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

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Abstract

Neurotoxicity studies are important in the preclinical stages of drug development process, because exposure to certain compounds that may enter the brain across a permeable blood brain barrier damages neurons and other supporting cells such as astrocytes. This could, in turn, lead to various neurological disorders such as Parkinson's or Huntington's disease as well as various dementias. Toxicity assessment is often done by pathologists after these exposures by qualitatively or semiquantitatively grading the severity of neurotoxicity in histopathology slides. Quantification of the extent of neurotoxicity supports qualitative histopathological analysis and provides a better understanding of the global extent of brain damage. Stereological techniques such as the utilization of an optical fractionator provide an unbiased quantification of the neuronal damage; however, the process is time-consuming. Advent of whole slide imaging (WSI) introduced digital image analysis which made quantification of neurotoxicity automated, faster and with reduced bias, making statistical comparisons possible. Although automated to a certain level, simple digital image analysis requires manual efforts of experts which is time-consuming and limits analysis of large datasets. Digital image analysis coupled with a deep learning artificial intelligence model provides a good alternative solution to time-consuming stereological and simple digital analysis. Deep learning models could be trained to identify damaged or dead neurons in an automated fashion. This review has focused on and discusses studies demonstrating the role of deep learning in segmentation of brain regions, toxicity detection and quantification of degenerated neurons as well as the estimation of area/volume of degeneration.

KEYWORDS

convolutional neural network, deep learning, digital image analysis, neurotoxicity, whole slide imaging

1 | INTRODUCTION

A promising and emerging field of artificial intelligence (AI) interacting with neuroscience is the deep learning approach (Marblestone, Wayne, & Kording, 2016; Richards et al., 2019). Deep learning is a machine learning method which is a subsystem of AI (Bini, 2018; LeCun, Bengio, & Hinton, 2015). It is an artificial neural network with several hidden layers, and these hidden layers are placed between input and output layers of the network. Deep learning neural network models are trained or learned to do specific computation. Larger artificial neural networks can be trained with this approach and thus are very useful for larger data sets (Benke & Benke, 2018; De Cnudde,

Ramon, Martens, & Provost, 2019; Hey, Butler, Jackson, & Thiyagalingam, 2020). Nowadays, deep learning approach is very popular with researchers working on behavioral and neurophysiological data to tap into representations of neural activity in the brain (Phan, Dou, Piniewski, & Kil, 2016; Vahid, Mückschel, Neuhaus, Stock, & Beste, 2018). As the popularity and effectiveness of deep learning approach have increased, researchers have started using this approach to automate procedures which require manual efforts that has limited our efforts to analyse larger data sets. Deep learning neural networks are feedforward neural network (FNN), recurrent neural network (RNN), or convolutional neural network (CNN) (Emmert-Streib, Yang, Feng, Tripathi, & Dehmer, 2020) (Figure 1). CNNs have become popular in recent times because of their low computational cost and their applications in various areas such as image recognition, image classification, image segmentation, drug design and drug discovery. CNNs are motivated by similar networks in the brain and particularly from the findings from the cells in the visual cortex that respond to edges and lines as demonstrated by Hubel and Wiesel (1962). CNNs are effective because of their weight sharing approach. They perform convolution, pooling and nonlinearity operations through their convolutional and pooling layers between the output and input and extract features from the image that was fed as an input. Before the convolutional operation is performed, filters (Kernels) are introduced to detect different features in the input image. Parameters such as filter size and number are fed to the CNN. Training allows the CNN to learn filter values.

AI/deep learning integration in pathology and histology was introduced with advances in digital imaging (Brachtel & Yagi, 2012; Ghaznavi, Evans, Madabhushi, & Feldman, 2013; Gurcan et al., 2009; Aeffner et al., 2019; Ameisen, Naour, & Daniel, 2012; Bhargava & Madabhushi, 2016; Hanna et al., 2019; Webster & Dunstan, 2014; Ying & Monticello, 2006; Zarella et al., 2019; Laurinavicius et al., 2012; Pantanowitz, 2010; Park, Pantanowitz, & Parwani, 2012). One of the digital imaging modalities which has great advantages is whole slide imaging (WSI). It requires commercially available scanners such as the ones from Olympus (Olympus corporation, USA) and Aperio (Leica Biosystems Inc, USA) to digitally convert glass slides with histological sections into high resolution digital images which can be later viewed and analysed with the help of specific software. Deep learning approaches are now increasingly getting attention in the field of digital image analysis (Kraus et al., 2017; Fuyong, Yuanpu, Hai, Fujun, & Lin, 2018; Dimitriou, Arandjelović, & Caie, 2019; Shen, Wu, & Suk, 2017; Moen et al., 2019; Litjens et al., 2017). Digital image analysis using deep learning adds classifiers to identify objects in an image using different classification steps in which deep learning models are trained (Janowczyk & Madabhushi, 2016; Komura & Ishikawa, 2018; Madabhushi & Lee, 2016). These trained models are then used to extract objects in an automated fashion from similar kind of images. Most of the efforts involving deep learning in digital image analysis have been done on histopathological sections mainly from oncology (Bug, Feuerhake, Oswald, Schüler, & Merhof, 2019; Sikpa et al., 2019; Tang, Zawaski, Francis, Qutub, & Gaber, 2019), toxicology

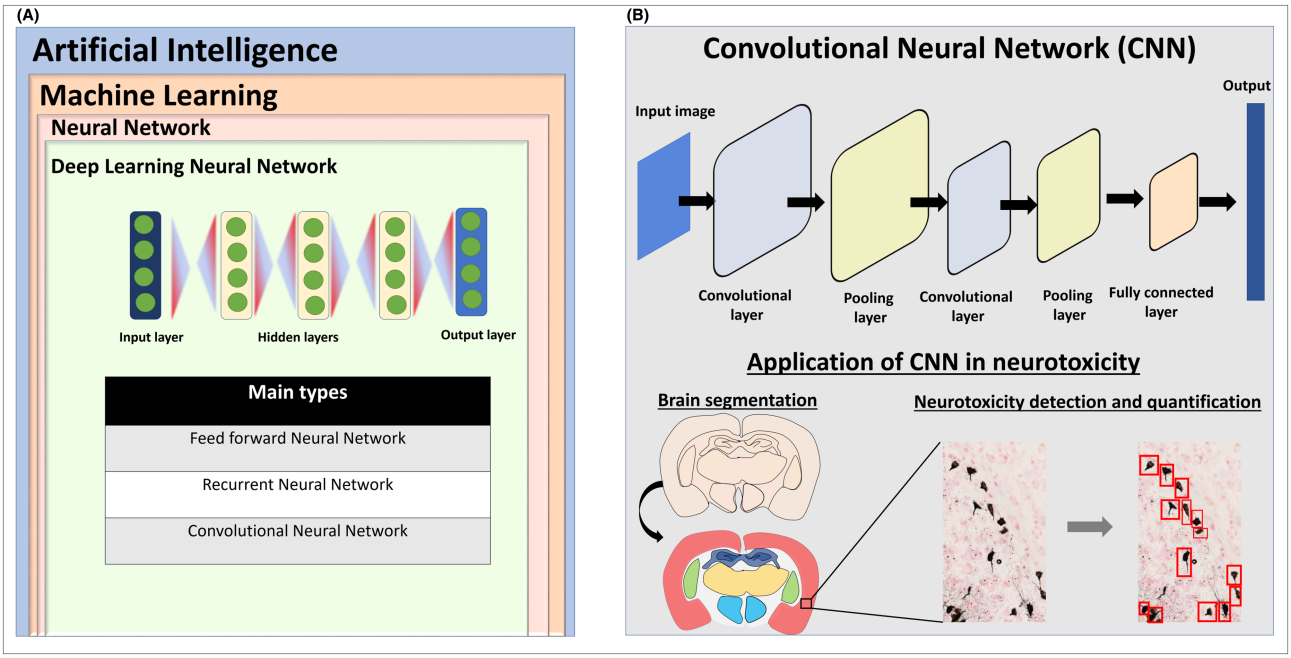

FIGURE 1 Schematic representation of convoluted neural network (CNN). (A) Figure shows CNN as a subsystem of machine learning which itself is subsystem of artifical intelligence. (B) Upper panel shows schematic representation of convolutional and pooling layers between input image and output in a typical CNN. Lower panel shows role of CNN in brain segmentation, neuroxicity detection and analysis. Red box shows schematic example of detection of degenerated neurons (black) in AmCuAg stained section

(Hu et al., 2020; Turner et al., 2020) and neuropathology (Signaevsky et al., 2019) studies.

