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Quantification of Neurons of Human Nucleus Accumbens by Fractal Analysis

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ABSTRACT

Background: Analysis of volumetric and morphological neuronal data has been of keen interest to neurologists and neuroscientists because of its implications in pathological conditions such as schizophrenia, autism, obsessive compulsive disorder etc. One such part of human brain which has been explored in recent years is nucleus accumbens, a part of ventral striatum leaning against septal nuclei. An easier, freely accessible and cost effective technique to measure neurons of nucleus accumbens is the use of Image J -Fiji software. One of the applications of software is Fractal box analysis. This technique helps in analysis of the Euclidean geometry of neurons (Parameters such as length and breadth which are not good characteristics of multipolar neurons). Aim and Objectives: The present study was undertaken to study and analyze images of morphology of neurons of nucleus accumbens using Image J as an automated image analysis technique. **Methods & Results:** A qualitative cross sectional study was done using fifty five serial sections of nucleus accumbens. The 4 µ tissue sections were stained with hematoxylin and eosin. Freely downloadable Image J software was installed, images of serial sections were imported to Image J, processed and fractal box analysis was done. Fractal Box analysis of image of neurons of nucleus accumbens revealed statistically significant value (D= 1.99). **Conclusion:** Results of the present study can be extrapolated to correlate with pathological conditions associated with emotional and behavioral disorders involving nucleus accumbens Image J is cost effective software which is beneficial to identify and measure neurons of Nucleus accumbens.

Keywords: *Nucleus Accumbens, Fractals, Image Fiji*

INTRODUCTION

The component of basal ganglia bridging ventral part of caudate nucleus and globuspallidus caudal to anterior limb of internal capsule is known as Fundus striati. Nucleus accumbens is an integral part of fundus striate.[1] Nucleus Accumbens is a part of ventral striatum. As a subdivision of basal ganglia, it iswell known among neurological studies for emotion, planned motor activities related to food and drug reward, motivation, substance abuse, sexual arousal, stress response and neuropsychiatric disorders. Theodor Meynert, an Austrian psychiatrist in 1872 coined the term Nucleus accumbens meaning "Nucleus leaning against the septum". It is said to serve as Limbic – motor interface, an area where neuronal fibers of limbic system gain access to motor system. Mogenson in 1980 proposed the feature that major inputs for nucleus accumbens is from amygdala and hypothalamus. The former in turn projects to globuspallidus thence to ventrolateral motor nucleus of thalamus, somato-motor area of cerebral cortex as well as brain stem motor nuclei.[2,3]

Histologically, nucleus accumbens consists of 95% of GABAergic neurons and 5% cholinergic neurons. GABAergic neurons are medium spiny neurons.[4] Nucleus accumbens is divided by two different sets of criteria: 1) Mosaic arrangement of patch matrix organization. The patches are dense µ- opiate receptor

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binding sites and matrix has high acetyl cholinesterase activity, strong calcium binding protein immunoreactivity and rich plexus of somatostatin fibers.2) Core and shell compartments is anatomical and morphological classification. Core has pyramidal like neuron with spines on secondary branches and shell has fusiform and multipolar neurons.[5]

Need for the present study

The main drive for histological assessment of nucleus accumbens emanated from the requirement of a robust and costeffective method to explore anatomy of nucleus accumbens so that the latter can be considered for deep brain stimulation in various disorders such as Tourett's syndrome, obsessive compulsive disorder, depression, obsessive compulsive disorder and Alzheimer's disease.[6] Modern neurohistological research is dependent on use of sophisticated cellular, molecular laboratories and digital imaging methods. A resource poor setting demands a simple, fast and cost effective way of neuroscience research. One such easier method is fractal analysis using Image J software. Nucleus accumbens can be easily explored using its histological sections, images can be processed and analyzed in a cost effective manner.[7,8]

Overview of Image J software

Image J is public domain image processing and analyzing software. It is free open source software for automated image analysis. Image J, a Java image processing and analysis program is inspired by NIH Image for the Macintosh .The application needs installation of Java 1.5 or later version.It can read image formats including TIFF, GIF, JPEG, BMP, DOCOM, FITS and raw. The downloadable distribution is available for Windows, Mac OS X and Linux. Uses of the application are to process images (display, edit, analyze, process, save and print 8- bit, 18- bit and 32- bit) and analyze image (calculate area and pixel value statistics, measure distances and angles, create density histograms). Fiji is a distribution of Image J together with Java. The main focus of Fiji is on life sciences wherein image stitching, segmentation, feature extraction and 3D visualization can be done.[9,10]

What is Fractal analysis?

