committee, CSIR.
9. Member, Monitoring Committee of Network Project , CSIR.
10. Member, Programme Advisory Committee in Health Sciences, Department of Science and Technology, India.
11. Fellow, National Academy of Medical Sciences, India.
12. President, Indian Academy of Neuroscience, India
13. Chairman, Building Committee, Institute of Life Sciences, Bhuvaneswar.
14. Chairman, SRF & RA Selection Committee in Medical Sciences, CSIR, India.

Membership of Editorial Board of Journals:

1. Member, Editorial Board of the International Journal, "Neurotoxicity Research", USA.
2. Member, Editorial Board, Anti-oxidants and Redox Signalling.
3. Member, Editorial Board, Indian Journal of Biochemistry and Biophysics.
4. Member, Editorial Board, Current Science.

N. R Jana:

Invited as a visiting scientist to work at Structural Neuropathology Laboratory of

RIKEN Brain Science Institute, Japan during May, 2005.

V. Rema:

Invited to review grants as a member of the review panel committee for The US Army Medical Research and Material Command, Congressionally Directed Peer Reviewed Medical Research Program.

S. Mani:

Member, Stem Cell Task Force, Department of Biotechnology.

N. Jain:

Member, Editorial Board, Journal of Biosciences.

Co-Editor, Annals of Neurosciences.

LIST OF RESEARCH PROJECTS FUNDED BY EXTERNAL AGENCIES

Nihar Ranjan Jana

Molecular mechanism of the pathogenesis of the CAG repeats neurodegenerative diseases (DBT).

Molecular mechanism of the pathogenesis of polyglutamine diseases (RIKEN Brain Science Institute, Japan).

Vijayalakshmi Ravindranath

Cytochromes P450 dependent metabolism of drugs in brain (NIH-RO1 - MH70054)

Evaluation of the molecular basis of the pharmacological action of traditional medicinal preparations used in the treatment of dementia (DBT)

Shyamala Mani

Regulation of neurogenesis in the cerebellum (FIRCA-NIH grant as well as intramural support)

To investigate the mechanisms by which embryonic stem cells differentiate into distinct neuronal subtypes (DBT as well as intramural support)

Prabodha Swain

Effect of Neural Gene(s) in the differentiation of retinal cells. (Part of the project is funded by DBT)

Prasun K. Roy

Application of Stochastic Resonance and Stability analysis for Brain Imaging and Therapy [Defence Ministry (DRDO)]

Aditya Murthy

Neural Control of Action by Basal ganglia Network (DBT)

Probing the control of action using saccadic eye movements (DST, India)

Brain Mechanisms of Action Control in Humans (DST)

Neeraj Jain

Brain Reorganization following Spinal Cord Injuries (Wellcome Trust, UK)

V. Rema

Effect of cortical injuries on the neurophysiological, molecular and behavioural functions. (Wellcome Trust, UK)

Soumya Iyengar

Emergence of primary and non-primary auditory cortical areas during late foetal and early postnatal ages in humans (DBT)

Abbreviations:

DBT: Department of Biotechnology, Govt. of India.

DST: Department of Science & Technology, Govt. of India.

FIRCA: Fogarty International Research Collaboration Award, NIH USA.

VARIOUS CORE FACILITIES AT NBRC

DISTRIBUTED INFORMATION CENTRE (DIC)

Objective

To play an active role in the development of neuroinformatics and to develop tools for analysis of different kinds of data related to neuroscience.

The Infrastructure

DIC has acquired modern technology in networking, hardware and software. It has also expanded its internal network by adding several desktops/workstations loaded with heterogeneous operating systems, establishing interconnectivity between buildings by using a high-speed backbone and adding servers, keeping in mind the increasing needs of NBRC researchers. VSAT has also been set up in this context. It has merged the bandwidth of different Internet connections as a single powerful entity to provide uninterrupted high-speed connectivity.

DIC has set up a high speed computing facility in the form of fast T300 storage arrays that run the web server on a Solaris 8 operating system. The T300 storage array is used as a repository for neural data and high-resolution graphics. The IBM server, which provides file & print services, runs the Windows 2003 Server Operating System and acts as the primary domain controller. An exchange 2000 server has been hooked on to LAN to provide e-communication. All the servers and PCs are hooked on to the network to access and share resources.