2 | QUANTITATIVE NEUROTOXICITY

Quantification in histopathology has been widely used in recent times because of its advantages in terms of precision, reduced bias and generalizability and provides a window for statistical comparisons. Several studies have been performed which involve quantification of histopathological findings (Horai et al., 2019; Bishop & Robinson, 2001; Horai, Kakimoto, Takemoto, & Tanaka, 2017; Vandenberghe et al., 2016; Majeed et al., 2019). Neurotoxicity studies are an essential part of the regulatory regulations for drug development. Safety and efficacy studies are conducted before the clinical trials. Neurotoxicity evaluation in the preclinical stages is normally performed by board certified pathologists. Pathologists grade the severity of toxicity by evaluating brain sections on a 4- or 5-point grading scale (Schafer et al., 2018). Such a histopathological assessment is the gold standard, but it takes a significant amount of time and is mainly qualitative or semiquantitative (Meyerholz & Beck, 2018).

Because toxicity in the nervous system depends on several factors such as dose of the toxic compound, age and gender of the animal and exposure time to the dose, it becomes very important to quantify neurotoxicity. Quantification of toxicity provides a better understanding of the toxic effects of compounds and drugs. Various areas of the brain have significant differences in vulnerabilities to various toxic agents because of factors such as nonuniform distribution of receptors, oxidative stress and differences in synaptic connections. Quantification of neurotoxicity is an excellent tool to illustrate these differences in vulnerabilities by statistically comparing extent of degeneration in different brain regions. Also, being able to get a value for global toxicity is a far more powerful approach than just reporting qualitative changes in different areas because the additive effect of a large number of small changes (global effects) is highly significant in evaluating the overall comprehensive damage to the brain. It provides data for better statistical comparison of toxic effects to support the traditional histopathological analysis of toxicity done by pathologists.

Quantification of toxicity mainly involves measuring degenerated area in the brain and/or total degenerated neuron counts in the brain after exposure to toxic compounds. Degenerated area and degenerated neurons in the brain can be visualized by various staining methods such as amino cupric silver (AmCuAg) staining (Baloyannis, 2015; de Olmos, Beltramino, & de Lorenzo, 1994; Switzer, 2000) and Fluoro-Jade stains (Schmued & Hopkins, 2000; Schmued, Stowers, Scallet, & Xu, 2005). Development of digital image analysis tools has opened efficient ways of quantifying neurotoxicity in brain of animals treated with various neurotoxic compounds as well as in neurodegenerative conditions (Johnstone et al., 2018; Jensen et al., 1993; Kneynsberg, Collier, Manfredsson, & Kanaan, 2016; Srivastava et al., 2020; Scallet, Pothuluri, Rountree, & Matthews, 2000). Image analysis tools have automated certain steps in the workflow (Figure 2) for the quantification of toxicity, but there are still limitations that make the procedure time-consuming. In this

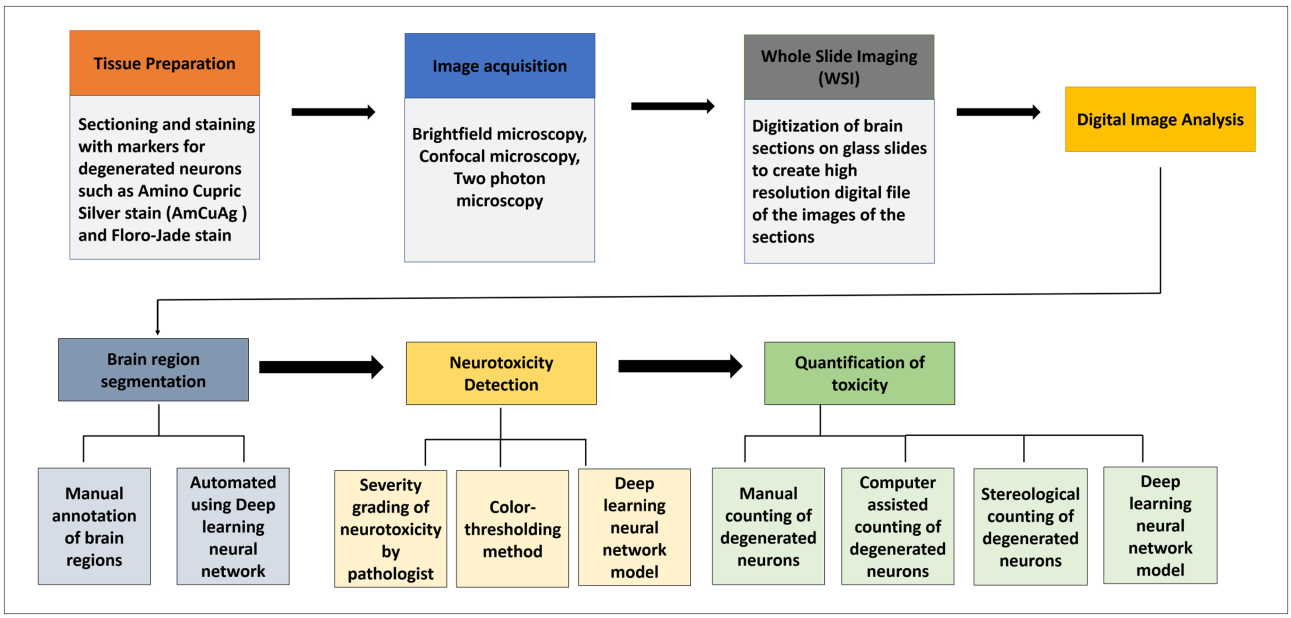

FIGURE 2 Workflow for neurotoxicological analysis. Several steps are involved in the analysis of neurotoxicity starting from tissue preparation which involves sectioning and staining of brain sections with markers of neuronal degeneration. Image acquisition of the stained sections is next step in the workflow followed by whole sliding imaging to produce high resolution digital images of the sections. High resolution digital images are a great source for further analysis which could be either manual or by image processing tools which also provides automated solutions. Neurotoxicity analysis involves brain segmentation into various brain regions for detection and quantification of neurotoxicity for better understanding of extent of toxicity and sensitivity of different areas to neurotoxic compounds/drugs

update on advances in deep learning in digital image analysis, we have discussed the time-consuming steps in the process of quantification of neurotoxicity data and where a deep learning approach is contributing as well as the potential for transforming the way we analyse and interpret the neurotoxicity data.

3 | AI/DEEP LEARNING IN AUTOMATED BRAIN SEGMENTATION INTO DIFFERENT REGIONS

One of the major time-consuming steps in the quantification of toxicity is the manual procedure to mark regions of interest for analysis on the brain sections. It basically involves segmentation of brain into regions of interest (by drawing annotation layers [Figure 3]). Annotation layers are manually drawn within the regions of interest in the brain sections. It takes significant amount of time and, as a result, limits the whole brain analysis with respect to changes in different areas of the brain. Also, because of the time-consuming manual procedure, it limits the number of experimental animals analysed. Segmentation of the brain into different regions is important for quantitative neurotoxicity analysis because neurons in different areas of the brain show differential vulnerability to toxic compounds. Segmenting of brain regions will allow quantification of different areas separately, which will help in statistical comparison of degeneration between all the areas of the brain rather than just focusing on regions of interest. This is a process too time-consuming for individuals to perform manually.

