

Algorithmic Estimation of Cortical Autolysis in Post-Mortem Adult Rat Brains: Summary

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1. Introduction

The brain is a delicate organ whose ultrastructural organization is particularly important in the life of the organism. For this reason it is also specially important as an object of cryopreservation for the purpose of eventual resuscitation of the organism. It thus is desirable be able to estimate the extent of damage to the brain that may expected postmortem before preservation in some form (including tissue fixation as well as cryopreservation) can be effected. This article briefly summarizes efforts to estimate computationally the extent of degradation in cortical rat brain tissue as a function of postmortem, warm ischemic exposure. (A more detailed report of this study should eventually appear.) The efforts involved analysis of electron-microscope (EM) images of cortical rat brain tissue that had been exposed to different periods of warm ischemia prior to fixation. A “learning” algorithm was developed using Mathematica which could be “trained” on a series of achromatic images expressed as numerical arrays to estimate the corresponding ischemic times. The algorithm would then, in theory, be able to estimate the ischemic time for other, not previously processed images without any prior knowledge of the samples that were imaged. In this way it would be able to assess the extent of damage, expressed in units of ischemic time at constant, body temperature, and the correctness of its assessment would be reflected in how accurately it was able to estimate the ischemic exposure. It was hoped that insight would be gained into the process of tissue deterioration under ischemia, as well as a method being obtained to assess the quality of any preservative method. The method in fact achieved only limited success, but some useful insight was obtained, and an algorithmic procedure was developed that might possibly find other uses beyond its original, intended scope.

2. Methods

A master algorithm was given a batch of EM images, “primary training images” (PTIs) in a standardized format covering tissue samples exposed to different ischemic times. The master algorithm then constructed a working algorithm which, given an image expressed as a numerical array, would crunch the array down to a single number giving the ischemic time in hours. The working algorithm in turn had two parts whose functions respectively were (1) to process the image into a “signature” vector of coefficients, and (2) to inner-product the vector with a second, “scaling” vector to obtain the final (scalar) output, the estimated ischemic time. Processing the image involved reducing it to a one-dimensional signature by a power spectrum analysis of its 2-dimensional fourier transform followed by a further reduction to products of powers of coefficients of an approximating polynomial. The scaling vector in turn was to be determined by the master algorithm from a coefficient matrix whose rows were the signature vectors of the PTIs in a chosen order.

The problem for the master algorithm then became that of finding a scaling vector whose product with the coefficient matrix would fit the known ischemic times in a least-squares sense. In practice a very good-fitting scaling vector could be obtained in this way, but the vector itself was ill-conditioned (contained very large-magnitude entries) making it useless for processing other images the master algorithm had not seen before. In short the working algorithm was too “strong” and had to be weakened and otherwise adapted so it would apply to other images besides the PTIs. This was accomplished by a method that used singular value decomposition of the coefficient matrix, with Tikhonoff regularization for reduction in size of the reciprocal-singular values.[1] A working algorithm could then be obtained that would produce a least-squares fit to an expanded set of images including, besides the original, PTIs, a representative sampling of “secondary training images” (STIs) that the master algorithm had not seen before. In practice the fitting to the original images was no longer nearly perfect, but by way of compensation was much better on the STIs. The working algorithm could then be applied to other, similar but previously unseen “followup test images” (FTIs) with results comparable to those obtained for the PTIs and STIs.

3. An Illustration

To illustrate the above principles and verify that the program was working correctly, a series of random, cloud-pattern images was generated mathematically and subjected to varying amounts of simple forms of degradation. In this way sets of PTIs, STIs and FTIs were numerically created for the master algorithm. Figure 1 shows an image, dimensioned 256 x 256, that is similar to one of the original, undegraded images, and the same image with two forms of degradation, blurring (gaussian filter) and noise (normal distribution), both by 50 arbitrary units corresponding subjectively to a heavy effect.