Various Software Packages

- **SUN Enterprise 420R server with 4 processors**
- **Silicon Graphics Work station**
- **Red Hat Enterprise Linux**
- **Windows 2003 Server**
- **Apache Web Server on SUN Solaris Ver.8**
- **Perl, CGI, PHP**
- **Oracle 9i, MS Access, Mysql, ASP.Net, Visual Basic. Net**

Support in Research Activities

DIC software personnel provide support for various research activities and help in image processing, data analysis and warehousing.

DNA Microarray Analysis

As part of the analysis of raw data generated from microarray experiments done by Scientists/ Students, DIC does clustering, t-tests, ANOVA and other statistical tests using various statistical software packages

Complete information about every experiment, specification of samples and Slide-Tech data are recorded in Oracle 9i installed on the Sun Solaris system.

DNA Sequencing

NBRC uses the MegaBase™ 1000 DNA Analysis system to perform DNA sequencing. Raw data generated by this system is processed using a sequence analyser to determine the order of base pairs in DNA samples. Base calling algorithms are used to analyse the raw data. The accuracy and length of the sequence generated depends on quality and quantity of the DNA prepared. Once the analyzed sequence is obtained, the sequence is subjected to a BLAST search to verify the cloned gene.

Data analysis

Image processing for enhancement of MRI images using image processing tools and sound analysis of various speech samples obtained in the respective research laboratories of NBRC is also carried out in collaboration with personnel from DIC.

Bio-Grid

DIC also managed the private network called Virtual Private Network (VPN) on behalf of Department of Biotechnology (DBT) until August 2004. This was aimed at integrating scientific research among Indian research institutes, and connected 12 DBT locations through which scientific information and data was shared across the country.

Services for the Students

DIC manages PCs, the software setup, and user environment creation in common rooms and laboratories at NBRC configured for their respective research areas with 24 hrs Internet facility.

Services for the Scientists

NBRC provides high-end PCs, Laptops and external hard disks for each scientist. A Neuroscience community portal is being developed to enhance associations amongst scientists.

Conducted Training Programmes / Workshops

A Computational Neuroscience Workshop was conducted in October 2004 at the University of Hyderabad and was attended by several students from all over India.

The Joint workshop on Neuroinformatics and Neurobiology (NBNI – 2004) was organized

by NBRC. Scientists from four participating countries – Japan, China, Korea and India participated bringing together mathematicians, experimentalists and computer scientists

Future Plans

To set up a videoconference facility to telecast in-house lectures to various networked centres on demand basis.

Developing a Neuroscience Portal as a one-stop site for all neuroscience activities in and around India.

Developing a high speed computing facility to share knowledge across NBRC networked centres.

ANIMAL FACILITY

The animal facility of NBRC meets all national and international standards. Its central air-conditioning facility with an air cycle of 12-15 times per hour, 100% fresh air changes, remote monitoring of the temperature and humidity and controlled light cycle provides a uniformly comfortable environment to animals. It also hosts a state of the art surgical operation theatre equipped with gas anaesthesia machine, heart rate monitor, pulse oximeter, surgical microscope, intensity controlled surgical lights, ultrasonic instrument cleaner and provision for sterilization of instruments using steam or ethylene oxide.

Beside a well-equipped surgical suite, the animal facility has a necropsy room with a laminar flow hood, deep freezer, fire alarm system and incinerator. The outdoor play area for non-human primates has six large interconnected enclosures that provide a flexible layout for optimising enrichment and social interactions. There are special arrangements for housing transgenic mice comprising of filter top cages in separate enclosures. The facility currently houses four strains of mice, three strains of rats, rabbits, zebra finches, two strains of transgenic mice and a few non-human primates.

DIGITAL LIBRARY

The NBRC Library plays a vital role in the collection, development and dissemination of scientific and technical information to meet the present and future needs of the centre, and also provides facilities and support to the scientists, researchers, students, staff and its networked centres.

The NBRC library has a good collection of Journals, books and other relevant research material on Neuroscience, Biochemistry, Genetics, Molecular Biology, Immunology & Microbiology, Pharmacology & Toxicology, Psychology, Physics, Mathematics, Computer Science and general subjects. The NBRC Library currently subscribes to 466 journals, amongst which 292 are online journals and others are in printed hard copy format. The library also subscribes to newspapers and newsletters. The main aim of the Library is to acquire a large collection of books and journals on neuroscience and related areas.

To provide optimum services to all users, the list of collections available at NBRC is digitized and full access is given to the users. We use the LSEASE software for the digitization of collections. It also helps in efficient library operations viz. administration, acquisition, circulation, serial control, cataloguing, information retrieval etc.

