Quantitative neurotoxicology: Potential role of artificial intelligence/deep learning approach

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Abstract
The quantification of neurotoxicity contributes to a better understanding of the global scope of brain injury and aids qualitative histological examination. Although the process takes time, stereological techniques, including the use of an optical fractionator, offer an objective measurement of the neuronal damage. With the introduction of whole slide imaging (WSI) and digital image processing, neurotoxicity could now be quantified automatically, more quickly, and with less bias, enabling statistical comparisons. Simple digital picture analysis requires the manual labour of professionals, even though it can be automated to some extent. This limits the analysis of huge datasets and takes time. A deep learning artificial intelligence model combined with digital image analysis offers a viable substitute for laborious stereological and basic digital analysis. Deep learning algorithms could be trained to automatically recognise damaged or dead neurons. The works that show how deep learning is employed in brain region segmentation, toxicity detection, neuronal degeneration quantification, and area/volume estimation of degeneration have been the main emphasis of this paper.

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1. Introduction

Models of deep learning neural networks are taught or trained to do particular computations. This method may be used to train larger artificial neural networks, which makes them highly beneficial for larger data sets.[1] In order to access representations of neural activity in the brain, researchers are increasingly using the deep learning approach these days, working with behavioural and neurophysiological data. [2] Our ability to evaluate bigger data sets has been hampered by academics' use of deep learning techniques to automate tasks that formerly required human labour as their popularity and efficacy have grown. [3] The three types of neural networks used in deep learning are given in (Figure 1). [4] Due to its inexpensive computational cost and wide range of applications, including drug design and discovery, image identification, classification, and segmentation, CNNs have gained popularity in recent years.

In order to identify distinct features in the input image, filters, also known as kernels, are introduced before to the convolutional procedure. [5] The CNN is provided parameters like number and filter size. The CNN can learn filter values through training.[6] Digital image analysis is a topic that is now paying more and more attention to deep learning techniques. [7] Using various classification processes where deep learning models are developed, deep learning-based digital image analysis adds classifiers to identify items in a picture.

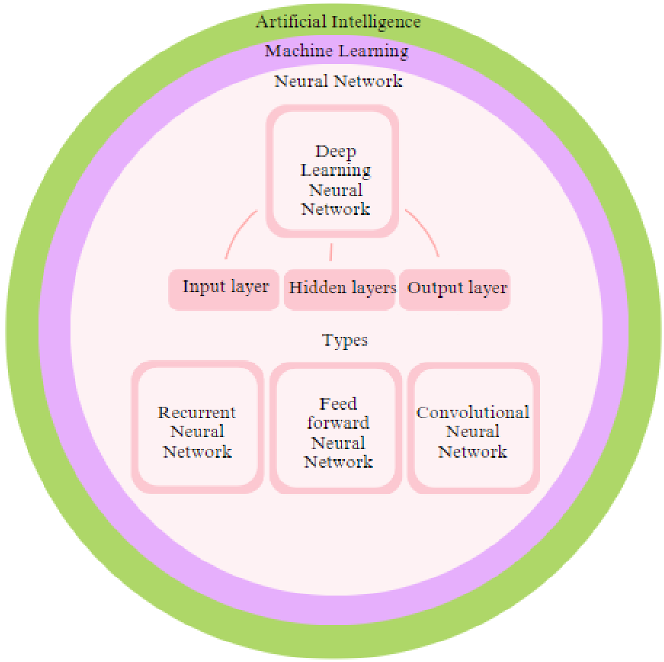

Figure 1: diagram illustrating the convoluted neural network (CNN). CNN is depicted in Figure (A) as a machine learning subsystem, which is a subsystem of artificial intelligence.

[8] Then, using these trained models, objects are automatically extracted from similar types of photos. (Figure 2) Histopathological sections from cancer, toxicology, and other fields have been the focus of the majority of deep learning efforts in digital image processing.

2. Quantitative Neurotoxicity

Numerous investigations involving the quantification of histopathological findings have been carried out. [9] Studies on neurotoxicity are a crucial component of the rules governing the development of new drugs. Clinical trials are preceded by safety and efficacy investigations. [10] Pathologists who have earned board certification typically perform neurotoxicity evaluations in the preclinical stages. [11] Using a 4- or 5-point grading system, pathologists assess brain slices to determine the degree of toxicity. The gold standard is a histopathological assessment of this kind, but it is primarily qualitative or semiquantitative and requires a substantial amount of time.