Fractal geometry is the field of mathematics which deals with analysis of irregular patterns made of elements that are similar to each other. Benoit B Mandelbrot (1924 -2010) developed fractal theory concept, also termed the term fractal meaning to break or fragment. In relation to neurons, fractal dimension refers to statistical measure of neurons that correlates with morphological and structural complexity.[11] This complexity can be quantified by Fractal dimension value(FD). FD value of a neuron indicates how densely the neuronal branching pattern occupies a portion of metric space in which it is embedded. This infers that we can obtain quantitative information about how a particular neuron occupies a certain portion of space.[12,13]

Aim and Objectives

Aim of the present study was to explore morphology of neurons of nucleus accumbens. Objective was to analyze neuronal structure using fractal analysis of Image J software.

MATERIALS AND METHODS

Methodology

A cross- sectional / descriptive study design was taken for quantification of neurons of nucleus accumbens for a period of eight months (February 2017 – September 2017) at department of anatomy at our institution.

Ethical Considerations: Image J being open source public domain software has four essential freedoms[8]

1. To run the program, for any purpose,
2. To study how the program works and change it to make it do what we wish
3. To redistribute copies so that our neighbor can be helped and lastly
4. To improve program and release program to public

Sample

Five formalized human brain specimens used for routine educational dissection were chosen for pilot study to determine the sample size.

Inclusion Criteria

Adult human formalized brains were considered for the study. For analysis of images, Image J software Fiji version was chosen as it can process bio formats easily and analysis of nervous tissue would be easier.

Exclusive criteria[9]

Human brains with distorted cerebral hemispheres, foetal and infant brains were excluded from the study. There are many freely accessible softwares which make biological imaging cost effective such as TIMWIN downloadable from Garbo, SIGMASCAN

downloadable from SYSTAT, Image Pro Plus downloadable from Media Cybernetics, DA Cell Counter from Yamato. These softwares were not considered as they were not modular. They involve complex steps for image processing and analysis.

Frontal / coronal section of the selected brain specimens was done at the level of optic chiasma. Nucleus Accumbens was identified as grey matter of brain tissue (Fundus striati) lateral to anterior horn of lateral ventricle, caudal to anterior limb of internal capsule connecting caudate nucleus and globuspallidus [Figure 1].[14]

Three cubic centimeter tissue block from all the five brain specimens were dissected and processed, paraffin embedded and 4 micrometer thick sections were made using microtome. These tissue sections were brought to water, stained with hematoxylin and eosin stain and mounted with DPX. Fifth paraffin tissue block was chosen for serial sections after examination of all the sections of all tissue blocks, as features were well demonstrated from fifth block. Fifty five serial sections were taken from fifth tissue block. The photographs of nucleus accumbens were obtained using Labomed microscope under 40 x objective and saved in JPG/ JPEG (Joint Photographic Expert Group) format in the SD(Secure digital)memory card.[Figure 2]

The images were transferred to Laptop into a folder and analysed using Image J software.

Image J software was downloaded and installed from the website: http://imagej.nih.gov/ij/download.html.

Image J application installation[7]

Images of nucleus accumbens to be analyzed wereimported to Image J window and processed.