The NBRC Library has set up 20 IBM PC-Pentium-IV Computers with ISDN Internet facility to provide services for researchers and students at NBRC in the common rooms. The Library provides access to the most current reference sources available in order to ensure the accuracy of information. The Library has also been providing electronic access to subscribed journals within the campus portal and maintains digital archives and newspaper clippings about the centre.

A total of 170 registered users including scientists, researchers, students and other staff uses the NBRC library facilities. The NBRC Library also provides the facility of "Inter Library Loan" to 45 Networked Centres all over India. Researchers, scientists and students across the country send their requirements for research materials or journal articles through e-mail to NBRC Library (library@nbrc.ac.in), which is downloaded and sent to the requestors by the library staff free of cost. The library entertains an average of approximately 350 articles every year and requests are increasing day-by-day.

The NBRC Library regularly evaluates its information services to ensure that the Institution's requirements are met. It also promotes cooperation among different institutional libraries by providing an efficient and reliable means of resource sharing, that is, the inter-library loan facility to maximize resources and provide copies of documents that are not available in other libraries.

The Main Activities of NBRC Library

1. Book Acquisition
2. Acquisition of Periodicals
3. Selective Dissemination of Information (SDI),
4. Current Awareness Services (CAS)
5. Inter Library Loan
6. Resource Sharing
7. Circulation services
8. Reference Services, Bibliographic services
9. Indexing and Special Services
10. Collects, maintains, stores and retrieves information and data, keeping in the view the evolving needs of its researchers
11. Providing help to Network Centres.

The main aim of the NBRC library staff is to provide excellent services to the scientists, researchers, research associates and students of NBRC and all centres associated with the institute.

NATIONAL FACILITIES

DNA Micro Array Facility

Genomic micro-array R&D programs on infectious diseases and neurological disorders.

The National Brain Research Centre has set up a core microarray program especially for the neuroscience research community in India. This facility is available to all scientists interested in using this technique for asking neuroscience-related questions the institute and collaborating research centers within and outside the NBRC network.

The micro-array facility was commissioned in September 2002 at the NBRC interim facility at Gurgaon. A robotic liquid handling system with PCR setup has also been included in the facility which is designed to minimize handling errors and perform high throughput research. During the last year, different laboratories at NBRC have used the microarray facility to address different questions in neuroscience. NBRC has also provided access to the microarray facility for faculty and students of other scientific institutes including NCCS, Pune, to perform their experiments. We have also provided hands-on training for using microarray technology to trainees from abroad and summer students interested in microarray techniques. Trained personnel from the DIC at NBRC now provide most of the services like data acquisition, normalizing data sets and subsequent analysis.

DNA SEQUENCING FACILITY

A centralized DNA sequencing facility has been set up by NBRC for strengthening the infrastructure for cellular and molecular neurobiologists at the institute as well as for investigators in the Indian neuroscience community. Currently, NBRC has the MegaBACE™ 1000 DNA Analysis system, a high-throughput, fluorescence based DNA sequencing system utilizing capillary electrophoresis with up to 96 capillaries operating in parallel. The fully automatic system performs sample injection, gel matrix replacement, DNA separation, detection and data analysis. This system has the ability to sequence 96 samples in just two hours and performs up to 9 runs per day. The average read length with dye primer chemistry is 550bp. This system can also be used for genotyping and SNP analysis.

