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INSTITUTE OF HIGHER EDUCATION AND RESEARCH
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Ref. No.SMS-2018-O-08

Date: 20.02.2019

TO

Mr. Rajesh
Professor/Anatomy
BIHER

Thro: Concern Head of the Department

Greetings!!!

We are happy to announce that the Research Advisory Committee has approved your proposal for Seed Money Scheme-2018 which was presented by you. You are requested to complete the proposal and send the progress report to the Dean Research in the prescribed time period.

Title of the Project: Extrinsic factors in regulation of adult hippocampal neurogenesis

Seed Money Amount: Rs.1, 00,000/- (Rupees One Lakh Only)

Approved on: 05.02.2019

Payment details:

Voucher No.53 **Dated: 27.02.2019**

With Regards

Dean-Research

Bharath University

SELAIYUR, CHENNAI - 600 073, TAMIL NADU, INDIA.

CASH / PAYMENT VOUCHER

Date 27/02/2019

V.No. 53

Debit _____ Amount

Rs.

PAID TO Dr. B. Rajesh

RUPEES One Lakh Only

TOWARDS Seed Money Scheme -2018

BHARATH UNIVERSITY CHENNAI 600 073. INDIA.

Authorised by **Finance Manager** **Cashier/Accountant** **Payee's Signature**

PROPOSAL SUBMISSION

1. **Details of Principal Investigator**

Name	: Dr. B Rajesh
Designation	: Professor
Highest Qualifications	: Ph.D.
Department	: Anatomy
E-mail	: anat_rajesh@rediffmail.com
Contact no	: 9345575143
Date of Joining	: 14.06.2010

2. **Details of Principal Investigator**

Name	: N.Anandaramajayan
Designation	: Assistant Professor
Highest Qualifications	: M.Sc.,
Department	: Anatomy
E-mail	: anandaraman2006@gmail.com
Contact no	: 9894343517
Date of Joining	: 1-12-2010

Technical details

1. Introduction:

Neuronal mitosis is considered as a prenatal phenomenon until the proliferation of granule cells was observed in cerebellar cortex of young animals. Pioneering work of Altman and Das (1965) found the thymidine labeled granule cells in the dentate gyrus (DG) of the hippocampus of adult rats and cats.[1] The sub-ventricular zone (SVZ) of lateral ventricle and sub-granular zone (SGZ) of the DG are the two regions of the brains where the process of adult neurogenesis could be observed.[2] Pioneering work on postmortem hippocampal tissue obtained from the cancer patients who got BrdU infusion for diagnostic purpose revealed the BrdU got incorporated in DNA of the cells that undergo division.[3] This demonstrates that SVZ of lateral ventricle and SGZ of hippocampus in humans are the potential zones for adult neurogenesis. However, the functionality of the newly generated neurons could not be elucidated by this study. Growing evidence of functionality was established as the adult-born granule cells of hippocampal DG made glutamatergic synapses with inter-neurons of hilus and pyramidal cells of CA3 region.[4] Therefore, the new neurons are the fully functional unit of hippocampal circuitry.

THE PROCESS INVOLVED IN ADULT NEUROGENESIS

The neural stem cells (NSCs) are retained in the adult brain for self-renewal for maintaining function such as learning and memory. The NSCs were observed in SVZ of lateral ventricles and SGZ of the DG of the hippocampus.[5] The NSCs undergo asymmetrical divisions to become neural progenitor cells (NPCs). The NPCs mature during their migration to assigned sites to either become neurons or glial cells. The NPCs of SVZ mature to become neurons in the olfactory bulb [6] and NPCs of SGZ of DG mature to become newlyborn adult neurons and get migrated to the granular cell proper.[7]