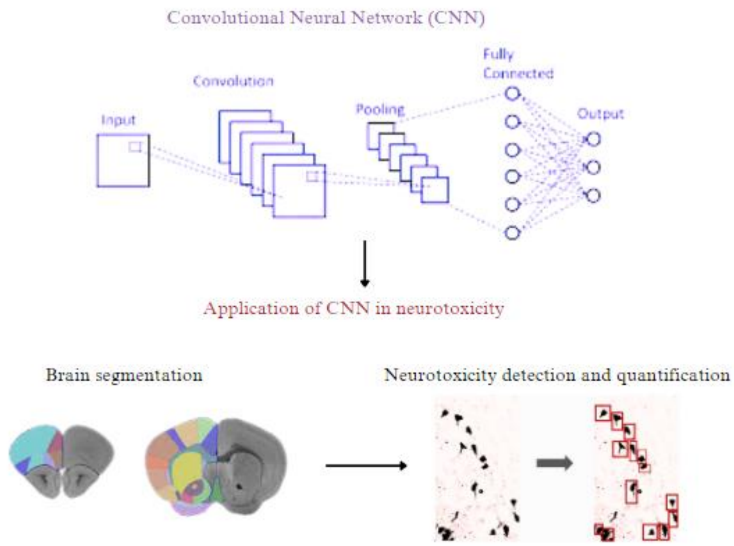

Figure 2: The upper panel schematically depicts the pooling and convolutional layers that connect the input and output images in a standard CNN. CNN's function in brain segmentation, neuroxicity analysis, and detection is seen in the lower panel.

Quantifying neurotoxicity becomes crucial since the nervous system's toxicity is contingent upon multiple elements, including the toxic compound's dose, the animal's age and gender, and the length of time the animal is exposed to the dose. [12] A deeper comprehension of the harmful effects of substances and medications is possible through the quantification of toxicity. [13] Different regions of the brain are significantly more or less vulnerable to different toxic substances due to a variety of reasons, including variances in synaptic connections, oxidative stress, and nonuniform receptor distribution.

Furthermore, obtaining a global toxicity value is a far more potent strategy than merely summarising qualitative changes in various domains, as the cumulative impact of numerous minor alterations, or global effects, plays a major role in assessing the total amount of extensive brain damage. [14] It offers information for more accurate statistical comparison of harmful effects, which bolsters pathologists' conventional histopathological toxicity studies.

Measuring the amount of brain degeneration and/or the total number of degenerated neurons in the brain following exposure to hazardous substances are the primary methods used to quantify toxicity. The brain's deteriorated areas and neurons can be seen using staining techniques like amino cupric silver (AmCuAg) staining. [15] The development of digital image processing technologies has made it possible to measure neurotoxicity in the brains of animals treated with different neurotoxic chemicals and in neurodegenerative diseases in an effective manner. [16] Although several phases in the workflow for the assessment of toxicity have been automated by image analysis tools (Figure 3), there are still constraints that make the process labor-intensive.

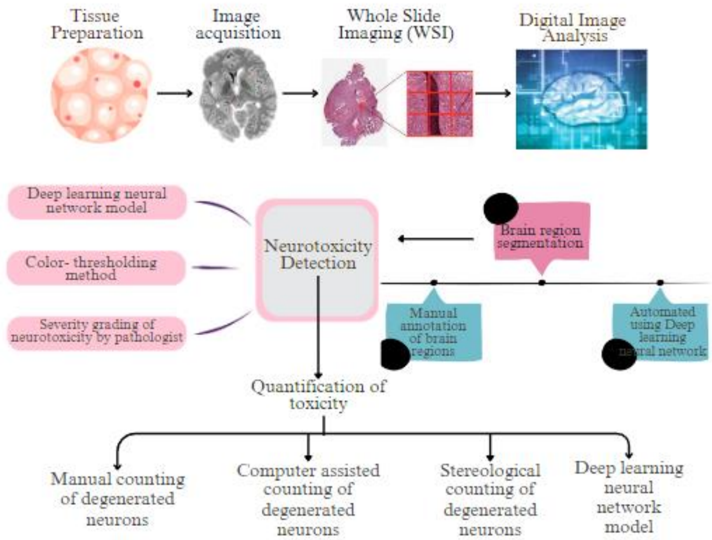

Figure 3: Workflow for neurotoxicological analysis.