ACADEMIC COURSES

Ph. D Neuroscience

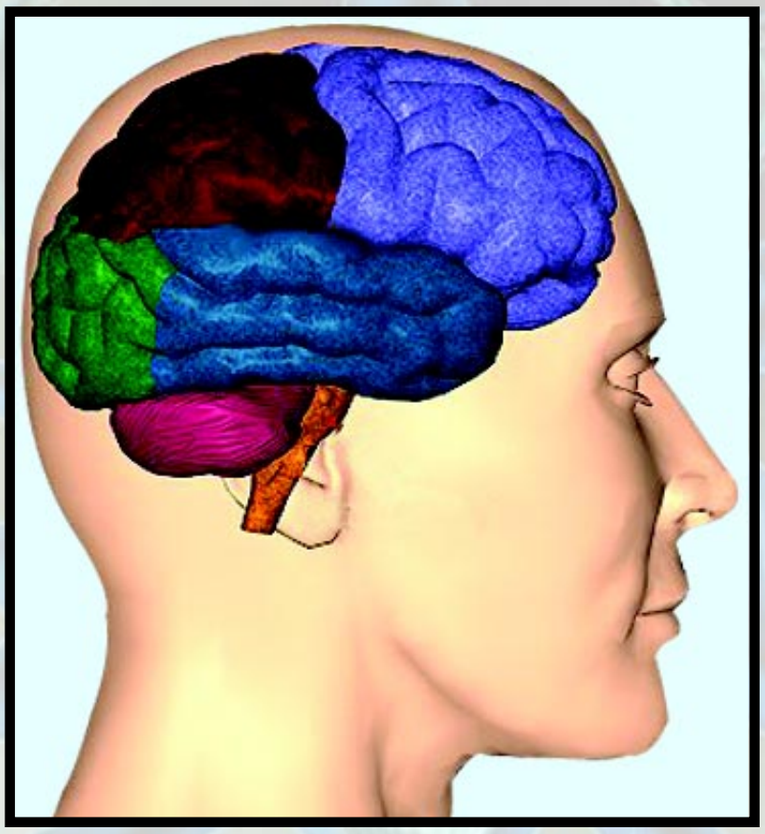
NBRC was granted Deemed University status in 2002 by the Ministry of Human Resource Development and since then, has been recruiting students from diverse backgrounds including master's degrees in any subject related to neuroscience, MBBS, BE or B.Tech. for its Ph.D. programme. Thirteen students were admitted to NBRC for its PhD programme in 2004. The admission to neuroscience is publicised through advertisements in widely circulated national newspapers and on the NBRC website from which forms can be downloaded. Students are chosen with great care, after a rigorous screening process and two rounds of interviews by all members of the faculty to ensure that only the most deserving are chosen for the Ph.D. programme. The first year of the programme consists of course-work encompassing different aspects of neuroscience as diverse as genetics, cell biology, developmental biology, neurochemistry, systems neuroscience, neuroanatomy, cognitive and clinical neuroscience, biostatistics, computational neuroscience and membrane biophysics. Most of these courses are taught by NBRC faculty and wherever required, experts in different areas are also invited to teach part of the course-work. Students also do three-month long laboratory rotations during this period in three labs, choosing from amongst faculty working in the major areas of molecular, systems and computational neuroscience. In addition to didactic lectures, students also complete written assignments and present seminars as part of their course-work. Throughout their tenure at NBRC, Ph.D. students are required to attend as well as present scientific papers related to neuroscience at the weekly Journal Club, which is assessed by NBRC faculty. When first year Ph.D. students successfully complete their coursework and a comprehensive viva-voce examination conducted by a panel of external examiners and NBRC faculty members, they can register for obtaining their Ph.D. degrees. Students then join different laboratories at NBRC to complete their dissertation research work. The aim of the Ph.D. program is to produce human resource trained in different aspects of neuroscience and to promote excellence in the field.

M. Sc. Neuroscience

NBRC introduced its M.Sc. Neuroscience course in 2004 with a view to accomplish its mandate of developing trained manpower having a broad overview of different aspects of neuroscience. Ten students from various backgrounds such as MBBS/BE/B.Sc./B.A. have been selected for this two-year course in order to impart a broad perspective of the major disciplines of neuroscience. The first year comprises of course-work taught mainly by NBRC faculty in neuroanatomy,

neurophysiology, neurochemistry, molecular neurobiology, systems neuroscience, computational and theoretical neuroscience, cognitive and clinical neuroscience as well as practical knowledge in the above aspects. Experts in different fields from other institutions and universities are also invited to teach students part of the course work. Students join different laboratories in their second year and work on a research project under guidance of NBRC faculty in partial fulfillment of their masters' degree. Students are provided hostel accommodation and a stipend of Rs 1000/- per month. The manpower trained under this programme would be a highly skilled human resource for the existing Ph.D. programme in specific disciplines of neuroscience that are currently being carried out in the country.

EXTRAMURAL ACTIVITIES



EXTRAMURAL ACTIVITIES

EXTRAMURAL ACTIVITIES

Networking Activities

One of the major goals of NBRC is to network existing neuroscience groups / institutions in the country and promote multidisciplinary research in neuroscience. This networking of existing neuroscience centres with NBRC not only prevents unnecessary duplication of already existing work and facilities but also helps in sharing of expertise and available infrastructure for mutual benefit.