Deep learning/AI can be extremely useful in segmentation of the brain regions. The possibility of annotating an area (such as claustrum or substantia nigra) in an image or set of images (coronal brain section) and training the algorithm to identify and segment that brain structure in all the remaining sections in that animal and for all the sections in other experimental animals is an important advance. This will help in significantly reducing the time spent in segmentation of the brain areas and will make it possible to run the image processing algorithm in all the animals in these segmented brain areas at the same time. Researchers have been trying to create such segmentation algorithms through an automated AI approach, but it is an evolving area of research. Several studies have been done to automate image segmentation using deep CNN (Haberl et al., 2018; Kraus, Ba, & Frey, 2016). CNN is an artificial neural network model with an input and output layers and several hidden layers. Hidden layers in CNN consist of convolutional layers (Yamashita, Nishio, Do, & Togashi, 2018). In a study by Kraus et al. (2016), deep CNNs with multiple instance learning (MIL) have been used to classify and segment images (breast cancer

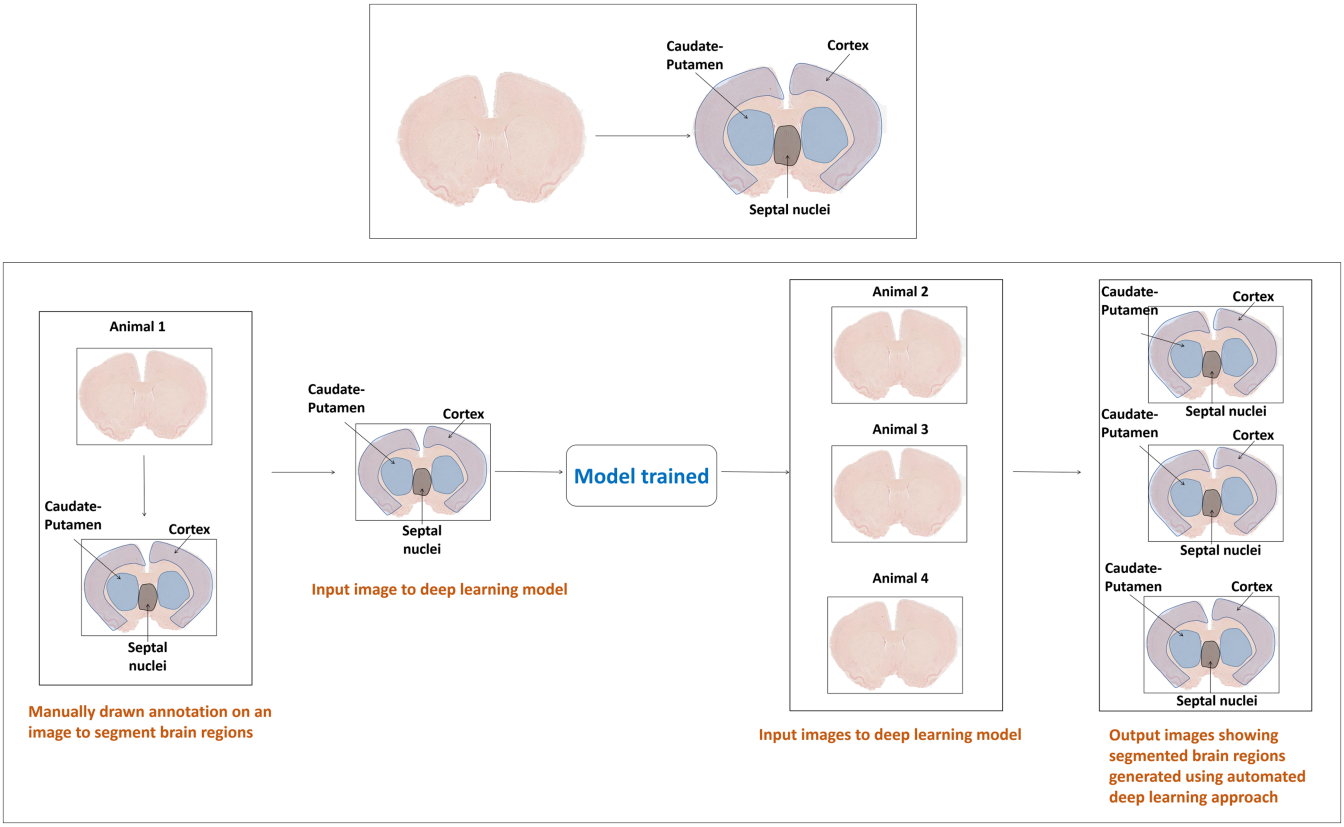

FIGURE 3 Schematic shows segmentation of a coronal section of the rat brain. Upper panel shows manual annotations on the brain image showing segmentation into different brain areas. Lower panel show how deep learning approach could automate the process of brain segmentation. Input image which is manually annotated is fed to deep learning model which is later trained. Similar images from other animals are fed to the model for the segmentation into different brain areas. Output shows images with segmentation performed by deep learning model

cells data set) which contained different channels with fluorescent markers such as for DNA and actin filaments. Haberl et al. (2018) also used deep CNN on images obtained from light, X-ray and electron microscopy for image segmentation which is known as CDeep3M. It is a cloud-based ready to use tool using a neural network model for image segmentation. A study by Tan et al. (2020) used a framework known as DeepBrainSeg to segment brain wide regions using CNN training. In this study, brain images from mouse brain imaged using various imaging methods such as fluorescence microscopy, microoptical sectioning tomography, serial two photon system and MRI (T2*) were used. Images were used to manually delineate boundaries to develop a training set, and a neural network was trained on that. Other processing stages such as registration and prediction by the trained neural network were performed to get segmented regions. One recent study has demonstrated automated segmentation of the mouse brain using deep neural network-based method known as SeBRe (Iqbal, Khan, & Karayannis, 2019). In this study by Iqbal et al. (2019), a brain image from a mouse brain labelled with a neuronal marker was taken as an input image. The image was manually annotated for different brain regions. It was fed into a deep brain neural network model and then processed using several processing stages. The model was trained and applied to a similar image from different animals. Output was imaged with segmented regions as classified in the input image. To measure its efficiency, SeBRe was applied on images with different neuronal markers. Segmentation applied to subregions such as CA1, CA2 and CA3 and the dentate gyrus of the hippocampus were isolated using SeBRe suggesting that fine scale segmentation could also be done with this neural network model.

Numerous studies have used MRI as a tool to determine neurotoxicity (Csernansky, 2001; Hanig et al., 2014; Johnson et al., 2014). Segmenting brain regions in the MRI images and neurotoxicity analysis in the segmented region is important for better understanding of the extent and sensitivity of different areas to the toxic compounds or drugs. There are many studies that have proposed using automated brain segmentation using MRI images (Feo & Giove, 2019; Liu, Unsal, Tao, & Zhang, 2020; Oguz, Zhang, Rumple, & Sonka, 2014). Several studies (Guha Roy, Conjeti, Navab, & Wachinger, 2019; Kushibar et al., 2018; Mehta, Majumdar, & Sivaswamy, 2017) have used deep learning approach for segmenting the brain regions using MRI image data. Some studies such as Iqbal et al. (2019) have shown effectiveness of their deep learning model by training them on different imaging modalities such as images of the labelled (neuronal marker) sections obtained from the mouse brain as well as on MRI brain data sets, but such studies are very scarce.

There are not enough neurotoxicity studies which have employed this approach of segmenting the brain regions to quantify neurotoxicity in different brain regions to understand the sensitivity and vulnerability of these regions to toxic compounds or drugs.

4 | AI/DEEP LEARNING IN AUTOMATED DETECTION AND ANALYSIS OF TOXICITY IN DIFFERENT REGIONS OF BRAIN

Cell detection is a crucial step in quantitating histopathological results (Xing & Yang, 2016). For instance, some image processing softwares such as ImageJ (NIH, Maryland, USA), Fiji (Schindelin et al., 2012) and Halo (Indica labs, NM, USA) have colour thresholding (Fermin, Gerber, & Torre-Bueno, 1992) tools (detect structures with specific colour) which are used to detect silver stained degenerated neurons that appear black in brain sections. This approach has its limitations. Artefacts such as nonspecific staining as well as debris of degenerated neurons are also picked up by this method which adds to the total estimated degeneration in the brain sections. An AI approach has a considerable potential to address this limitation. Models can be trained using a deep learning approach to detect degenerated neurons based on their size, colour and shape as shown (manually classified) on the input image fed to the model. Deep CNN model has been used to detect neuronal damage automatically in rat cerebral ischaemic-reperfusion model (Wang et al., 2020). Images of label-free brain sections were obtained using two photon microscopy, and a deep learning algorithm was applied to it to detect injured neurons. Pathologists identified damaged neurons on two photon microscopic and H&E images. Brain sections were stained by NeuN and H&E to compare this approach to standard histology in detecting neuronal damage. This study suggested that deep learning application on two photon images provides a tool to automatically detect neuronal damage on label-free brain sections.