Prerequisites for Fractal analysis are: - Image must be 8 bit, binary (black and white images, wherein objects are black and background is white). Hence images were processed for 8 bit type (3366x 2255 pixels) and binary was made.A binary image is one consisting of only two pixel values, usually black (0) and white (1). The most subjective step in this process is deciding which parts of the original image will contribute to the binary image. Grid was applied for binary image and was further analyzed by fractal box method. [Figure 3,4]

RESULTS

The histological section of slide of nucleus accumbens showed collection of varied shapes and sizes of neurons. Most of the neurons were pyramidal, fusiform, few were ovoid and angular. They could be identified as neurons because their nuclei were heterochromaticand nucleolus was well defined on the contrary neuroglia appeared as small sized, euchromatic cells. The serial 4 micron thick sections displayed collection of neurons upto 49th section suggesting the approximate depth of nucleus accumbens must be 196 microns/ 1.96 mm.

Results of Fractal analysis[11]

Fractal dimension of neurons of nucleus accumbens were calculated in three main forms [Figure 5-7]

a. Whole binary image(black and white)- measured space filling property of neuron
b. Binary outlined image- assessed irregularity of shape of neuron
c. Binary skeletonized image – helped in calculating FD of dendrites and to summarize degree of dendritic aberrationsfrom straight lines

In fractal analysis, D is the parameter that describes the relationship between size of neuron and measuring scale. In the present study, fractal dimension “D“value was 1.99.“D” quantifies variation in length, area, volume with changes in the measuring scale. It is an indicator of how space filling a structure is. The fractional part of exponent indicates the complexity of the neuron. Objects such as a ball can be approximated to earth because of similarity and characteristic diameter. This infers that if the surface of earth becomes smoother, magnification will be decreased. Fractal objects cannot be represented by any combination of shapes and length. For certain structures such as neurons whose diameter and length are complex measurements, Euclidean geometry (diameter or length of neurons as not good descriptors of complexity), suffice its characteristics. So, special features of fractal objects are characteristic length, self- similarity and complexity. Neuronal tissue obeys these laws of fractal analysis. Complexity can be attributed to neuronal surface irregularity and intricate branching pattern.[12]

If the fractional part of the exponent is 0, D would be equal to the Euclidean dimension for a line. If the line becomes more space filling, the fractional part of the exponent increases towards 2.[12,13] Fractal dimension is indicator of how space filling the neuron. A dimension of 1.4 would indicate less space filling compared to 1.8. In the present case, a value of 1.9946 infers that neurons are statistically more space filling. The plot description is illustrated below. [Figure 8] The log of size on X- axis and log count of Y- axis, the data fitted on a straight line.

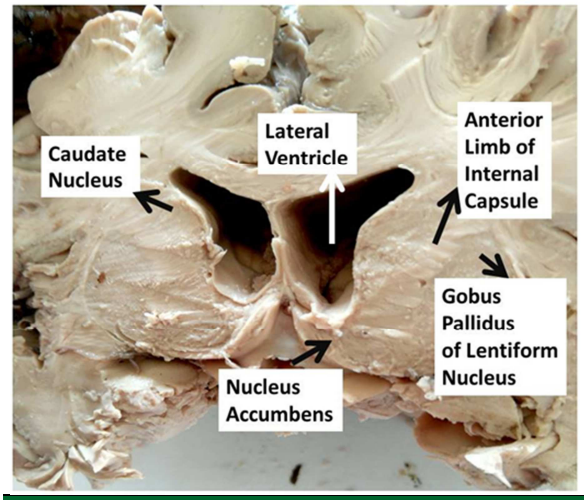

Figure 1: Coronal section of human brain at the level of Optic chiasma to locate Nucleus Accumbens