The networking of NBRC with other existing neuroscience groups/ institutions brings together researchers from varying backgrounds to pursue common objectives that may be beyond the capacity of an individual investigator, group or institution. This is important since the major achievements in neuroscience are being made through a multidisciplinary approach bringing together scientists working in different disciplines into the mainstream of neuroscience and brain research activity.

The following institutions/ universities are member of our network activities so as to facilitate neuroscience research across the country.

List of Network Centres:

1. All India Institute of Medical Sciences (AIIMS), New Delhi.
2. Banaras Hindu University (BHU), Varanasi.
3. Bangur Institute of Neurology, Kolkata.
4. Centre for Behavioural and Cognitive Sciences (CBCS), University of Allahabad, Allahabad.
5. Centre for Cellular & Molecular Biology (CCMB), Hyderabad.
6. Central Drug Research Institute (CDRI), Lucknow.
7. Centre for DNA Fingerprinting and Diagnostic, Hyderabad.
8. Central Food and Technological Research Institute (CFTRI), Mysore.
9. Cochin University of Science and Technology, Cochin.
10. Department of Biotechnology. New Delhi.
11. Delhi University, South Campus, Delhi.
12. Dr. A. L. Neurosurgical Centre, Chennai.
13. Indo American Hospital Brain and Spine Center, Kerala.
14. Institute of Cybernetics, Systems and Information Technology, Kolkata.

15. International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi.

16. Institute of Genomics and Integrative Biology (IGIB), Delhi.

17. Institute of Human Behaviours & Allied Sciences (IHBAS), Delhi.

18. Indian Institute of Information Technology (IIIT), Allahabad.

19. Indian Institute of Technology (IIT), Mumbai.

20. Indian Institute of Technology (IIT), Delhi.

21. Indian Institute of Technology (IIT), Kanpur.

22. Indian Institute of Science (IIS), Bangalore.

23. Indian Institute of Chemical Biology (IICB), Kolkata.

24. Institute of Nuclear Medicine and Allied Sciences (INMAS), New Delhi.

25. Industrial Toxicology Research Centre (ITRC), Lucknow.

26. Indian Statistical Institute, Kolkata.

27. International School of Photomics, Cochin.

28. Jawaharlal Nehru University (JNU), New Delhi.

29. Jawaharlal Nehru Centre for Advance Scientific Research (JNCASR), Bangalore.

30. Jiwaji University, Gwalior.

31. M.S. University of Baroda (Dept. of Microbiology and Biotechnology Centre), Baroda.

32. National Centre for Biological Sciences (NCBS), Bangalore.

33. National Informatics Centre (Medical Informatics and Telemedicine Division), (NIC) New Delhi.

34. National Institute of Mental Health & Neuroscience (NIMHANS), Bangalore.

35. Nizams Institute for Medical Sciences (NIMS), Hyderabad.

36. National Neuroscience Centre (NNC), Kolkata.

37. Sanjay Gandhi Post-Graduate Institute of Medical Sciences (SGPGIMS), Lucknow.

38. School of Information Technology, West Bengal University.

39. Shree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvanthapuram.

40. Sri Venkadeswara Institute of Medical Sciences, Tirupati.

41. Tata Institute of Fundamental Research (TIFR), Mumbai.

42. University College of Medical Sciences (UCMS), Delhi.

43. University of Hyderabad, Hyderabad.

44. University of Calcutta, Kolkata.

45. Vidyasagar Institute of Mental Health and Neuroscience (VIMHANS), New Delhi.

TRAINING

Summer Training and Short-Term Programmes

Continuing the training programmes of previous years, nine students from different academic institutions and universities throughout the country were accepted for project training as part of their post-graduate training and for summer training programmes at NBRC. Their applications were screened and they were selected based on availability of space in different laboratories at NBRC. Each student completed a short project with an NBRC faculty member and learned research methodologies pertaining to a specialized area of neuroscience. Summer trainees were also encouraged to attend seminars and journal clubs organized at the institute during their tenure. The summer training projects give students an exposure to neuroscience and to encourage them to consider it as a future career option.

INTERNATIONAL COLLABORATIONS

International collaborations help building bridges between Indian neuroscientists and the international neuroscience community through exchange programs and promote excellence in science. Despite having been established recently, NBRC has made great strides in establishing such collaborations with various prestigious neuroscience institutions in different countries.