Exclusion of artefacts could also be incorporated in the AI workflow for detection of degenerated neurons. Exposure to a neurotoxic drug leads to inflammatory responses such as activated microglia. Detection of activated microglia could be crucial in understanding toxicity of a compound. Deep learning could play a very important role in detection of activated microglia in an automated fashion based on some basic features such as number, area and length of the processes of activated microglia which are different from nonactivated microglia (Heindl et al., 2018). Similarly, a deep learning model could be trained to detect morphological changes in astrocytes after exposure to toxic compounds (Kayasandik, Ru, & Labate, 2020; Suleymanova et al., 2018). Degenerative changes in white matter could also be detected with trained deep learning models. In one study, brain wide analysis for neuron detection has been performed with a deep learning method (DeNeRD—detect neurons in different brain regions during development) (Iqbal, Sheikh, & Karayannis, 2019). Human experts marked neurons by annotation on the brain images, and then a deep neural network model was created to learn the features of the neurons during the training sessions. Images of mouse brain section were fed into the DeNeRD neural network as an input image, and neurons were detected as an output in the preregistered brain areas after completion of a set of preprocessing steps. Different brain markers such as CAMKIIa, GAD1 and VGAT were used in the study by Iqbal et al. (2019) to detect neurons in the whole brain.

Another generic deep learning-based solution to cell detection, cell segmentation and morphometry is U-Net (Falk et al., 2019). Performance of U-Net was demonstrated on 2D images from fluorescence microscopy and 3D bright-field images for the detection of microglial cells tagged with fluorescent-protein. Neurite segmentation was also performed using U-Net in electron microscopy images stack. U-Net also runs as a plugin as an interface with the popular and freely available ImageJ software and provides cloud service for easy accessibility from a remote computer. Because it can be trained on new data sets, U-Net has an advantage over other software such as CellProfiler (Carpenter et al., 2006; Jones et al., 2008) where training on new data is not allowed. CellProfiler software was developed by the Carpenter lab at the Broad Institute of Harvard and MIT. CellProfiler 3.0 (McQuin et al., 2018) provides deep learning (ClassifyPixels-UNet module) AI solutions for image segmentation and volume analysis demonstrated on 3D hiPSCs (human induced pluripotent stem cell) images. Some of the commercial softwares such as HALO (Indica labs, NM, USA) and Aivia 6 (DRVISION, Bellevue, WA, USA) also provide AI solutions to detect rare events and cells. HALO AI solutions are mainly based on VGG, MiniNet and DenseNet neural networks for tissue segmentation and quantification of images of H&E-stained, Silver stained, ISH and IHC stained sections. Aivia 6 (DRVISION, Bellevue, WA, USA) provides AI solutions in the form of pretrained neural network models which are based on CNN architectures such as DenseNet, UNet and 3D-UNet for image segmentation in 3D images obtained from electron microscopy.

Another important step in quantitative neurotoxicology is determining the estimate of the overall damage in the brain caused by the exposure agent. This estimate could be in the form of total degenerated area, degenerated neuron cell counts and white matter damage. Detection of degenerated neurons should be counted in order to get estimates of correct neuron numbers which are lost due to the toxic effect of a compound or due to neurodegenerative disease. This is important as it can help in understanding the effect of drug at different stages of exposure to a compound or progression of a neurodegenerative disease. Cell/neuron counting can be done manually, aided by a computer, stereologically and by digital image analysis. Manual counting of degenerated neurons has its advantages in terms of its adaptability and flexibility because an expert can detect artefacts and could classify objects more accurately during counting process. Disadvantages of manual counting are in the form of bias and variation when for instance more than one expert is involved in the counting process on same dataset. Also, manual counting is time-consuming and limited by number of experts performing the task. Computer assisted counting allows counting with faster pace but lacks adaptability.

Stereological counting of neurons can be done using various methods such as optical fractionator and cell profiling (Gundersen, Bendtsen, et al., 1988b; Gundersen & Jensen, 1987; Gundersen, Jensen, Kiêu, & Nielsen, 1999; Gundersen, Bagger, et al., 1988a; Caicedo et al., 2017; Golub et al., 2015; Gundersen, 1986; Schmitz & Hof, 2000, 2005; Schmitz, Korr, & Heinsen, 1999; West, 1999; Olesen, Needham, & Pakkenberg, 2017; Herculano-Houzel, von Bartheld, Miller, & Kaas, 2015; Ip, Cheong, & Volkmann, 2017; Larsen, 2017). Sampling biases could be avoided by stereological techniques such as optical fractionator unlike in nonstereological techniques and profile counting. Assumption of features such as size, shape and orientation is not done in stereology technique. In optical fractionator technique, sampling and counting of all the objects/cells have equal probability which makes this technique unbiased. Optical fractionator involves optical dissector and fractionator for quantification (mainly counting) which is mainly done for 3D structures.

Stereological counting of neurons has an advantage over manual as well as over counting done with help of a computer, but is still time-consuming and most of it requires a lot of manual labour. Such techniques require neuron counting for each region in a brain section separately. For the whole brain analysis, every brain section in an animal is separately analysed for neurodegeneration which requires significant manual effort. Some studies have employed both manual counting and stereological counting to report neurotoxicity. One such study by Bukhatwa et al. (2009) reported dopaminergic neuron (Flurogold stained) loss in substantia nigra after administration of proteasomal inhibitor using manual counting and compared it with stereological counting to confirm neuron loss. Significant loss of neurons was observed with stereological counting when compared to manual counting reflecting the importance of the counting method used for neurotoxicity studies. Neurotoxicity analysis in 2D digital images became more meaningful with introduction of whole slide imaging and digital image analysis tools which permitted more comprehensive reporting of degenerated areas and neuron count. Advantages of digital image analysis can be appreciated as it allows quantification of axon density (area occupied by axons) and axon number to observe the effect of toxins on axonal loss (Johnstone et al., 2018; Mysona et al., 2020). Deep learning as an alternative approach to count neurons is becoming popular. In one of the studies, deep CNN was used to count tyrosine hydroxylase positive dopaminergic neurons in substantia nigra of mouse and rat brain (Penttinen et al., 2018). Stereological counting of neurons was also performed and compared to counts done by CNN using Aiforia™ platform (Aiforia Technologies, MA, USA), and results showed similar counts of neurons in 6-OHDA (6-Hydroxydopamine) lesioned as compared to intact rats and mice brains. Aiforia AI solutions are based on CNN and have demonstrated quantification of TH+ neurons in substantia nigra of rat brain and activated states of microglia and astrocytes which are important markers in neurotoxicity studies. Aiforia AI also provides solution for image segmentation for region- wise analysis in brain sections. Another study also reported automatic cell counting which was performed using deep learning approach and unbiased stereology (Alahmari et al., 2019). For the unbiased stereology counting of Neu-N (Neuronal Nuclei) positive neurons from mouse neocortex sections, an automated fractionator method was used which included counting rules which authors suggested were unbiased that avoided errors and false assumptions in the counting process. Deep learning training was done on accepted masks after unbiased stereology using an adaptive segmentation algorithm (ASA) (Mouton et al., 2017) and

examined on test data. In one of the recent studies, machine learning was used to predict neurotoxicity in organoids of midbrain dopaminergic neurons in which Parkinson's disease (PD) was induced by 6OHDA neurotoxin (Monzel et al., 2020). Organoids were derived from human iPSC (induced pluripotent stem cell) lines. 6OHDA exposure led to reduction in dopaminergic neurons as reflected by reduction in tyrosine hydroxylase (TH+) positive cells. A machine learning model which was based on random forest algorithm (Breiman, 2001) was used. A model was build using training data sets, and separate data sets were used to validate the model to predict neurotoxicity in organoids. Training models using deep learning methods have a significant potential to make degenerated neuron counting easier due to automation.

AI approaches such as deep learning will significantly reduce the time allotted to the analysis of the extent of neurotoxicity. This will contribute in increasing the number of brain sections that can be processed resulting in more experimental animals being analysed bringing reproducibility and reduced bias to the data. Some of the deep learning models such as CDeep3M and Aiforia platform available for image segmentation and quantification are cloud-based (Navale & Bourne, 2018) which makes them more effective for larger datasets in terms of training the algorithms and lowering the computing cost over other methods for neurotoxicity analysis. A list of some of the deep learning AI solutions for image segmentation and quantification are given in Table 1.