During the 1st week after neurogenesis, the adult-born neurons in the SGZ migrate to the granule cell layer proper of DG. Growth of dendritic spines is the sign of the establishment of the synapses. These neurons are immature, shown irregular cell body with or without short processes and lack afferent inputs and not integrated into the hippocampal circuitry. [8,9] During the 2nd week post-neurogenesis, the 14 days old adult-born neurons show a marked rise in DCX. During the 4th week of post-neurogenesis, the final steps of maturation of the adult-born neurons take place. These cells express NeuN. The dendritic spines grow and reach as far as the outer molecular layer of DG, indicating the formation of glutamatergic synapses.[8] The connectivity established by the adult-born neurons includes axo-somatic, axo-dendritic, and axo-spinous inputs. This makes it evident that, integration of these new neurons in the hippocampal circuitry.[10] Neurogenesis plays an essential role during brain development and in the aged brain. Alterations in the degree of adult neurogenesis were observed in neurodegenerative disorders, mood disorders, and drug abuse. [11,12] It was also suggested that adult neurogenesis act as a potential target in treatment of neurobehavioral disorders. [13,14] Hence, this review

focuses on the extrinsic factors that affect the neurogenesis and its role in the treatment of neurobehavioral disorders.

ENVIRONMENTAL ENRICHMENT (EE)

The mice exposed to a stressful situation such as social conflict followed by environmentally enriched housing by keeping nesting material, running wheels, and paper tubes, reduced stress-induced behaviors and showed an increase in adult-born neurons, as it is evident from increased BrdU and DCX positive cells. Conversely, the study group in which conditional ablation of adult neurogenesis in mice exposed to stress followed by housing in EE, does not alter the stress induced behavior indicating that EE requires adult neurogenesis to get recovered from stress-induced behavior. Besides these, DCX positive neuroblasts were absent in their hippocampus and reduced BrdU cell counts reveal less proliferation in SVZ of lateral ventricle and SGZ of the DG of the hippocampus.[15] EE increases the level of expression of proteins, namely, phosphorylated cyclic adenosine monophosphate response element binding protein (pCREB), stromal cell-derived factor 1, brain derived neurotrophic factor (BDNF), and C-X-C motif chemokine receptor 4. These increased levels of expression are associated with adult neurogenesis.[16] Furthermore, it is observed that the EE reduces debilitated neurogenesis following surgery by preserving BDNF expression.[17]

Besides protection from infectious agents, the immune system is also involved in neurobehavioral processes.[18] Typically rodents and humans have T cell in their central nervous system.[19] Hippocampal dependent learning and memory, and neurogenesis are severely impaired in severe combined immune deficiency mice, and CD4+ depleted mice. C57BL6/J female mice injected with anti-CD8α (i.p) resulted in the depletion of CD8+ T cells. This depletion deterred EE-induced raise in BrdU+ cells in DG. Hence, the EE induced neurogenesis partially depends on CD8+ T cells.[18] Inducing temporal lobe epilepsy in adult Wistar rats caused a decrease in BrdU/DCX positive neurons in DG. Exposing these rats to EE raised cell proliferation and improved cognition.[20] Hippocampal Aβ pathology is reduced in 5xFAD mice housed in EE. Surprisingly, the mice have shown significant improvement in cell proliferation and adult neurogenesis in the DG of the hippocampus.[21] EE deterred the age-related decline in spatial memory, where the rodents were tested at different time points of age up to 20 months (young, middle-aged, and aged). The spatial memory was preserved throughout life by active levels of VEGF, which in turn increased neurogenesis.[22]