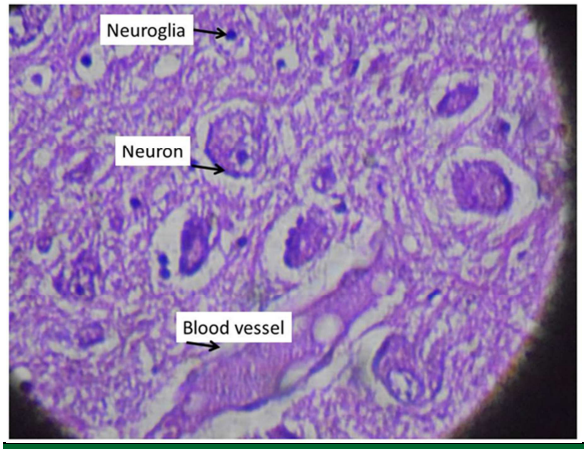

Figure 2: Hematoxylin and eosin stained 4 µ thick section of Nucleus accumbens

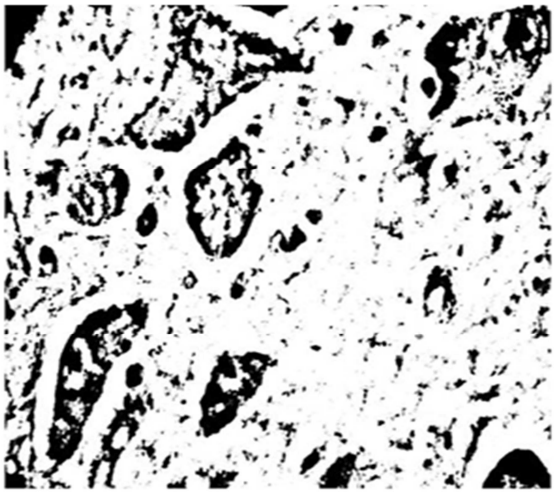

Figure 3: Binary (Black and white) image

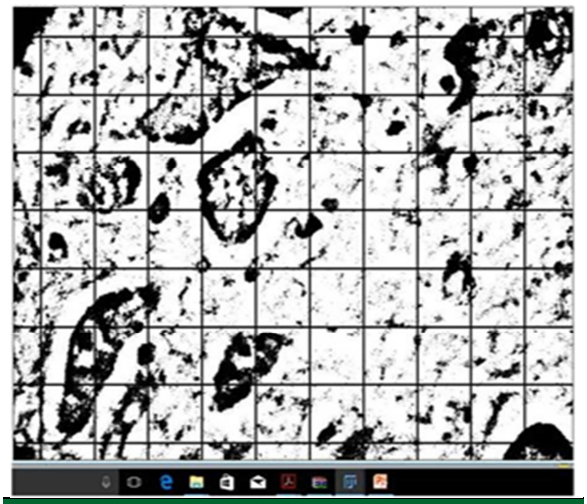

Fig 4: Binary image with grid

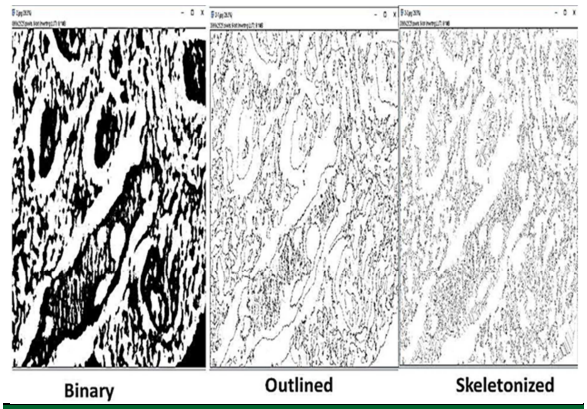

Figure 5,6,7: Binary, Outlined and skeletonized formats of Image of section of nucleus accumbens

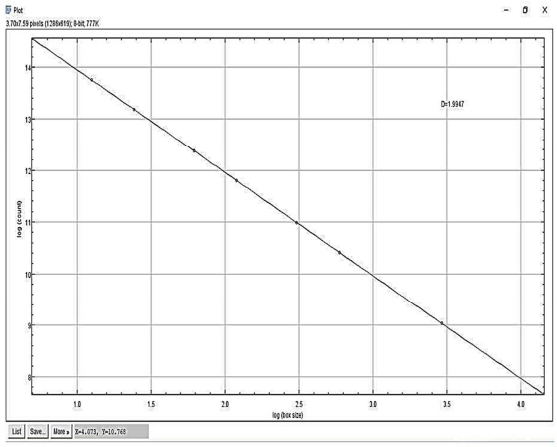

Figure 8: Graph 1: Log-log plot between the numbers of squares (N) in y axis and square size (r) in x axis, fitted by a straight line. The fractal dimension D is calculated from the slope of the straight line. R is the corresponding correlation coefficient. Fractal dimension value is 1.9947.