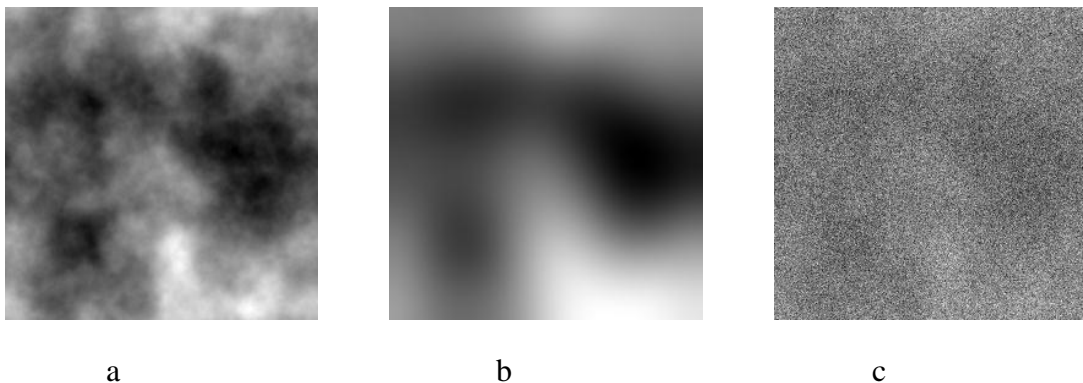


Fig. 1. A random cloud image: (a) original (undegraded); (b) heavily blurred; (c) not blurred, but with heavy added noise.

Figure 2 shows results of running the program which in this case is attempting to estimate how much degradation an image received, for the two cases of blurring and noise. Results for PTIs are represented by green dots, STIs cyan dots, FTIs red dots. In each case the PTIs and STIs cover degradation exposures from 0-50 spaced at intervals of 5 (11 exposure points in all). For the FTIs the degradation amounts are spaced at intervals of 2.5 (21 points in all) so there are FTIs both at the same exposures as the PTIs and STIs, and at the midpoints between these exposures. In this way the working algorithm can be tested both for images it has never seen (the FTIs in general) and also for degradation exposures it was not trained on, to see if it will still make the correct estimates. For all types of images there are three images per exposure point with all images different from one another, starting out as random cloud patterns (129 images in all). Errors in the working algorithm's estimates are shown as deviations from the blue reference line. As can be seen, there is at least a rough fit for all classes of images and both types of degradation, with a better fit for noise than for blurring.

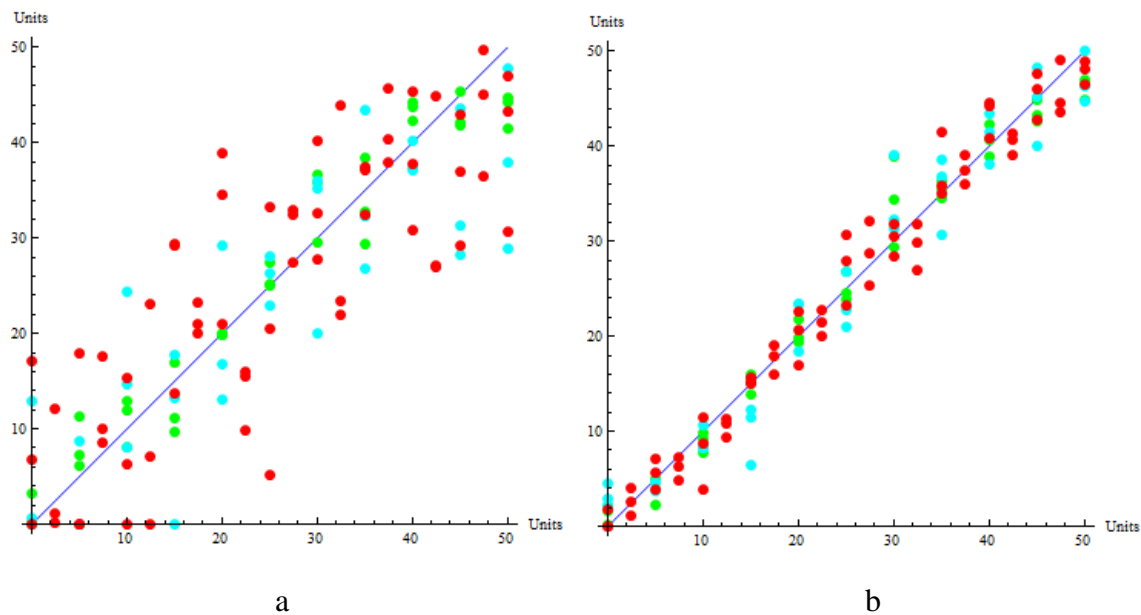


Fig. 2. Performance of working algorithms for estimating image degradation for (a) blurring and (b) noise. X-axis is actual amount of degradation, Y-axis is estimated amount; errors are shown as deviations from blue reference line. Green dots correspond to primary training images, cyan (blue-green) to secondary training images, and red to followup test images.