TABLE 1 Examples of deep learning AI solutions for image processing

Name	Method	Application
U-Net (Falk et al., 2019)	❖ Generic deep-learning-based ❖ Convolutional neural network ❖ Interface with ImageJ through Plugin ❖ Cloud-based	❖ Cell detection (e.g., fluorescent-protein-tagged microglial cells) ❖ Cell segmentation (e.g., 2D images from fluorescence microscopy and 3D bright-field images) ❖ Neurite segmentation (electron microscopy stacks) ❖ Morphometry
DeNeRD (Iqbal, Sheikh, & Karayannis, 2019)	❖ Convolutional neural network method (fast-RCNN)	❖ Images of mouse brain sections used were obtained from open source database (Allen Institute for Brain Science) ❖ Neural density could be calculated of a brain area.
CDeep3M (Haberl et al., 2018)	❖ Deep convolutional neural network ❖ Cloud-based	❖ Image segmentation Images from light, X-ray, and electron microscopy
SeBRe (Iqbal, Khan, & Karayannis, 2019)	❖ Convolutional neural network ❖ Deep ResNet101 and FPN architectures (first five stages)	❖ Segmentation of brain images (e.g., images from mouse brain) ❖ MRI (T1-weighted) images of human brain obtained from internet brain segmentation repository (IBSR)
HALO (Indica Labs, USA)	❖ VGG, MiniNet, and DenseNet neural networks ❖ Cloud-based	❖ Tissue segmentation ❖ Quantification after classification (e.g., H&E-stained, silver stained, ISH, and IHC stained sections)
Aiforia (Aiforia Technologies, Cambridge, MA, USA)	❖ Convolutional neural networks ❖ Cloud-based	❖ Quantification of TH + neurons, activated states of microglia, astrocytes in images of rat brain, ❖ Image segmentation based on anatomical regions
Cell Profiler (McQuin et al., 2018)	❖ Deep learning model ❖ ClassifyPixels-Unet module ❖ Cloud-based	❖ Image segmentation ❖ Volume analysis (e.g., 3D hiPSCs [human induced pluripotent stem cell] images)
Aivia 6 (DRVISION, Bellevue, WA, USA)	❖ Pretrained neural network models ❖ Based on CNN architectures such as DenseNet, UNet and 3D-UNet ❖ Cloud-based	❖ Image segmentation ❖ 3D images from electron microscopy

5 | CONCLUSION

Use of AI in neuroscience is a rapidly evolving field with its application noticed in various areas under the umbrella of brain research. One such area where its application could be very useful is quantification of toxicity in brain. Whole slide digital imaging has made a significant contribution to histopathological analysis, and it is becoming very useful in neurotoxicity analysis. Various image processing tools have been developed after the introduction of whole slide digital imaging. These tools are very effective in detecting degenerated areas and neurons but have limitations in terms of excluding artefacts/noise in the data. A deep learning approach addresses these issues and has been a very powerful tool as it reduces bias and increases accuracy in the data interpretation. Deep learning is showing promise in terms of automated and precise brain segmentation, automated detection of degenerated neurons and counting which are crucial for quantification of neurotoxicity. Although a deep learning approach has been applied to specific tasks such as brain segmentation, there is scarcity of workflow available in any commercial software where automated brain segmentation and automated degenerated neuron detection/analysis using AI/deep learning could be accomplished utilizing a single platform.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

DISCLAIMER

The content of this publication represents solely the authors' views and may not reflect any position of the U.S. Government or the Food and Drug Administration.

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REFERENCES

Aeffner, F., Zarella, M. D., Buchbinder, N., Bui, M. M., Goodman, M. R., Hartman, D. J., … Bowman, D. (2019). Introduction to digital image analysis in whole-slide imaging: A white paper from the digital pathology association. *Journal of Pathology Informatics*, *10*, 9. https://doi.org/10.4103/jpi.jpi_82_18

Alahmari, S. S., Goldgof, D., Hall, L., Phoulady, H. A., Patel, R. H., & Mouton, P. R. (2019). Automated cell counts on tissue sections by deep learning and unbiased stereology. *Journal of Chemical Neuroanatomy*, *96*, 94–101. https://doi.org/10.1016/j.jchemneu.2018.12.010

Ameisen, D., le Naour, G., & Daniel, C. (2012). Whole slide imaging technology: From digitization to online applications. *Médecine Sciences*, *28* (11), 977–982. https://doi.org/10.1051/medsci/20122811017

Baloyannis, S. J. (2015). Staining of dead neurons by the Golgi method in autopsy material. *Methods in Molecular Biology (Clifton, N.J.)*, *1254*, 167–179. https://doi.org/10.1007/978-1-4939-2152-2_13

Benke, K., & Benke, G. (2018). Artificial intelligence and big data in public health. *International Journal of Environmental Research and Public Health*, *15*(12). https://doi.org/10.3390/ijerph15122796

Bhargava, R., & Madabhushi, A. (2016). Emerging themes in image informatics and molecular analysis for digital pathology. *Annual Review of Biomedical Engineering*, *18*, 387–412. https://doi.org/10.1146/annurev-bioeng-112415-114722

Bini, S. A. (2018). Artificial intelligence, machine learning, deep learning, and cognitive computing: What Do these terms mean and how will they impact health care? *The Journal of Arthroplasty*, *33*(8), 2358–2361. https://doi.org/10.1016/j.arth.2018.02.067

Bishop, G. M., & Robinson, S. R. (2001). Quantitative analysis of cell death and ferritin expression in response to cortical iron: Implications for hypoxia-ischemia and stroke. *Brain Research*, *907*(1–2), 175–187. https://doi.org/10.1016/s0006-8993(01)02303-4

Brachtel, E., & Yagi, Y. (2012). Digital imaging in pathology—Current applications and challenges. *Journal of Biophotonics*, *5*(4), 327–335. https://doi.org/10.1002/jbio.201100103

Breiman, L. (2001). Random forests. *Machine Learning*, *45*, 5–32. https://doi.org/10.1023/A:1010933404324

Bug, D., Feuerhake, F., Oswald, E., Schüler, J., & Merhof, D. (2019). Semi-automated analysis of digital whole slides from humanized lung-cancer xenograft models for checkpoint inhibitor response prediction. *Oncotarget*, *10*(44), 4587–4597. https://doi.org/10.18632/oncotarget.27069

Bukhatwa, S., Iravani, M. M., Zeng, B. Y., Cooper, J. D., Rose, S., & Jenner, P. (2009). An immunohistochemical and stereological analysis of PSI-induced nigral neuronal degeneration in the rat. *Journal of Neurochemistry*, *109*(1), 52–59. https://doi.org/10.1111/j.1471-4159.2009.05956.x

Caicedo, J. C., Cooper, S., Heigwer, F., Warchal, S., Qiu, P., Molnar, C., … Carpenter, A. E. (2017). Data-analysis strategies for image-based cell profiling. *Nature Methods*, *14*(9), 849–863. https://doi.org/10.1038/nmeth.4397

Carpenter, A. E., Jones, T. R., Lamprecht, M. R., Clarke, C., Kang, I. H., Friman, O., … Sabatini, D. M. (2006). CellProfiler: Image analysis software for identifying and quantifying cell phenotypes. *Genome Biology*, *7*(10), R100. https://doi.org/10.1186/gb-2006-7-10-r100

Csernansky, J. G. (2001). Structural MRI as a tool for the study of neurotoxicity and neurodegenerative disorders. *Journal of Analytical Toxicology*, *25*(5), 414–418. https://doi.org/10.1093/jat/25.5.414

De Cnudde, S., Ramon, Y., Martens, D., & Provost, F. (2019). Deep learning on big, sparse, behavioral data. *Big Data*, *7*(4), 286–307. https://doi.org/10.1089/big.2019.0095

de Olmos, J. S., Beltramino, C. A., & de Lorenzo, S. D. (1994). Use of an amino-cupric-silver technique for the detection of early and semiacute neuronal degeneration caused by neurotoxicants, hypoxia, and physical trauma. *Neurotoxicology and Teratology*, *16*(6), 545–561. https://doi.org/10.1016/0892-0362(94)90033-7

