Automated Assessment of Necrotic Neurons and Dark Artifactual Neurons in Transgenic Mouse Model of Alzheimer's Disease Using Deep Learning

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INTRODUCTION

Neuronal necrosis is an end-stage, non-reversible cellular response to injury. Neuronal necrosis is considered as an adverse finding and therefore identifying the presence of necrotic neurons is an important endpoint in routine preclinical toxicology studies, animal model phenotyping, or in specialized neurotoxicity studies.

The main differential for neuronal necrosis in hematoxylin and eosin (HE)-stained brain sections are dark neuron artifacts, which are common histological artifacts in immersion-fixed brains. These can be difficult to differentiate from true necrotic neurons. Therefore, fluoro-Jade C (FJC) staining is often used to aid pathologists in the differentiation, since FJC identifies apoptotic, necrotic and degenerating neurons but not artifactual dark neurons.

In this study, we developed artificial intelligence (AI) models, using convolutional neural networks (CNN) to detect necrotic neurons and differentiate them from dark neuron artifacts. In addition, the AI model generated an accurate heat map to the most affected brain areas.

MATERIALS AND METHODS

Two supervised deep learning AI models were developed using Aiforia software and scanned whole slide images (WSIs) of coronal brain sections from 20 transgenic mice (TG) and 3 age-matched wildtype controls (Overview, **Figure 1**).

The first AI model consisted of 2 object feature classes that were trained to detect hypereosinophilic necrotic neurons (necrotic neuron type I) and dark neuron artifacts on HE-stained brain sections. Degenerate vacuolated neurons in early stages of necrosis (necrotic neuron type II) or end-stage necrotic faded neurons (ghost neurons or necrotic neuron type III) were not included in the AI training.

The second AI model included 1 object feature class, trained to detect necrotic neurons (showing fluorescent positivity) on FJC-stained brain sections. The analysis results from the 2 models (**Figure 2**) were visually evaluated by a board-certified veterinary pathologist experienced in neuropathology. Necrotic neurons were most commonly found in the hippocampal region of the brain (**Figure 3**), and their occurrence in this area was compared between HE-stained and FJC-stained sections.

RESULTS

The AI model accurately identified type I necrotic neurons, clearly distinguishing them from artifactual dark neurons in HE-stained sections and from autofluorescent cells in FJ-stained sections, with low error rates (**Figure 4**). The number of necrotic neurons in the brains of transgenic mice, along with dark artifactual neurons in both transgenic and wildtype brains, was substantial. Their spatial distribution matched the brain regions typically associated with these changes in the literature and was consistent with the pathologist's evaluation.

False positive cells in HE-stained sections were rare and primarily arising from out of focus nuclei next to red blood cells or from hypertrophied microglia. False positive cells in the FJC-stained sections were very rare and mainly originated from autofluorescent red blood cells. Degenerate vacuolated neurons and pale end-stage necrotic neurons (types II and III) were rare and excluded from the AI model's training data, so the model did not identify them in HE-stained sections.

Both AI models not only identified necrotic and artifactual dark neurons as individual objects but also incorporated low-magnification heatmaps (**Figure 2**). These heatmaps, generated from specific object detection results, guided the pathologist in identifying the most affected regions and the spatial distribution of lesions.

CONCLUSIONS

The AI-based detection of necrotic neurons in HE and FJC-stained sections closely matched the pathologist's evaluation, accurately distinguishing type I necrotic neurons from artifactual dark neurons in HE-stained sections. The few false positives—caused by autofluorescent red blood cells in FJC-stained sections or out-of-focus cells in HE-stained sections—could be further minimized with additional training data and by reducing autofluorescence levels during the preanalytical phase.

The transgenic mice in this study's training and analysis set had an unusually high density of necrotic neurons. Typically, the number of true necrotic neurons is lower, thus AI-supported analysis of brain sections could greatly enhance pathologist screening efficiency in more typical cases. This is particularly due to the heatmap-guided rapid detection of the most affected areas during the initial review of whole-slide images.

The AI models assist pathologists in locating and quantifying necrotic neurons in HE-stained sections and differentiating them from artifactual dark neurons. This support enhances objectivity in neuropathology studies by saving time and reducing variation between pathologists.

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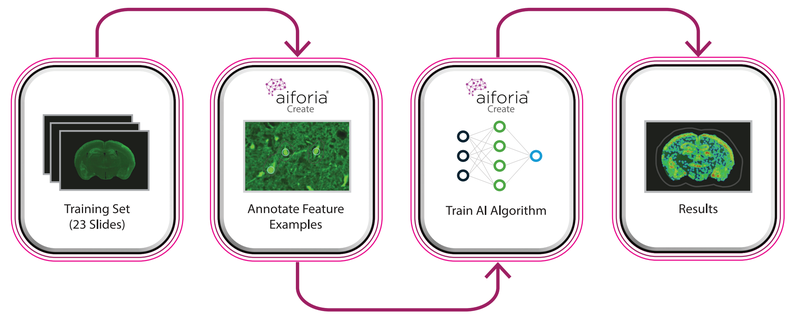

Figure 1. The AI model training and performance evaluation process.