SOCIAL BEHAVIOR

Adult neurogenesis has increased following chronic sexual experience in male rats.[23] Juvenile rats (22 days old) socially isolated by individually housing them in the cages for 4 or 8 weeks have shown reduced neurogenesis compared to group-housed rats. However, the rats isolated for the first 4 weeks followed by group housing them for the next 4 weeks have shown more BrdU-positive cells suggestive of group housing, increased neurogenesis in DG of those rats experienced the previous social isolation.[24] Similarly, adult female prairie voles (90–120 days of age) exposed to social isolation for 6 weeks have shown a reduction in adult

neurogenesis, cell survival, and neuronal differentiation in the DG of hippocampus.[25] C57BL/6 mice subjected to chronic social defeat stress (by introducing them individually in the home cage of aggressive CD1 mouse), and decreased the survival of adult-born dentate granule cells (DGCs). However, it promotes synaptic maturation of adult-born DGCs.[26] Early maternal separation suppressed adult neurogenesis and reduced plasticity in rats.[27] However, the consequences of paternal separation on adult neurogenesis in offsprings were inconspicuous. He et al. (2018) has shown that the paternal deprivation decreased the BrdU/DCX positive cells in DG of mandarin vole's indicative of reduced neurogenesis.[28] Social isolation of mice during adolescence reduced the hippocampal neurogenesis. However, the exercise paradigm overcomes the deficit in neurogenesis.[29]

2. Review of status of Research and Development in the subject

Lee JS, Kim HG, Lee HW, Han JM, Lee SK, Kim DW, et al. Hippocampal memory enhancing activity of pine needle extract against scopolamine-induced amnesia in a mouse model. Sci Rep 2015;5:1-10.

Phytochemicals such as Glehnia littoralis, grape seed extract, platycodon grandiflorum, and pine needle extract were found to increase the cell proliferation and neuroblast differentiation in SGZ of DG as shown by an increase in Ki67 and DCX positive cells in rodents.[50-53] Out of these phytochemicals, Glehnia littoralis and pine needle extract shown an increase in BDNF expression.[50,53] These shreds of evidence suggest that the hippocampal adult neurogenesis is increased by the administration of phytochemicals in rodents.

2.1. International Status:

Neurogenesis is an endogenous process that involves coordinated proliferation, differentiation, and migration of neural precursor cells [1]. It determines brain formation during embryonic development and persists in localized regions of the adult brain or neurogenic niches. As a result of aging, brain injury, and genetic mutations, the progressive loss of structure, function, and depletion of neural precursors may contribute to neurodegenerative disorders including Alzheimer's disease (AD) and Parkinson's diseases (PD) [2]. For years, the occurrence of neurogenesis in the adult brain and the capacity to generate new neurons has been debated [3, 4], however, a number of studies have provided clear evidence of neurogenesis in the subgranular zone (SGZ) and subventricular zone (SVZ) of the adult brain [1, 5]. At the SVZ, neural stem cells (NSCs) migrate along the rostral migratory stream (RMS) and differentiate into interneurons in the olfactory bulb (OB). NSCs located in the SGZ give rise to granular neurons that integrate into functional circuits in the hippocampus [6]. Studies have revealed important determinants that enhance neurogenesis in the adult brain. These determinants broadly include intrinsic and extrinsic factors. The intrinsic factors include neurotrophic factors [7], transcriptional programs [8], inflammatory cytokines [9], neurotransmitters and hormones [10]. The extrinsic factors include physical activity [10], dietary intake [11] and stem cell

transplantation [12]. This review will discuss our current state of understanding of how these factors regulate adult neurogenesis and their potential application towards neurorestorative approaches.

2.2. National Status:

NIL

3. Progress/ achievement so far, if any

a) Reference papers was collected.
b) Literature survey was studied.
c). Materials and methods were designed