In order to create high quality digital images of the sections, whole slide imaging is the next step in the procedure after image acquisition of the stained sections. Digital photographs with high resolution are an excellent resource for additional analysis, which can be done manually or with the aid of image processing software that offers automated solutions. To detect and quantify neurotoxicity and gain a better knowledge of the level of toxicity and the vulnerability of different brain regions to neurotoxic compounds/drugs, neurotoxicity analysis entails segmenting the brain into separate brain regions. An update on the developments in deep learning for digital image analysis has been provided.

3. Automated Brain Segmentation into Different Regions using AI/Deep Learning

The manual process of marking brain sections' regions of interest for examination is one of the most laborious and time-consuming processes in the quantification of toxicity. In essence, it entails segmenting the brain into interest areas by the creation of annotation layers (Figure 3). Within the brain sections' regions of interest, annotation layers are manually drawn. It takes a long period, which restricts the investigation of the entire brain in terms of alterations in various brain regions. Additionally, the number of experimental animals that may be examined is limited due to the laborious manual technique.

Instead of concentrating only on areas of interest, (Figure 4) brain region segmentation will enable quantification of various areas independently, facilitating statistical comparisons of degeneration across the entire brain. The process takes too long for one person to complete by hand.

By doing this, the time required to segment the brain areas will be greatly decreased, and all of the animals in the segmented brain areas will be able to use the image processing method simultaneously. Although this is still an emerging field of study, researchers have been attempting to develop such segmentation algorithms using an automated AI approach. Deep CNN has been used in a number of research to automate image segmentation. An artificial neural network model called CNN has multiple hidden layers in addition to input and output layers. Convolutional layers make up CNN's hidden layers. Deep CNNs with multiple instance learning (MIL) have been used to segment and categories images (such as those of breast cancer), which included several channels with fluorescent markers.

The rat brain's coronal region is segmented, as seen in the schematic. The brain image has been manually annotated in the upper panel to demonstrate its segmentation into several brain regions. The bottom panel illustrates how brain segmentation could be automated using a deep learning approach. A carefully annotated input image is fed into a deep learning model, which is then trained. The model is fed similar images from other animals in order to segment it into distinct brain areas. Images segmented using a deep learning model cells data set are displayed in the output.

Image segmentation using deep neural networks (DNN) on pictures from light, X-ray, and electron microscopy is called CDeep3M. It is a ready-to-use cloud-based solution that uses an image segmentation neural network model. CNN training is used in the DeepBrainSeg framework to separate brain-wide areas. The study included mouse brain images obtained using a variety of imaging techniques, including serial two photon system, fluorescence microscopy, microoptical sectioning tomography, and MRI (T2*). Boundaries were manually drawn on images to create a training set, which was then used to train a neural network.

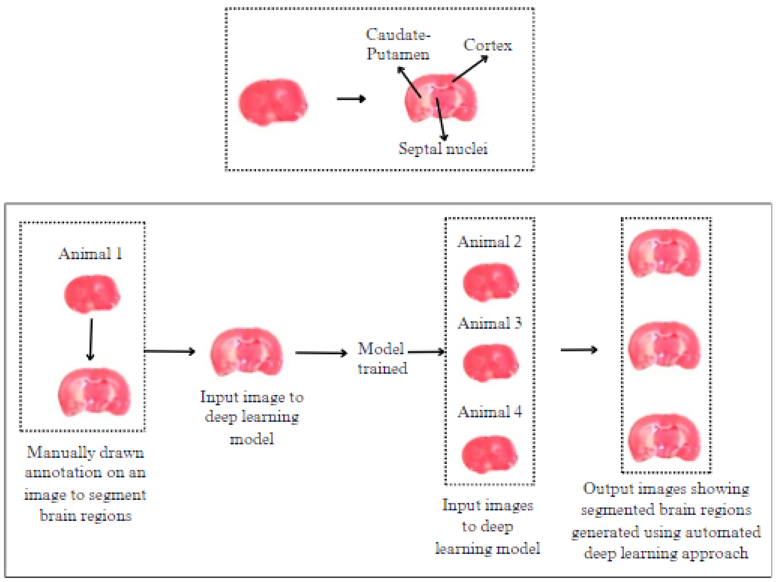

Figure 4: Schematic shows segmentation of a coronal section of the rat brain.

