

NATIONAL INSTITUTE OF MENTAL HEALTH AND NEURO SCIENCES, BENGALURU

राष्ट्रीय मानसिक स्वास्थ्य एवं तंत्रिका विज्ञान संस्थान, बेंगलुरु

ರಾಷಟರೀಯ ಮಾನಸೆಕ ಆರೋಗಯ ಮತತು ನರ ವೆಜಞಾನ ಸಂಸಥೆ, ಬೆಂಗಳೂರು Institute of National Importance । राष्ट्रीय महत्व का संस्थान । ರಾಷಟೆರೀಯ ಮಹತವದ ಸಂಸ

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Department of Biophysics

27 September 2021

TO WHOMSOEVER IT MAY CONCERN

I, Dr. Indrani Datta, Associate Professor, Department of Biophysics, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, Karnataka would like to certify that Aishwarya Raj is a PhD student under my guidance in the Department of Biophysics, NIMHANS. She had joined on July 02, 2018 and bears the student ID number - PhBps201803. This is also to certify that the research work that she has submitted for the Sun Pharma Science Foundation Science Scholar Award was done by her solely.

This work is titled, "Effect of monomeric and aggregated wild type and A30P/A53T double mutant α-synuclein on anti-oxidant mechanism and glutamate metabolic profile of cultured astrocytes". The work aims to assess the effect of various forms of α-synuclein on anti-oxidantmechanism and glutamate-metabolism.

The work involves use of purified primary rat astrocyte culture, isolated from rat pup (post-natal day 2-4) midbrain. Astroglial biology has to be studied in the rat-midbrain astrocyte cultures upon extracellular exposure of Wild-type and A53T/A30P double-mutant α-synuclein, both in monomeric and aggregated-forms (α-synuclein forms characterized by spectrofluorimetry, dynamic light scattering and atomic force microscopy). α-synuclein in its different forms was fluorescently tagged and its engulfment and/or association with the astrocytes (GFAP immunopositive cells) was quantified using flow cytometry and visualized using fluorescence microscopy. Survival was assessed by MTT assay followed by its detection using spectrophotometry; fluoroprobes were used for detection of oxidative and nitrative-stress and measured using spectrofluorimetry and flow cytometry respectively; glutathione and glutamatecontent by spectrophotometry; expression of enzymes associated with oxidative-stress and glutamate metabolism, glutamate and glutathione transporters assessed by qPCR, flow cytometry, immunofluorescence and live cell imaging. Indrani Datta

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