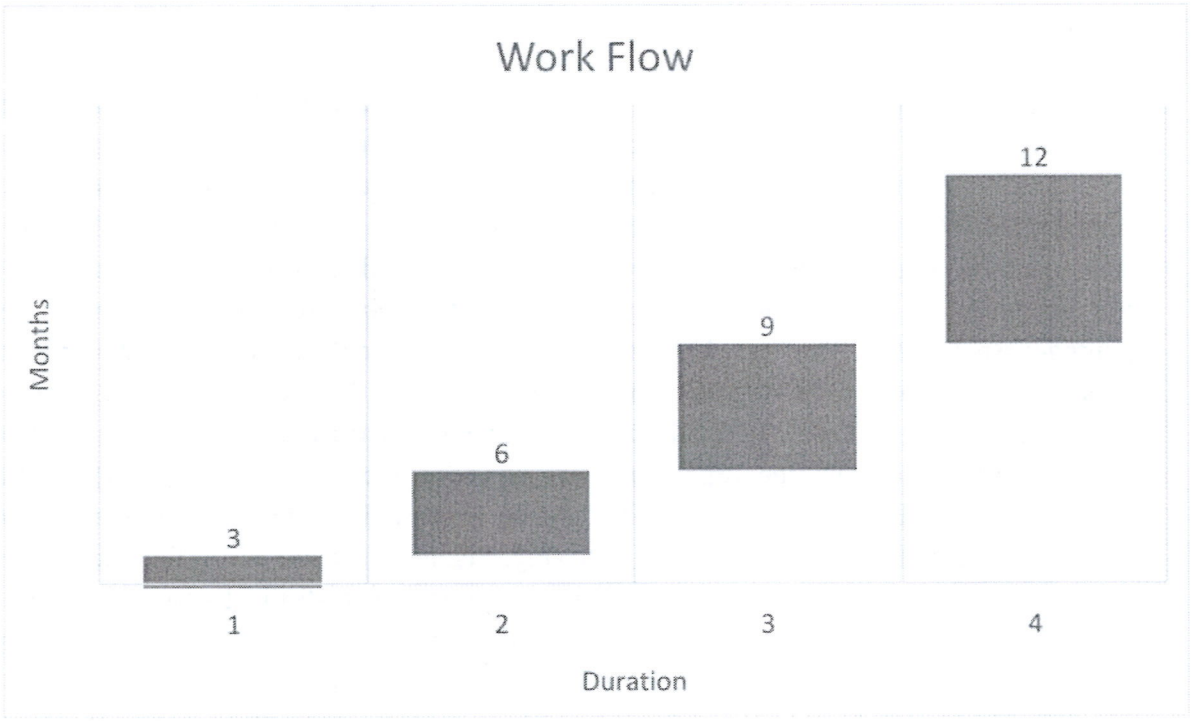
4. Work plan

4.1 Methodology

At the transcriptional level, transcription factors (TFs) regulate the expression of regulatory proteins that play an essential role in promoting adult neurogenesis. Under normal conditions, TFs orchestrate both the proliferation and the differentiation of NSCs into either neuronal or glial lineages [8]. They control the self-renewal of type 1 NSCs in SGZ (Fig. Fig.1b1b) and type B NSCs in SVZ (Fig. Fig.1b1b) which develop into type 2 cells and type C cells, respectively and the sequence and expression profile of TFs essential for NSC fate determination in adult neurogenesis [60, 61]. The best characterized TFs that have defined functions in regulating adult neurogenesis include: SRY-related high-mobility-group box 2 (Sox2), paired box gene 6 (Pax6), T-box brain gene 2 (Tbr2), RE1 silencing transcription factor (REST), achate-schute complex homolog-like 1 (Ascl1), the orphan nuclear hormone receptor tailless (TLX), cyclic AMP response element-binding protein (CREB), and neurogenic differentiation 1 (NeuroD1) [62–69]. Microglia in the alternatively-activated state (Fig. Fig.4c4c) release anti-inflammatory cytokines such as IL-4 and IL-10 as well as transforming growth factor-β (TGF- β), IGF-1 and BDNF, which in turn enhance neurogenesis [91, 98, 106]. Several studies reported that these anti-inflammatory cytokines mediate neuronal differentiation, migration and ultimately neurogenesis [91, 98]. The ectopic expression of the anti-inflammatory cytokine TGF- β via adenoviral vector delivery into the SVZ increased BrdU- and doublecortin-positive cells indicating an enhancement in neurogenesis [107]. Similar findings were seen in neuroprogenitor cell cultures when co-cultured with microglia stimulated with IL-4 [91]. Interestingly, the expression of IGF-1, which has a well-known role in neurogenesis, from microglia was found to increase after being stimulated with IL-4 [108].