4. Main Results

The main study focused on damage to brain tissue caused by warm ischemia, as shown in EM images. The images themselves were not degraded but the tissue they recorded was; it was hoped that the extent of this degradation could be estimated in much the same way as with the practice examples above.

The study commenced with preliminary work using a limited number of EM images that were obtained in a previous study and kindly made available to the author.[2] This in turn used male Wistar rats approximately 100 days old and weighing approximately 400g. The rats were euthanized and their cadavers stored at room temperature. Their brains were dissected at 1, 3, 6, 9, 12, and 24 hours postmortem and EM images in 1024 x 1024 format were prepared showing cortical tissue at various magnifications ranging down to a pixel size of about 2 nm. The images were downsized as necessary to a standard pixel length of 6.15 nm and cropped to 512 x 512 format. Results of a typical calculation are shown in Figure 3 (only PTIs and STIs; 3 PTIs and one STI per exposure point). Though the fitting appears rather good, there were significant shortcomings in obtaining the results, such as the absence of temperature control and the need to numerically process some of the images to obtain a large enough number in a desired format. These deficiencies led to followup, original work using a larger number of samples with formats standardized and ischemic temperature controlled.

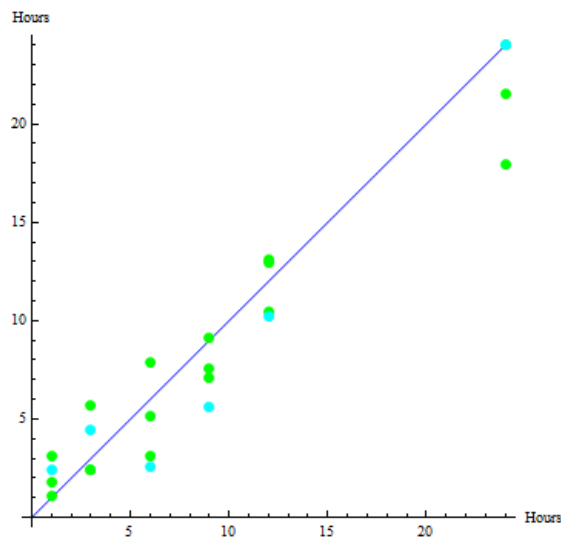


Fig. 3. Results obtained with algorithm for estimating ischemic times using images from a previous study, with ischemic exposure starting at rat body temperature. X-axis is actual ischemic time in hours, y-axis estimated ischemic time. Only PTIs (green, three per exposure) and STIs (cyan, one per exposure) are shown.

The followup work used male Sprague-Dawley rats approximately 100 days old and weighing approximately 400 g. The rats were euthanized and their cadavers maintained at rat body temperature (37°C) for 0.00, 1.00, 2.25, 3.86, 6.00, 9.00, 13.50, 21.00, 36.00, and 81.00 hours before their brains were fixed, dissected, and samples prepared for EM imaging. The choice of exposure times followed a simple formula which it was thought might simplify mathematical analysis and did in fact have some significance in presenting the results (see below). There was an additional exposure at 24.00 hours which provided at least one data point for the FTIs that was not found in the PTIs or STIs. (In fact these samples used cold, bloodless ischemic exposure, with storage temperatures between 0° and 4°C, thus were not strictly comparable to the other images. They were used because they were the only available images with nonstandard exposures.) The images that were used were in 1024 x 1024 format with pixel length of 3.0nm (figure 4). Typical results are shown in figure 5, a rather noisy fit somewhat in contrast to figure 3. In 5(a) the ischemic times are fit directly where in 5(b) an “exposure index” u is used for fitting, where u is defined in terms of ischemic time t by $u = 10t/(9+t)$. This results in equal spacing for the chosen exposure points. The problem defined in this way was significantly different from that defined in the more straightforward case in which ischemic times were used directly but differed widely in spacing. As can be seen, the fitting is still noisy but rather resembles the earlier case of fitting blurred images (figure 2a), if two extreme outlier points on the x-axis are ignored. Over all it is clear that some fitting is occurring but it is crude, and not as impressive as the preliminary results obtained earlier (figure 3). It should be noted too that for this case each 1024 x 1024 image was split into 16 equal components dimensioned 256 x 256 each and results were averaged over the 16 cases to obtain the fitting for one exposure point. This appeared to improve the overall fitting a bit, though not substantially. (It will also be noted that the FTIs corresponding to the 24-hour exposure [$u=7.27$] fit well with the other cases despite the noted difference in the ischemic protocol. It is not clear how to interpret this very limited result but it does appear interesting.)