Indo-US

NBRC signed an MOU with the National Institute of Mental Health (NIMH), USA. Since then, significant progress has been made by exchanging ideas and expertise between scientists from the two countries. An NIH-RO1 grant has also been funded.

Indo-Japanese

Dr. Nihar R. Jana's laboratory at NBRC is collaborating with Dr. Nobuyuki Nukina of the RIKEN Brain Science Institute, Japan to study the pathogenesis of the CAG.

Indo-Russian

Dr. Neeraj Jain, Dr. V. Rema, Dr. Nihar R. Jana, Dr. Aditya. Murthy and Dr. Prasun K.Roy have established a research collaboration entitled "Spinal cord plasticity and rehabilitation after spinal cord injuries" with their Russian counterparts to study different aspects of CNS disorders and neural plasticity.

Indo-French

Dr. Pierre Gressens and Dr. Shyamala Mani have been awarded an INSERM-ICMR collaborative grant to study the effect of maternal malnutrition on the developing brain of the fetus and also plan to use stem cells for neonatal neuroprotection. In addition, Dr. Hicks and Dr. Prabodha Swain have written a grant proposal for funding under Indo-French programs to facilitate their research collaborations.

Indo-Italian

An Indo-Italian agreement on neuroinformatics has been signed under the auspices of which research will be initiated in memory and language impairments in the early stages of Alzheimer's Disease using multimodal imaging techniques (PET, SPET; MRI) and the development of grid computing networks in research areas of common interest.

MEETINGS AND WORKSHOPS

Computational Neuroscience Workshop – 2004

A ten-day short course on Computational Neuroscience was held at University of Hyderabad from October 11th to 21st and was organized by Department of Computer and Information Sciences with financial assistance from National Brain Research Centre (NBRC) and the Universities with Potential for Excellence (UPE) grant from University of Hyderabad. Computational Neuroscience is a new and emerging inter-disciplinary area of research that combines insights from various disciplines such as computer science, neuroscience, mathematics, statistics, artificial intelligence (AI) and psychology.

The aim of this course was to provide students with an introduction to basic concepts in computational neuroscience. The course addressed basics of neuroscience as well as computational methods. Experiments and computational models addressing information processing at various levels such as cellular, systems and cognitive were the central theme of this course. Additionally, related topics in artificial neural networks, neuroimaging, philosophy of mind, cybernetics, machine learning and AI were also covered. Tools such as GENESIS, Matlab and Neural Network toolkits were used for demonstrations and exercises to gain a deeper understanding of the concepts and methods introduced in the course. Participants were assigned mini-projects involving computer simulation to be completed by the end of the course. Eminent researchers from various institutions such as NBRC, NCBS, NIMHANS, IISc, IIT Delhi, IIT Madras, IIT Kanpur, IIIT Hyderabad and University of Hyderabad delivered lectures. NBRC, Manesar, and Department of Computer and Information Sciences, University of Hyderabad, jointly organized this 10-day course. This course was the fourth in the series. Earlier courses were held at NCBS, IIT-Delhi and IIT-Kanpur.

70 students with Bachelors/Masters/ Doctoral students in all field of engineering/ sciences/mathematics had participated in this workshop.

Indo-French Workshop in Neuroscience held at the National Brain Research Centre, Manesar, India, February 14th and 15th, 2005.

An inter-disciplinary approach to understanding brain function in an integrative manner is a relatively new initiative in India. It is realized that scientists with training in diverse fields such as mathematics and psychology, together with clinicians interested in research in the neurosciences are needed to understand the complexity of the brain. To this end we have brought together scientists from France and

India working in diverse areas of neuroscience. The scientists from France were chosen because they had expressed interest in collaborating with scientists from India and seeking out new avenues of cooperation. Our goal was to facilitate this interaction and help promote collaborations between the French and the Indian scientists and generate new research proposals.

The two-day meeting was organized so that there was sufficient time for interaction and further discussions. The following are the outcomes of the meeting –

- Dr. Pierre Gressens and Dr. Shyamala Mani have been awarded a collaborative grant to study the effect of maternal malnutrition on the developing brain of the fetus. However, several other avenues where the research interests of the two laboratories overlap and where the two laboratories bring in complimentary expertise are being explored. One of the major areas is in the field of stem cell biology and the use of stem cells in the field of neonatal neuroprotection. Several pilot experiments were discussed and planned during the meeting.