Time Schedule of activities giving milestones through BAR diagram. (Maximum of 1/2 pages)

S. No	Activity/ mile stolen	1st Year			
1	Literature review	**1-3 month**			
2	Analysis of existing work	-	**4-6month**		
3	Designing & work initiated	-	-	**7-9 month**	
4	Statistics & Discussion with results	-	-	-	**10-12**



4.2 Expected outcome within the time period of See Money Scheme

There are many therapeutic interventions for treating patients with learning and memory deficits. The role of adult neurogenesis has not yet been studied extensively. The extrinsic factors such as EE, social behavior, exercise, yoga, and phytochemicals will be of great use in the treatment of patients suffering memory deficits associated with hippocampus.

5. Suggested Plan of action stating the name of funding agency where the project will be communicated for financial support within the time period of project.

Nil

6. Bibliography: Nil

Nil

7. List of Projects submitted/implemented by the Investigators (Separate for Pi and Co-PI)

7.1 Details of Projects submitted to various funding agencies:

S.No	Title	Cost in Lakhs	Month of Submission	Role as PI/Co-PI	Agency	Status
	NA	NA	NA	NA	NA	NA

7.2 Details of Projects under implementation

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
	NA NA	NA	NA	NA	NA

7.3 Details of Projects completed during the last 5 years

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
	NA	NA NA	NA	NA	NA

8. List of publications published by the Investigators, if any:

a) Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (issue)	Page no.	Year
1.	Hena Chandran, Jayanthi K., Prabavathy S., Renuka K. and **Rajesh Bhargavan**	Effectiveness of video assisted teaching on knowledge, attitude and practice among primary caregivers of children with autism spectrum disorder	Advances in Autism			2019
2.	Arivalagan Arunkumar, **Bhargavan Rajesh,** V. Tamilalagan	Variations among foramen transversarium in cervical vertebrae and its clinical significance	Indian Journal of Anatomy	7(2)	144-148	2018
3.	Mary Hydrina D'Silva1, Rijied Thompson Swer2, J. Anbalagan3, **Bhargavan Rajesh 4**	Effect of Radiofrequency Radiation Emitted from 2G and 3G Cell Phone on Developing Liver of Chick Embryo – A Comparative Study	Journal of Clinical and Diagnostic Research	11(7)	AC05 - AC09	2017
4.	M. Senthil Murugan,[1,*] R. Sudha,[1] and **Rajesh Bhargavan[2]**	Clinical Significance of an Unusual Variation Anomalous additional belly of the sternothyroid	Sultan Qaboos University Med J,	16(4)	e491–494,	2016

		muscle				
5.	Mary Hydrina D'Silva,1 Rijied Thompson Swer,1 J. Anbalagan,1 and **Rajesh Bhargavan2**	Effect of Ultrahigh Frequency Radiation Emitted from 2G Cell Phone on Developing Lens of Chick Embryo: A Histological Study	Advances in Anatomy	10(2)	1-9	2014
6.	Vasudev Anand Rao, Subashini Kaliaperumal, Thanikachalam Subramanyan, Kotapalli Rachandra Rao, **Rajesh Bhargavan**	Goldenhar's sequence with associated juvenile Glaucoma in turner's syndrome	Indian Journal Of Ophthalmology	53(4)	267-268	2005