Dimitriou, N., Arandjelović, O., & Caie, P. D. (2019). Deep learning for whole slide image analysis: An overview. *Frontiers in Medicine*, *6*, 264. https://doi.org/10.3389/fmed.2019.00264

Emmert-Streib, F., Yang, Z., Feng, H., Tripathi, S., & Dehmer, M. (2020). An introductory review of deep learning for prediction models with big data. *Frontiers in Artificial Intelligence*, *3*(4). https://doi.org/10.3389/frai.2020.00004

Falk, T., Mai, D., Bensch, R., Çiçek, Ö., Abdulkadir, A., Marrakchi, Y., … Ronneberger, O. (2019). U-Net: Deep learning for cell counting, detection, and morphometry. *Nature Methods*, *16*(1), 67–70. https://doi.org/10.1038/s41592-018-0261-2

Feo, R., & Giove, F. (2019). Towards an efficient segmentation of small rodents brain: A short critical review. *Journal of Neuroscience Methods*, *323*, 82–89. https://doi.org/10.1016/j.jneumeth.2019.05.003

Fermin, C. D., Gerber, M. A., & Torre-Bueno, J. R. (1992). Colour thresholding and objective quantification in bioimaging. *Journal of*

Microscopy, *167*(Pt 1), 85–95. https://doi.org/10.1111/j.1365-2818.1992.tb03221.x

Fuyong, X., Yuanpu, X., Hai, S., Fujun, L., & Lin, Y. (2018). Deep learning in microscopy image analysis: A survey. *IEEE Transactions on Neural Networks and Learning Systems*, *29*(10), 4550–4568. https://doi.org/10.1109/tnnls.2017.2766168

Ghaznavi, F., Evans, A., Madabhushi, A., & Feldman, M. (2013). Digital imaging in pathology: Whole-slide imaging and beyond. *Annual Review of Pathology*, *8*, 331–359. https://doi.org/10.1146/annurev-pathol-011811-120902

Golub, V. M., Brewer, J., Wu, X., Kuruba, R., Short, J., Manchi, M., … Reddy, D. S. (2015). Neurostereology protocol for unbiased quantification of neuronal injury and neurodegeneration. *Frontiers in Aging Neuroscience*, *7*, 196. https://doi.org/10.3389/fnagi.2015.00196

Guha Roy, A., Conjeti, S., Navab, N., & Wachinger, C. (2019). QuickNAT: A fully convolutional network for quick and accurate segmentation of neuroanatomy. *NeuroImage*, *186*, 713–727. https://doi.org/10.1016/j.neuroimage.2018.11.042

Gundersen, H. J. (1986). Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. *Journal of Microscopy*, *143*(Pt 1), 3–45. https://doi.org/10.1111/j.1365-2818.1986.tb02764.x

Gundersen, H. J., Bagger, P., Bendtsen, T. F., Evans, S. M., Korbo, L., Marcussen, N., … Pakkenberg, B. (1988). The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, *96*(10), 857–881. https://doi.org/10.1111/j.1699-0463.1988.tb00954.x

Gundersen, H. J., Bendtsen, T. F., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., … Vesterby, A. (1988). Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, *96*(5), 379–394. https://doi.org/10.1111/j.1699-0463.1988.tb05320.x

Gundersen, H. J., & Jensen, E. B. (1987). The efficiency of systematic sampling in stereology and its prediction. *Journal of Microscopy*, *147*(Pt 3), 229–263. https://doi.org/10.1111/j.1365-2818.1987.tb02837.x

Gundersen, H. J., Jensen, E. B., Kiêu, K., & Nielsen, J. (1999). The efficiency of systematic sampling in stereology—Reconsidered. *Journal of Microscopy*, *193*(Pt 3), 199–211. https://doi.org/10.1046/j.1365-2818.1999.00457.x

Gurcan, M. N., Boucheron, L. E., Can, A., Madabhushi, A., Rajpoot, N. M., & Yener, B. (2009). Histopathological image analysis: A review. *IEEE Reviews in Biomedical Engineering*, *2*, 147–171. https://doi.org/10.1109/rbme.2009.2034865

Haberl, M. G., Churas, C., Tindall, L., Boassa, D., Phan, S., Bushong, E. A., … Ellisman, M. H. (2018). CDeep3M-plug-and-play cloud-based deep learning for image segmentation. *Nature Methods*, *15*(9), 677–680. https://doi.org/10.1038/s41592-018-0106-z

Hanig, J., Paule, M. G., Ramu, J., Schmued, L., Konak, T., Chigurupati, S., … Liachenko, S. (2014). The use of MRI to assist the section selections for classical pathology assessment of neurotoxicity. *Regulatory Toxicology and Pharmacology*, *70*(3), 641–647. https://doi.org/10.1016/j.yrtph.2014.09.010

Hanna, M. G., Reuter, V. E., Hameed, M. R., Tan, L. K., Chiang, S., Sigel, C., … Sirintrapun, S. J. (2019). Whole slide imaging equivalency and efficiency study: Experience at a large academic center. *Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc*, *32*(7), 916–928. https://doi.org/10.1038/s41379-019-0205-0

Heindl, S., Gesierich, B., Benakis, C., Llovera, G., Duering, M., & Liesz, A. (2018). Automated morphological analysis of microglia after stroke. *Frontiers in Cellular Neuroscience*, *12*, 106. https://doi.org/10.3389/fncel.2018.00106

Herculano-Houzel, S., von Bartheld, C. S., Miller, D. J., & Kaas, J. H. (2015). How to count cells: The advantages and disadvantages of the isotropic fractionator compared with stereology. *Cell and Tissue Research*, *360*(1), 29–42. https://doi.org/10.1007/s00441-015-2127-6

Hey, T., Butler, K., Jackson, S., & Thiyagalingam, J. (2020). Machine learning and big scientific data. *Philosophical Transactions. Series a, Mathematical, Physical, and Engineering Sciences*, *378*(2166), 20190054. https://doi.org/10.1098/rsta.2019.0054

Horai, Y., Kakimoto, T., Takemoto, K., & Tanaka, M. (2017). Quantitative analysis of histopathological findings using image processing software. *Journal of Toxicologic Pathology*, *30*(4), 351–358. https://doi.org/10.1293/tox.2017-0031

Horai, Y., Mizukawa, M., Nishina, H., Nishikawa, S., Ono, Y., Takemoto, K., & Baba, N. (2019). Quantification of histopathological findings using a novel image analysis platform. *Journal of Toxicologic Pathology*, *32*(4), 319–327. https://doi.org/10.1293/tox.2019-0022

Hu, F., Schutt, L., Kozlowski, C., Regan, K., Dybdal, N., & Schutten, M. M. (2020). Ovarian toxicity assessment in histopathological images using deep learning. *Toxicologic Pathology*, *48*(2), 350–361. https://doi.org/10.1177/0192623319877871

Hubel, D. H., & Wiesel, T. N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *The Journal of Physiology*, *160*(1), 106–154. https://doi.org/10.1113/jphysiol.1962.sp006837

Ip, C. W., Cheong, D., & Volkmann, J. (2017). Stereological estimation of dopaminergic neuron number in the mouse substantia nigra using the optical fractionator and standard microscopy equipment. *Journal of Visualized Experiments: JoVE*, *127*. https://doi.org/10.3791/56103

Iqbal, A., Khan, R., & Karayannis, T. (2019). Developing a brain atlas through deep learning. *Nature Machine Intelligence*, *1*, 277–287. https://doi.org/10.1038/s42256-019-0058-8

Iqbal, A., Sheikh, A., & Karayannis, T. (2019). DeNeRD: High-throughput detection of neurons for brain-wide analysis with deep learning. *Scientific Reports*, *9*(1), 13828. https://doi.org/10.1038/s41598-019-50137-9

Janowczyk, A., & Madabhushi, A. (2016). Deep learning for digital pathology image analysis: A comprehensive tutorial with selected use cases. *Journal of Pathology Informatics*, *7*, 29. https://doi.org/10.4103/2153-3539.186902

Jensen, K. F., Olin, J., Haykal-Coates, N., O'Callaghan, J., Miller, D. B., & de Olmos, J. S. (1993). Mapping toxicant-induced nervous system damage with a cupric silver stain: A quantitative analysis of neural degeneration induced by 3,4-methylenedioxymethamphetamine. *NIDA Research Monograph*, *136*, 133–149; discussion 150-134. https://doi.org/10.1037/e495922006-008