- Dr. Gidrol had several one on one discussion with students, post-doctoral fellows and the bioinformatics staff working in the NBRC microarray facility at NBRC. The possibility of cooperation at several levels was discussed. One was the exchange of information on technical and experimental details of microarray experiments. The other was training of personnel in the microarray technique. It was proposed that senior students or post-doctoral fellows could be sent to Dr. Gidrol's laboratory for a short period for training. In addition India has expertise in informatics and post analysis of the data generated in a microarray experiment. This was an area where Dr. Gidrol felt it would be helpful for him if he could have such people in his laboratory since in France it was difficult to come across persons trained in mathematics and computer science who had an interest in applying their training to biology and genome analysis.

- Dr. Hicks and Dr. Prabodha Swain had very fruitful discussions during the visit and several areas of cooperation were discussed. They have written a grant proposal for funding under Indo-French programs to facilitate their cooperative research aims, which will include exchange visits of senior scientists and doctoral students.

- Dr. Shobini L. Rao of NIMHANS, Bangalore and Dr. Longo had interactions about the possibility of collaborating in the area of brain plasticity. Specifically the questions addressed were about the effect of the brain interacting with environment and how it affects brain functioning. Clinical evidence of brain plasticity in terms of the effects of cognitive retraining on brain damaged patients and the effect of education on cognitive functions were discussed and the manner in which the brain changes in response to the environment. Dr. Longo was interested in developing the mathematics for characterizing such interactions. This dialogue is being continued. Dr. Longo is trying to arrange Dr. Rao's visit to his laboratory and the avenue of visiting professorship is being explored.

- Dr. Kennedy thought that the strength of NBRC was the fact that we had expertise across different areas of neuroscience. He was interested in developing research

collaborations in the area of systems neuroscience and in isolating and working on stem cells from non-human primates.

Joint Workshop on Neurobiology and Neuroinformatics organized at NBRC, India – November 2004.

The Sixth China-India-Japan-Korea Joint Workshop on Neurobiology and Neuroinformatics was organized on November 19-20, 2004 at the Heritage Resort and NBRC, Manesar, India. This international workshop is an annual feature and provides a platform for students and scientists from four Asian countries (China-India-Japan-Korea) an opportunity to interact and exchange ideas Neurobiology and Neuroinformatics.

Scientists from India included those from NBRC and its network centres. By attending the workshop and scientific sessions, the scientists got a chance to present their work and receive inputs from the community. Mornings were devoted to joint sessions on talks, which would be of interest to the neuroscientists in general. There were parallel sessions on neurobiology and neuroinformatics in the afternoons, which were specialized and interactive. Scientists and students from China, Korea and Japan arrived in large numbers and we had a record number of 40 participants, the largest at any NBNI so far.

The Workshop mainly focused on neurobiology and neuroinformatics, which covered broad areas in brain research. The participants included neurobiologists, medical doctors, cognitive scientists, physicists, mathematicians, information scientists and various fields of engineering scientists. It provided inputs for a wide spectrum of topics such as Neural Stem Cells, fMRI, Plasticity and Neurotransmitters, Brain Computer Interface, Biologically-Motivated Artificial Vision, Principal Component Analysis, Artificial Auditory System, Computational Neural Models, Brain-based Cognition and Inference System, Brain Signal Measurement and Analysis, Brain-based Human Behaviour.

Laying of foundation stone for the National Neuro Imaging Facility Building by Hon'ble Minister Shri Kapil Sibal on Sept. 29, 2004

The Hon'ble Minister for Science and

Technology, Shri Kapil Sibal laid the foundation stone for the National Neuro Imaging Facility Building at NBRC on Sept.29, 2004. Speaking to the faculty, staff and students of the institute, he said that the faculty and students of NBRC have a major challenge and responsibility to the nation since healthy minds are needed in order to realize the dream of a developed India. Through the study of the normal brain and its diseases and disorders, brain research can enhance the development of children and help them to fulfill their potential, enrich adult life, and improve the likelihood of successful ageing.

He also said that since an epidemiological transition occurs from communicable to non-communicable disorders, it was imperative to gain a better understanding of brain functions in health and disease. He also felt that the mission statement of NBRC "to discover rational therapies and cures for brain disorders through brain research" needs to be realized with urgency. Shri Kapil Sibal concluded that NBRC had been created as a world-class facility and with the establishment of the functional Magnetic Resonance Imaging facility (fMRI), the institute would be at par with any of the leading brain research centres in the world. He hoped that the faculty and the students at NBRC who had the unique privilege of working in such an environment would do so with commitment and motivation. The Hon'ble Minister then visited different laboratories at NBRC and was informed about the research activities being carried out, followed by lunch with the Director and faculty of NBRC.