b). Co-Principal Investigator

S.No	Author names	Title of paper	Name of Journal	Vol (Issue)	Page No.	Year
1	**Anandaramajayan Nallathambi1,** Rajesh Bhargavan2	GC/MS Analysis of Bioactive Compounds in Aqueous Extract of Cynodon Dactylon	Indian Journal of Public Health Research & Development,	10(12)	55-59	2019
2.	Vijisha Phalgunan, **Anandaramajayan Nallathambi**	A Study on Bifurcation of Brachial Artery in South Indian Population (Tamil Nadu and Puducherry)	Indian Journal of Anatomy	7(1)	73-78	2018
3.	N.**Anandaramajayan***, K.C.Mallikarjuna.	Fused Typical Cervical Vertebra – A Case Report	Journal of Current Trends in Clinical Medicine & Laboratory Biochemistry	2(4)	64-66	2015
4.	N.**Anandaramajayan***, B.Rajesh.	Unilateral Renal Agenesis with variations in the vascular pattern of Testis, Supra Renal Gland and	Journal of Current Trends in Clinical Medicine &	2(2)	66-72	2014

		Diaphragm -A Case Report	Laboratory Biochemistry			
5.	B. Rajesh*, 2N. **Anandaramajayan**, V.Santhi, K.C. Mallikarjuna,S.I. Tolanur, R. Praveen Kumar	An abnormal radicle of Median Nerve from Musculocutaneous nerve in the Arm	Journal of Current Trends in Clinical Medicine & Laboratory Biochemistry	1(2)	34-36	2013

9. Budget

Sl. No	**Head**	**Amount in INR**
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	50000
2	Consumables (gels bottles, cotton, sprit, testing charges, tools, etc.)	5000
3	Travel support for the purpose of research work.	10000
4	Contingency	25000
5	Others consumables	10000
	Total	1,00,000

*In case of any joint proposal for purchasing a same equipment, each of the associated PLs is also required to give separate budget (without any clubbing) to avoid any ambiguity, if all the associated projects are not awarded by committee.

10. Name of at least two subject experts from the Institute and one from the outside Institute with their contact details:

1. **Dr. M. Sivakumar** Professor Dept. of Anatomy JIPMER, Puducherry **Mobile No**: 9994264019 **E-mail id:** sivakumar96@yahoo.com	2. **Dr. J Anbalagan** Professor of Anatomy Mahatma Gandhi Medical College and Research Institute, Pondicherry **Mobile No**: 9443500366 **E-mail id:** jayaramanbalagan@gmail.com

CERTIFICATE FROM THE INVESTIGATOR

Project Title: Extrinsic factors in regulation of adult hippocampal neurogenesis

It is certified that

1. I do hereby agree to submit a complete proposal for financial support to the external funding agency within the time period of SMS-2018.
2. I undertake that spare time on equipment procured in the project will be made available to other users.
3. I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in to.
4. I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involved field trails/experiments/exchange of specimens, human & animal materials etc.
5. I agree to abide by the terms and conditions of SMS-2018, BIHER, and Chennai.

Name and signature of
Principal Investigator

Name and signature of
Co-Principal Investigator

Date: 19.01.2019
Place: Pondicherry

Forwarded by Head of the Department

Signature of the Head

DEAN
SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSUDU, AGARAM VILLAGE,
KOODAPAKKAM POST,
PUDUCHERRY - 605 502

PROJECT EVALUATION FORMAT

Recommendation sheet

Name of the Principal Investigator	Dr. B Rajesh
Name of the Co-Principal Investigator	N.Anandaramajayan
Name of the Department	Anatomy
Title of project	Extrinsic factors in regulation of adult hippocampal neurogenesis
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	Recommended
Financial allocation recommended	Rs. 1,00,000/-

SI. No.	Head	Amount
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	50000
2	Consumables- Gel bottles, cotton, sprit, testing charges, tools, etc.	5000
3	Travel support for the purpose of research work.	10000
4	Contingency	25000
5	Others consumables	10000
	Total	**1,00,000**

Name and Signature of the Research Advisory Committee members with date

Internal Quality Assurance Cell * IQAC * Pondicherry-02 * SLIMS *

(Dr. G. Jayalakshmi).