To obtain segmented regions, additional processing steps including registration and prediction by the trained neural network were carried out. A recent study used a deep neural network-based technique called SeBRe to automate mouse brain segmentation. An input image was obtained by labelling a mouse brain with a neuronal marker. Several brain regions were manually marked on the image. It was processed through multiple processing stages after being fed into a deep brain neural network model. After training, the model was applied to an analogous image of other animals. Segmented portions from the input image were used to create the output image. SeBRe was used on pictures containing several neural markers in order to gauge its effectiveness. SeBRe segmentation was used to identify the hippocampus's dentate gyrus and subregions including CA1, CA2, and CA3.

4. AI/Deep Learning for Automated Toxicity Detection and Analysis in Different Brain Regions

One of the most important steps in quantifying histopathology data is cell detection. For example, certain image processing programmes, like ImageJ (NIH, Maryland, USA), have tools that can identify structures with a certain hue and are used to identify degenerated neurons stained with silver that appear black in sections of the brain. There are drawbacks to this strategy. This approach also picks up artefacts like nonspecific staining and the remnants of dying neurons, which raises the overall estimated amount of degeneration in the brain sections. This constraint has a great deal of potential to be addressed by an AI approach. As demonstrated, deep learning techniques can be used to train models to identify degeneration neurons based on their size, colour, and shape.

In the rat cerebral ischemia-reperfusion paradigm, the Deep CNN model has been utilised to automatically identify neuronal injury. Two photon microscopy was used to create images of label-free brain sections, and a deep learning system was then used to identify damaged neurons in those images. Two photon microscopy and H&E images were used by pathologists to identify damaged neurons. To compare this method of detecting neuronal injury to conventional histology, brain sections were stained with NeuN and H&E. According to this work, label-free brain regions may automatically identify neuronal injury using deep learning applied to two photon pictures.

Table 1 Comparison of Deep Learning Methods

Method	Merits	Demerits
Dropout	Overfitting is avoided	Converge required to no.of iterations increases
Stochastic Gradient Descent	Minimum trapping local to avoid	Convergence timeis longer, computationally expensive
Skip-gram	Can work on any raw text, Less memoryis required.	Softmax function is computationally expensive, High Training Time
Transfer learning	The second problem is rapid progress in training	Only works with similar problems
Backpropagation	Calculation of gradient	noisy data is sensitive
Learning Rate Decay	Reduces training time, Increases performance,	Computationally expensive
Batch Normalization	Increases stability of the network, Reduces covariant shift	During training computational overhead
Max-Pooling	Computational cost and reduced dimension	Considers only the maximum element

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One potential addition to the AI approach for identifying deteriorated neurons is the exclusion of artefacts. Anxiety brought on by a neurotoxic chemical exposure triggers inflammatory reactions, including activated microglia. Finding activated microglia may be essential to understanding a compound's toxicity. Based on some fundamental characteristics, such as the quantity, area, and duration of the processes that differentiate activated microglia from nonactivated microglia, deep learning may be crucial in the automated identification of activated microglia. Analogously, it is possible to train a deep learning model to identify morphological alterations in astrocytes subsequent to exposure to harmful substances. Trained deep learning models could also identify degenerative alterations in white matter. A deep learning technique called DeNeRD—which identifies neurons in various brain regions during development—was used to do brain-wide analysis for neuron detection.

U-Net is another general deep learning-based method for cell segmentation, morphometry, and detection. The effectiveness of U-Net was shown in the detection of fluorescent-protein-tagged microglial cells on 2D fluorescence microscopy pictures and 3D bright-field images. U-Net was also used to segment nitrite in the stack of electron microscopy images. In addition, U-Net offers cloud service for convenient connectivity from a remote computer and functions as a plugin as an interface with the well-known and openly accessible ImageJ software. U-Net has an advantage over other programmes like CellProfiler, where training on fresh data is prohibited, because it may be trained on new data sets.