DISCUSSION

Counting and analysis of histological sections of neural tissue using automated computational methods has greatly reduced observational/ manual errors. Neurons can be easily counted by counting pixels in spatial regions specified by the user in image analysis module or by analyzing color domain.[15]

There are fractal analytical tools available for estimation of relationship between scale of measurement and size/ mass of an object such as box counting, dilation, mass-radius as well as caliper method. Boxcounting or cumulative mass method is one of the conventional methods considered in the present study. The neurons would cover boxes at different scales. The boundary lines are statistically self-similar and can be analyzed characterizing the surface of neuron. This method establishes logarithmic relationship between scale and mass of neuron. The present study of nucleus accumbens relied on fractal analysis of neurons by box counting method for the same reason.[16]

The linearity of the plot infers selection of the measuring object. In the present case, D value was 1.99. This infers that the size of the box area was smaller than the line width of the neuron, meaning we were measuring area of neuron and the slope.[12]

Besides analysis of dimensions of neurons of histological sections, fractal analysis has been used in wide arrays of medical field. Studies have indicated that fractal analysis can be applied to study changes in branching pattern and density of retinal blood vessels. It has been recorded that fractal analysis of Magnetic resonance imaging of human cerebral cortex demonstrates fractal like organization which is also reflection of present study. Analysis of apical dendritic arborisation of pyramidal neurons of rat cerebral cortex showed that pyramidal neurons from lamina II-III had higher fractal dimension(FD) than lamina V.[16]

Clinical Significance

Application of fractal analysis in the field of neuroanatomical research is not only limited to neurons and glial cells. Variations of Amyloid deposits, criteria for diagnosis of Alzheimer's disease can be analyzed especially to differentiate various types of plaques. Histopathogical slides of Glioma's when analyzed by fractal method has shown that Grade 2 Glioma was more vascularized than Grade 3 Glioma 3.[15]

Fractal analysis would pave way for understating of molecular and cellular alteration in neurons of nucleus accumbens particularly medium spiny neurons. Four types of neurons were identified when morphology of neurons of humannucleus accumbens was assessed using Immunohistochemical expression of GAD 67 namely: Type 1- fusiform neurons, Type 2- fusiform neurons with lateral dendrites, Type 3- pyramidal neurons and Type 4- multipolar neurons. Our study could also demonstrate all these type of neurons with routine hematoxylin and eosin stained section processed for Image J software.[17]

Advantages and disadvantages of Image J software

The major advantage of Image J software is it's free access to installation. It is a standardized application so one can rely on statistically proven results. There is a provision for documentation of every step of application so that the latter can be easily reproducible.

On the contrary, the major disadvantage of Image J is slow start up. Though there are more than 360 built in Macro functions not all are usable. Knowledge of complex Image J and Java is required for effective analysis. There is no debugger and support for batch mode.[7]

Way forward

The present study focused on analyzing morphology of neurons of nucleus accumbens using fractal box analysis method of Image J software. Additional plugin of Image J software is Sholl analysis.[18] This plugin shall be installed and estimation of number of neurons in nucleus accumbens shall be taken up and comparison with other methods of neuronal number estimation shall be done in future.

As a next step of research, morphology of nucleus accumbens shall be extensively explored in various pathological conditions such as substance abuse, depressive psychosis, Tourett'ssyndrome. An attempt shall also be made to correlate findings of present study with functional magnetic resonance imaging of area of fundus striati particularly nucleus accumbens in normal individuals.

CONCLUSION

This simple automated image analysis of neurons of nucleus accumbens using fractal method gave an insight for better understanding of morphology of nucleus accumbens. Fractal analysis of neurons of nucleus accumbens resulted in a fractal dimension value of 1.99 indicating self-similarity. The present

study was a cost-effective, easily reproducible one as compared to expensive neurohistochemical techniques such as Golgi – Cox method and immunohistochemistry. Usage of freely downloadable version Of Image J Fiji is a boon to digital imaging in understanding various biological structures particularly analysis of structures with complexity such as neurons, retinal blood vessels, bronchial tree and renal vasculature.

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