Johnson, G. A., Calabrese, E., Little, P. B., Hedlund, L., Qi, Y., & Badea, A. (2014). Quantitative mapping of trimethyltin injury in the rat brain using magnetic resonance histology. *Neurotoxicology*, *42*, 12–23. https://doi.org/10.1016/j.neuro.2014.02.009

Johnstone, A. D., Hallett, R. M., de Léon, A., Carturan, B., Gibon, J., & Barker, P. A. (2018). A novel method for quantifying axon degeneration. *PLoS ONE*, *13*(7), e0199570. https://doi.org/10.1371/journal.pone.0199570

Jones, T. R., Kang, I. H., Wheeler, D. B., Lindquist, R. A., Papallo, A., Sabatini, D. M., … Carpenter, A. E. (2008). CellProfiler analyst: Data exploration and analysis software for complex image-based screens. *BMC Bioinformatics*, *9*, 482. https://doi.org/10.1186/1471-2105-9-482

Kayasandik, C. B., Ru, W., & Labate, D. (2020). A multistep deep learning framework for the automated detection and segmentation of astrocytes in fluorescent images of brain tissue. *Scientific Reports*, *10*(1), 5137. https://doi.org/10.1038/s41598-020-61953-9

Kneynsberg, A., Collier, T. J., Manfredsson, F. P., & Kanaan, N. M. (2016). Quantitative and semi-quantitative measurements of axonal degeneration in tissue and primary neuron cultures. *Journal of Neuroscience*

Methods, *266*, 32–41. https://doi.org/10.1016/j.jneumeth.2016.03.004

Komura, D., & Ishikawa, S. (2018). Machine learning methods for histopathological image analysis. *Computational and Structural Biotechnology Journal*, *16*, 34–42. https://doi.org/10.1016/j.csbj.2018.01.001

Kraus, O. Z., Ba, J. L., & Frey, B. J. (2016). Classifying and segmenting microscopy images with deep multiple instance learning. *Bioinformatics (Oxford, England)*, *32*(12), i52–i59. https://doi.org/10.1093/bioinformatics/btw252

Kraus, O. Z., Grys, B. T., Ba, J., Chong, Y., Frey, B. J., Boone, C., & Andrews, B. J. (2017). Automated analysis of high-content microscopy data with deep learning. *Molecular Systems Biology*, *13*(4), 924. https://doi.org/10.15252/msb.20177551

Kushibar, K., Valverde, S., González-Villà, S., Bernal, J., Cabezas, M., Oliver, A., & Lladó, X. (2018). Automated sub-cortical brain structure segmentation combining spatial and deep convolutional features. *Medical Image Analysis*, *48*, 177–186. https://doi.org/10.1016/j.media.2018.06.006

Larsen, K. B. (2017). Using the optical fractionator to estimate total cell numbers in the normal and abnormal developing human forebrain. *Frontiers in Neuroanatomy*, *11*, 112. https://doi.org/10.3389/fnana.2017.00112

Laurinavicius, A., Laurinaviciene, A., Dasevicius, D., Elie, N., Plancoulaine, B., Bor, C., & Herlin, P. (2012). Digital image analysis in pathology: Benefits and obligation. *Analytical Cellular Pathology (Amsterdam)*, *35*(2), 75–78. https://doi.org/10.3233/acp-2011-0033

LeCun, Y., Bengio, Y., & Hinton, G. (2015). Deep learning. *Nature*, *521*(7553), 436–444. https://doi.org/10.1038/nature14539

Litjens, G., Kooi, T., Bejnordi, B. E., Setio, A. A. A., Ciompi, F., Ghafoorian, M., … Sánchez, C. I. (2017). A survey on deep learning in medical image analysis. *Medical Image Analysis*, *42*, 60–88. https://doi.org/10.1016/j.media.2017.07.005

Liu, Y., Unsal, H. S., Tao, Y., & Zhang, N. (2020). Automatic brain extraction for rodent MRI images. *Neuroinformatics*, *18*(3), 395–406. https://doi.org/10.1007/s12021-020-09453-z

Madabhushi, A., & Lee, G. (2016). Image analysis and machine learning in digital pathology: Challenges and opportunities. *Medical Image Analysis*, *33*, 170–175. https://doi.org/10.1016/j.media.2016.06.037

Majeed, H., Keikhosravi, A., Kandel, M. E., Nguyen, T. H., Liu, Y., Kajdacsy-Balla, A., … Popescu, G. (2019). Quantitative histopathology of stained tissues using color spatial light interference microscopy (cSLIM). *Scientific Reports*, *9*(1), 14679. https://doi.org/10.1038/s41598-019-50143-x

Marblestone, A. H., Wayne, G., & Kording, K. P. (2016). Toward an integration of deep learning and neuroscience. *Frontiers in Computational Neuroscience*, *10*, 94. https://doi.org/10.3389/fncom.2016.00094

McQuin, C., Goodman, A., Chernyshev, V., Kamentsky, L., Cimini, B. A., Karhohs, K. W., … Carpenter, A. E. (2018). CellProfiler 3.0: Next-generation image processing for biology. *PLoS Biology*, *16*(7), e2005970. https://doi.org/10.1371/journal.pbio.2005970

Mehta, R., Majumdar, A., & Sivaswamy, J. (2017). BrainSegNet: A convolutional neural network architecture for automated segmentation of human brain structures. *Journal of Medical Imaging (Bellingham, Wash.)*, *4*(2), 024003. https://doi.org/10.1117/1.Jmi.4.2.024003

Meyerholz, D. K., & Beck, A. P. (2018). Fundamental concepts for semiquantitative tissue scoring in translational research. *ILAR Journal*, *59*(1), 13–17. https://doi.org/10.1093/ilar/ily025

Moen, E., Bannon, D., Kudo, T., Graf, W., Covert, M., & Van Valen, D. (2019). Deep learning for cellular image analysis. *Nature Methods*, *16*(12), 1233–1246. https://doi.org/10.1038/s41592-019-0403-1

Monzel, A. S., Hemmer, K., Kaoma, T., Smits, L. M., Bolognin, S., Lucarelli, P., … Schwamborn, J. C. (2020). Machine learning-assisted neurotoxicity prediction in human midbrain organoids. *Parkinsonism & Related Disorders*, *75*, 105–109. https://doi.org/10.1016/j.parkreldis.2020.05.011

Mouton, P. R., Phoulady, H. A., Goldgof, D., Hall, L. O., Gordon, M., & Morgan, D. (2017). Unbiased estimation of cell number using the automatic optical fractionator. *Journal of Chemical Neuroanatomy*, *80*, A1–a8. https://doi.org/10.1016/j.jchemneu.2016.12.002

Mysona, B. A., Segar, S., Hernandez, C., Kim, C., Zhao, J., Mysona, D., & Bollinger, K. E. (2020). QuPath automated analysis of optic nerve degeneration in Brown Norway rats. *Translational Vision Science & Technology*, *9*(3), 22. https://doi.org/10.1167/tvst.9.3.22

Navale, V., & Bourne, P. E. (2018). Cloud computing applications for biomedical science: A perspective. *PLoS Computational Biology*, *14*(6), e1006144. https://doi.org/10.1371/journal.pcbi.1006144

Oguz, I., Zhang, H., Rumple, A., & Sonka, M. (2014). RATS: Rapid automatic tissue segmentation in rodent brain MRI. *Journal of Neuroscience Methods*, *221*, 175–182. https://doi.org/10.1016/j.jneumeth.2013.09.021

Olesen, M. V., Needham, E. K., & Pakkenberg, B. (2017). The optical fractionator technique to estimate cell numbers in a rat model of electroconvulsive therapy. *Journal of Visualized Experiments*, *125*. https://doi.org/10.3791/55737

Pantanowitz, L. (2010). Digital images and the future of digital pathology. *Journal of Pathology Informatics*, *1*, 15. https://doi.org/10.4103/2153-3539.68332

Park, S., Pantanowitz, L., & Parwani, A. V. (2012). Digital imaging in pathology. *Clinics in Laboratory Medicine*, *32*(4), 557–584. https://doi.org/10.1016/j.cll.2012.07.006