Finding the estimate of the total amount of brain damage the exposure agent has caused is a crucial step in quantitative neurotoxicology. White matter damage, the overall deteriorated area, and the number of degenerated neuron cells might all be used to estimate this. To obtain an accurate estimate of the number of neurons destroyed as a result of a neurodegenerative illness or a toxic chemical, degraded neurons should be counted. This holds significance as it might aid in comprehending the impact of a medication at various phases of compound exposure or the advancement of a neurodegenerative ailment. Digital image analysis, stereology, computer assistance, and manual counting of cells and neurons are all possible methods. The versatility and adaptability of manual counting of degenerated neurons are advantages since an expert may identify artefacts and classify items more precisely throughout the counting process. When multiple experts count the same dataset, for example, there is a chance of bias and variation in the manual counting process. Furthermore, counting by hand takes a lot of time and is only possible with a certain number of professionals. Although counting can be done more quickly with computer assistance, it is less flexible.

There are several ways to count neurons stereologically, including cell profiling and optical fractionators. Unlike nonstereological approaches like profile counting, stereological techniques like the optical fractionator could help prevent sampling biases. In the stereology technique, assumptions about properties like size, form, and orientation are not made. The optical fractionator technique is impartial since sampling and counting every object or cell have the same probability. Optical fractionators are used mostly for 3D structures for quantification (counting) purposes. They combine an optical dissector and a fractionator.

While stereological neuron counting offers advantages over both manual and computer-assisted counting, it still takes a lot of time and labor-intensive physical labour. These methods necessitate counting neurons independently in each region of a brain chunk. Every part of an animal's brain is examined independently for neurodegeneration as part of the whole brain examination, which necessitates a large amount of manual labour. To report neurotoxicity, some studies have used combined stereological and manual counting. When comparing stereological counting to manual counting, a significant loss of neurons was seen, highlighting the significance of the counting technique employed in neurotoxicity investigations. With the advent of whole slide imaging and digital images, neurotoxicity analysis in 2D digital images gained greater significance. Benefits of digital image analysis include the ability to quantify axon density, or the area occupied by axons, and axon number, which can be used to see how toxins affect axonal loss. Deep learning is gaining popularity as a substitute method for counting neurons. In one study, the substantia nigra of the mouse and rat brains' tyrosine hydroxylase-positive dopaminergic neurons were counted using deep CNN. Additionally, a stereological count of neurons was carried out.

Deep learning and other AI techniques will drastically cut down on the amount of time needed to analyse the degree of neurotoxicity. This will help to increase the amount of brain sections that can be processed, which will enable the analysis of more experimental animals and improve data repeatability and reduce bias. When it comes to training algorithms and reducing computing costs, some deep learning models, like CDeep3M and Aiforia platform, which are available for image segmentation and quantification, are cloud-based. This makes them more effective for larger datasets than other methods for neurotoxicity analysis.

5. Conclusion

AI's application in neuroscience is a quickly developing field that falls under the general heading of brain research. The measurement of brain toxicity is one such area where its application can be very helpful. Digital imaging of entire slides has shown to be highly beneficial in neurotoxicity investigation and has played a major role in histopathology analysis as well. Since the advent of full slide digital imaging, numerous image processing tools have been created. These methods have limits in terms of removing noise or artefacts from the data, but they are quite good at identifying degraded areas and neurons. These problems are addressed using a deep learning strategy, which has proven to be an extremely effective tool since it decreases bias and improves data interpretation accuracy. In terms of automated and accurate brain segmentation, automated detection of degenerated neurons, and counting—all essential for quantifying neurotoxicity—deep learning is exhibiting promise. While deep learning has been used for certain tasks, like brain segmentation, there is a lack of workflow in commercial software that allows for automated brain segmentation and automated deep learning/AI detection/analysis of degenerated neurons using a single platform.

Abbreviation

AI	- Artificial Intelligence
WSI	- Whole Slide Imaging
CNN	- Convoluted Neural Network
MIL	- Multiple Instance Learning
DNN	- Deep Neural Networks

Competing interests

The authors declare that they have no competing interests.

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Authors' contribution

Author A supports to find materials and results part in this manuscript. Author B helps to develop literature part.

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