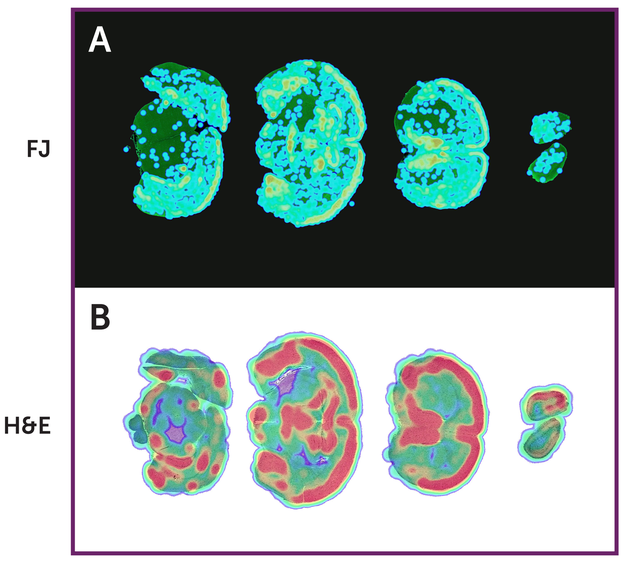

Figure 2. Low-power view of heat maps for necrotic neuron object detection, highlighting the most affected brain regions. **A.** Transgenic strain, FJC stained. **B.** Transgenic strain, H&E stain.

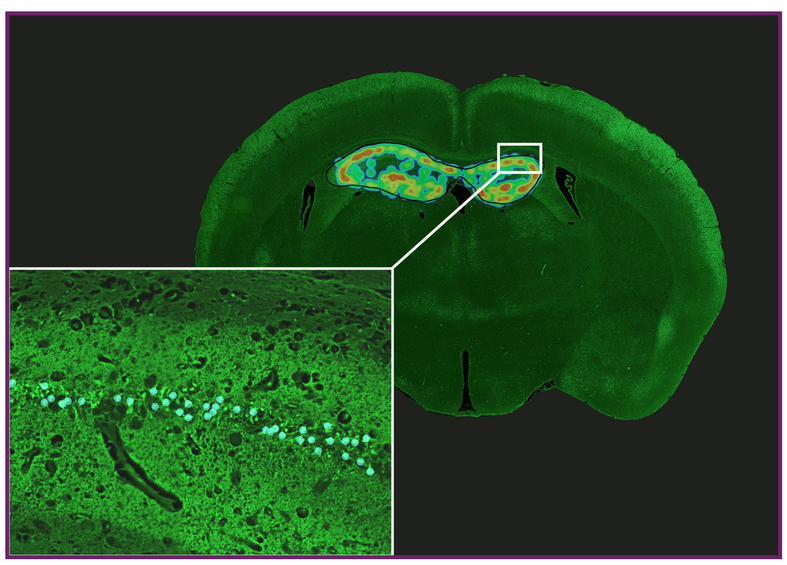

Figure 3. Low-power view of a FJC-stained TG rat brain section, with the region of interest focusing on the hippocampal area, displaying the heatmap of detected necrotic neurons. **Inset:** Close up view of object detection of necrotic neurons.

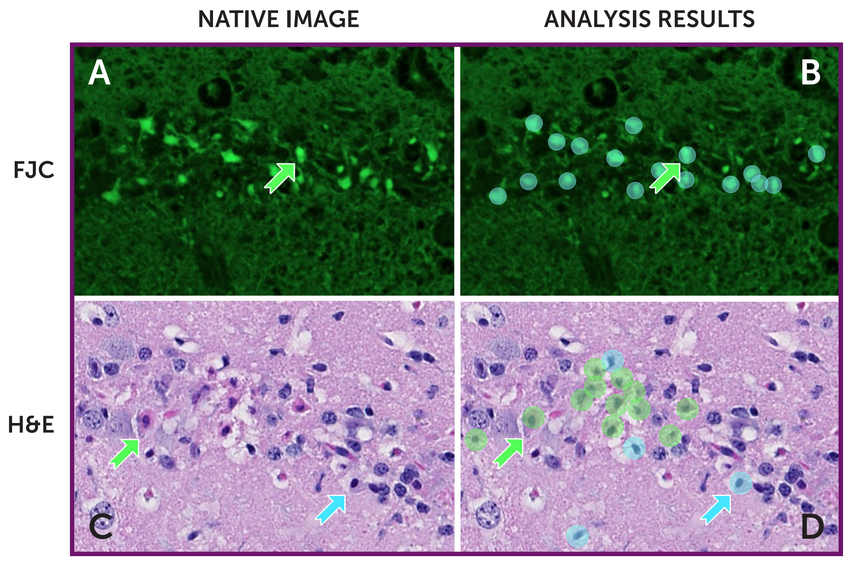

Figure 4. High power views of the hippocampal areas in serial TG rat brain sections. **A.** Native view of FJC stained section. **B.** Object detection overlay of the necrotic neurons. **C.** Native view of H&E stained section. **D.** Object detection overlay of the necrotic (Green dots, arrow example) and artifactal dark neurons (Blue dots, arrow example).