First Foundation Day Celebrations

The first Foundation Day was celebrated by NBRC on December 16, 2004 to mark the first anniversary of the dedication of NBRC to the Nation by the President of India, His Excellency, Dr A P J Abdul Kalam. In order to give a glimpse of cutting edge brain research, students from four schools of Haryana were invited to visit NBRC. The students were given a tour of the laboratories and were told about various research projects and the different approaches and tools to investigate the functioning of the brain in health and disease. Posters made by NBRC students were displayed to help explain the ongoing research activities at the centre to encourage school students to think of neuroscience research as a possible career option in the future. Research scholars of NBRC organized and conducted a quiz competition based on brain related questions in which teams from each of the schools participated. Awards were given to the winning teams and all the

Explaining the DNA Microarray technique

participants. The quiz competition was presided over by Mr. Sunil Ahuja, the Chief Secretary, Government of Haryana, who also addressed the budding scientists of the nation.

In the evening a public lecture entitled 'The Self Made Brain' was delivered by Prof Colin Blakemore, FRS, CEO, Medical Research Council, UK at the auditorium of Indian National Science Academy, New Delhi, which was followed by a reception.

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1. Prof. Vijayalakshmi Ravindranath
2. Dr. Neeraj Jain
3. Dr. Rema Velayudhan
4. Dr. P.K. Roy
5. Dr. Pankaj Seth
6. Dr. Aditya Murthy
7. Dr. Nandini C. Singh
8. Dr. Nihar Ranjan Jana
9. Dr. Prabodha K. Swain
10. Dr. Shyamala Mani
11. Dr. Soumya Iyengar
12. Dr. Anirban Basu
13. Mr. B. Satyananda Gupta

Post Doctorate Fellows

1. Dr. Renuka Ramachandran
2. Dr. Sajla Singh
3. Dr. K. Vani
4. Dr. Sarath Babu
5. Dr. R.C. Kumar
6. Dr. Sayali Ranade
7. Dr. Madhumita P. Ghosh

PhD Students

1. Mr. Reddy Peera. K.
2. Mr. Supriya Ray
3. Mr. Anand Goswami
4. Ms. Latika Singh
5. Mr. Sandeep Kumar
6. Ms. Priyanka Dikshit
7. Mr. Manoj Kumar
8. Ms. Rashmi Mishra
9. Ms. Smitha Karunakaran
10. Mr. Ziauddin
11. Ms. Chinmoyee Maharana
12. Mr. Alok Gupta
13. Mr. Leslee Lazar
14. Ms. Nazia Khurshid

15. Ms. Shalaka Mulherkar
16. Mr. Kh. Budhchandra Singh
17. Dr. Raka Maitra
18. Ms. Mamata Mishra
19. Mr. Arjun
20. Mr. Manoj Kumar Mishra
21. Mr. Niranjan A. Kambi
22. Mr. Anshul Shrivastava
23. Mr. Patel Dharmeshkumar
24. Mr. Shashank Tandon
25. Ms. Uzma Saeed
26. Mr. Amit Kumar Mishra
27. Ms. Varsha Agarwal
28. Ms. Manisha Chugh
29. Mr. Shailesh K.Gupta
30. Mr. S. Srirangan
31. Ms. Latha Diwakar

M. Sc (Previous) Students

1. Mr. Tanuj Gulati
2. Ms. Bhavana Shrivastava
3. Mr. Vishal Kapoor
4. Ms. Geethika Phukan
5. Ms. K.M. Sharika
6. Mr. Ayan Ghoshal
7. Mr. Pranav Oberoi
8. Mr. Rahul Chaudary
9. Mr. Dhrubjyothi Chowdhury
10. Mr. Sourojit Bhowmick

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13. Ms. Preeti Kohli
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15. Ms. Anindita Bir
16. Mr. Srikanth Ramaswamy
17. Ms. Sandhya Singh
18. Mr. Abhay Kumar Saini
19. Ms. Mohita Srivastava
20. Ms. Taruna Ladha
21. Ms. Nazia Rehman
22. Mr. Amol Vijay Ghadge

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11. Mr. Dil Bahadur Karki
12. Mr. Mahender Kumar Singh
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5. Ms. Pooja Gosain
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1. Mr. Santosh Kumar

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