Penttinen, A. M., Parkkinen, I., Blom, S., Kopra, J., Andressoo, J. O., Pitkänen, K., … Airavaara, M. (2018). Implementation of deep neural networks to count dopamine neurons in substantia nigra. *The European Journal of Neuroscience*, *48*(6), 2354–2361. https://doi.org/10.1111/ejn.14129

Phan, N., Dou, D., Piniewski, B., & Kil, D. (2016). A deep learning approach for human behavior prediction with explanations in health social networks: Social restricted Boltzmann machine (SRBM(+)). *Social Network Analysis and Mining*, *6*. https://doi.org/10.1007/s13278-016-0379-0

Richards, B. A., Lillicrap, T. P., Beaudoin, P., Bengio, Y., Bogacz, R., Christensen, A., … Kording, K. P. (2019). A deep learning framework for neuroscience. *Nature Neuroscience*, *22*(11), 1761–1770. https://doi.org/10.1038/s41593-019-0520-2

Scallet, A. C., Pothuluri, N., Rountree, R. L., & Matthews, J. C. (2000). Quantitating silver-stained neurodegeneration: The neurotoxicity of trimethlytin (TMT) in aged rats. *Journal of Neuroscience Methods*, *98*(1), 69–76. https://doi.org/10.1016/s0165-0270(00)00191-6

Schafer, K. A., Eighmy, J., Fikes, J. D., Halpern, W. G., Hukkanen, R. R., Long, G. G., … Francke, S. (2018). Use of severity grades to characterize histopathologic changes. *Toxicologic Pathology*, *46*(3), 256–265. https://doi.org/10.1177/0192623318761348

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682. https://doi.org/10.1038/nmeth.2019

Schmitz, C., & Hof, P. R. (2000). Recommendations for straightforward and rigorous methods of counting neurons based on a computer simulation approach. *Journal of Chemical Neuroanatomy*, *20*(1), 93–114. https://doi.org/10.1016/s0891-0618(00)00066-1

Schmitz, C., & Hof, P. R. (2005). Design-based stereology in neuroscience. *Neuroscience*, *130*(4), 813–831. https://doi.org/10.1016/j.neuroscience.2004.08.050

Schmitz, C., Korr, H., & Heinsen, H. (1999). Design-based counting techniques: The real problems. *Trends in Neurosciences*, *22*(8), 345–346. https://doi.org/10.1016/s0166-2236(99)01418-6

Schmued, L. C., & Hopkins, K. J. (2000). Fluoro-Jade B: A high affinity fluorescent marker for the localization of neuronal degeneration. *Brain Research*, *874*(2), 123–130. https://doi.org/10.1016/s0006-8993(00)02513-0

Schmued, L. C., Stowers, C. C., Scallet, A. C., & Xu, L. (2005). Fluoro-Jade C results in ultra high resolution and contrast labeling of degenerating neurons. *Brain Research*, *1035*(1), 24–31. https://doi.org/10.1016/j.brainres.2004.11.054

Shen, D., Wu, G., & Suk, H. I. (2017). Deep learning in medical image analysis. *Annual Review of Biomedical Engineering*, *19*, 221–248. https://doi.org/10.1146/annurev-bioeng-071516-044442

Signaevsky, M., Prastawa, M., Farrell, K., Tabish, N., Baldwin, E., Han, N., … Crary, J. F. (2019). Artificial intelligence in neuropathology: Deep learning-based assessment of tauopathy. *Laboratory Investigation; a Journal of Technical Methods and Pathology*, *99*(7), 1019–1029. https://doi.org/10.1038/s41374-019-0202-4

Sikpa, D., Fouquet, J. P., Lebel, R., Diamandis, P., Richer, M., & Lepage, M. (2019). Automated detection and quantification of breast cancer brain metastases in an animal model using democratized machine learning tools. *Scientific Reports*, *9*(1), 17333. https://doi.org/10.1038/s41598-019-53911-x

Srivastava, A., Liachenko, S., Sarkar, S., Paule, M., Negi, G., Pandey, J. P., & Hanig, J. P. (2020). Quantitative neurotoxicology: An assessment of the neurotoxic profile of kainic acid in Sprague Dawley rats. *International Journal of Toxicology*, *39*(4), 294–306. https://doi.org/10.1177/1091581820928497

Suleymanova, I., Balassa, T., Tripathi, S., Molnar, C., Saarma, M., Sidorova, Y., & Horvath, P. (2018). A deep convolutional neural network approach for astrocyte detection. *Scientific Reports*, *8*(1), 12878. https://doi.org/10.1038/s41598-018-31284-x

Switzer, R. C. 3rd. (2000). Application of silver degeneration stains for neurotoxicity testing. *Toxicologic Pathology*, *28*(1), 70–83. https://doi.org/10.1177/019262330002800109

Tan, C., Guan, Y., Feng, Z., Ni, H., Zhang, Z., Wang, Z., … Li, A. (2020). DeepBrainSeg: Automated brain region segmentation for micro-optical images with a convolutional neural network. *Frontiers in Neuroscience*, *14*, 179. https://doi.org/10.3389/fnins.2020.00179

Tang, T. T., Zawaski, J. A., Francis, K. N., Qutub, A. A., & Gaber, M. W. (2019). Image-based classification of tumor type and growth rate using machine learning: A preclinical study. *Scientific Reports*, *9*(1), 12529. https://doi.org/10.1038/s41598-019-48738-5

Turner, O. C., Aeffner, F., Bangari, D. S., High, W., Knight, B., Forest, T., … Sebastian, M. M. (2020). Society of toxicologic pathology digital pathology and image analysis special interest group article*: Opinion on the application of artificial intelligence and machine learning to digital toxicologic pathology. *Toxicologic Pathology*, *48*(2), 277–294. https://doi.org/10.1177/0192623319881401

Vahid, A., Mückschel, M., Neuhaus, A., Stock, A. K., & Beste, C. (2018). Machine learning provides novel neurophysiological features that predict performance to inhibit automated responses. *Scientific Reports*, *8*(1), 16235. https://doi.org/10.1038/s41598-018-34727-7

Vandenberghe, M. E., Hérard, A. S., Souedet, N., Sadouni, E., Santin, M. D., Briet, D., … Delzescaux, T. (2016). High-throughput 3D whole-brain quantitative histopathology in rodents. *Scientific Reports*, *6*, 20958. https://doi.org/10.1038/srep20958

Wang, S., Lin, B., Lin, G., Lin, R., Huang, F., Liu, W., … Chen, J. (2020). Automated label-free detection of injured neuron with deep learning by two-photon microscopy. *Journal of Biophotonics*, *13*(1), e201960062. https://doi.org/10.1002/jbio.201960062

Webster, J. D., & Dunstan, R. W. (2014). Whole-slide imaging and automated image analysis: Considerations and opportunities in the practice of pathology. *Veterinary Pathology*, *51*(1), 211–223. https://doi.org/10.1177/0300985813503570

West, M. J. (1999). Stereological methods for estimating the total number of neurons and synapses: Issues of precision and bias. *Trends in Neurosciences*, *22*(2), 51–61. https://doi.org/10.1016/s0166-2236(98)01362-9

Xing, F., & Yang, L. (2016). Robust nucleus/cell detection and segmentation in digital pathology and microscopy images: A comprehensive review. *IEEE Reviews in Biomedical Engineering*, *9*, 234–263. https://doi.org/10.1109/rbme.2016.2515127

Yamashita, R., Nishio, M., Do, R. K. G., & Togashi, K. (2018). Convolutional neural networks: An overview and application in radiology. *Insights into Imaging*, *9*(4), 611–629. https://doi.org/10.1007/s13244-018-0639-9

Ying, X., & Monticello, T. M. (2006). Modern imaging technologies in toxicologic pathology: An overview. *Toxicologic Pathology*, *34*(7), 815–826. https://doi.org/10.1080/01926230600918983

Zarella, M. D., Bowman, D., Aeffner, F., Farahani, N., Xthona, A., Absar, S. F., … Hartman, D. J. (2019). A practical guide to whole slide imaging: A white paper from the digital pathology association. *Archives of Pathology & Laboratory Medicine*, *143*(2), 222–234. https://doi.org/10.5858/arpa.2018-0343-RA

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