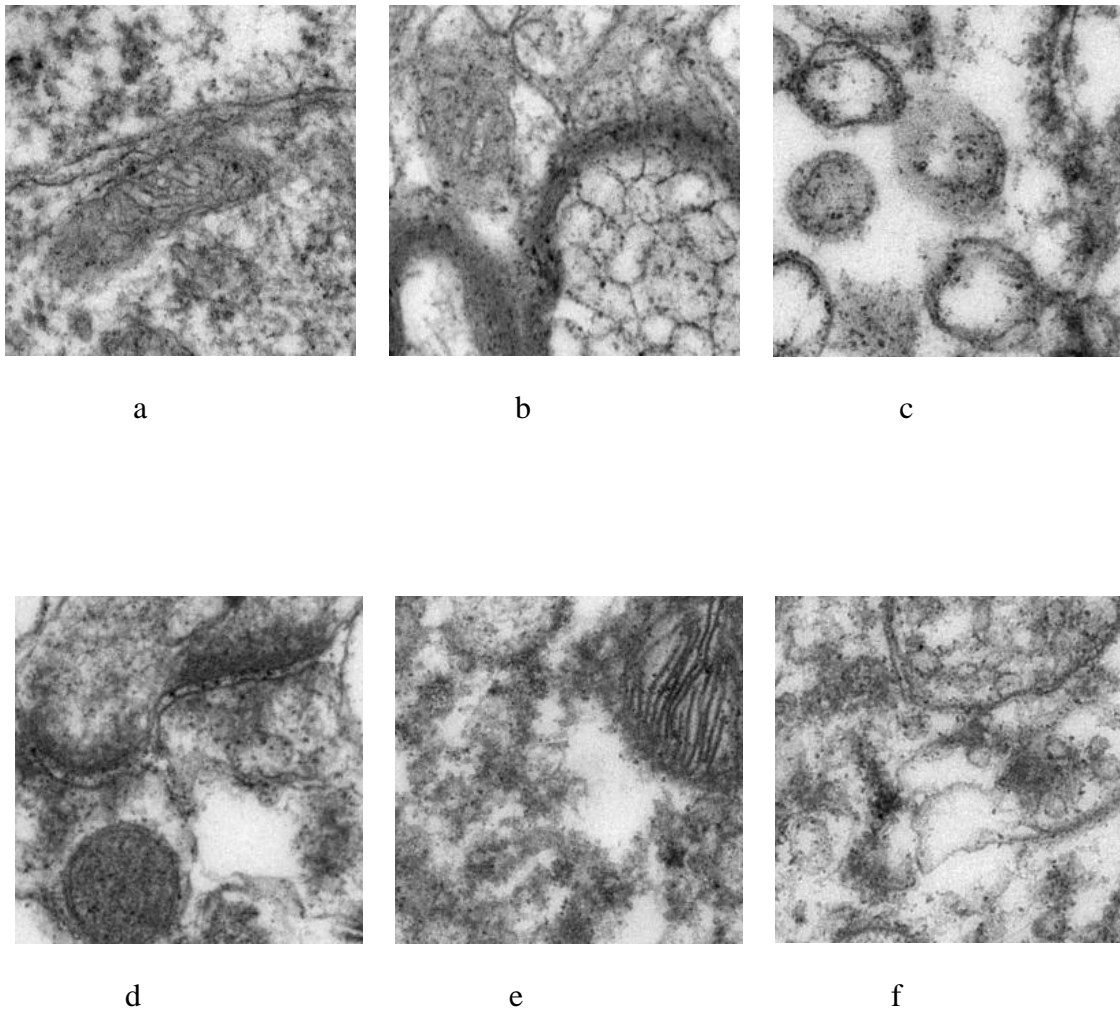


Fig. 4. Clippings from images used in the study showing rat brain cortical tissue with postmortem ischemic times of (a, b) 0, (c, d) 21, and (e, f) 81 hours. Each clipping is 256 x 256 pixels with 3.0 nm pixel width (about 750 nm total width) and corresponds to a central portion of the original, 1024 x 1024 images.

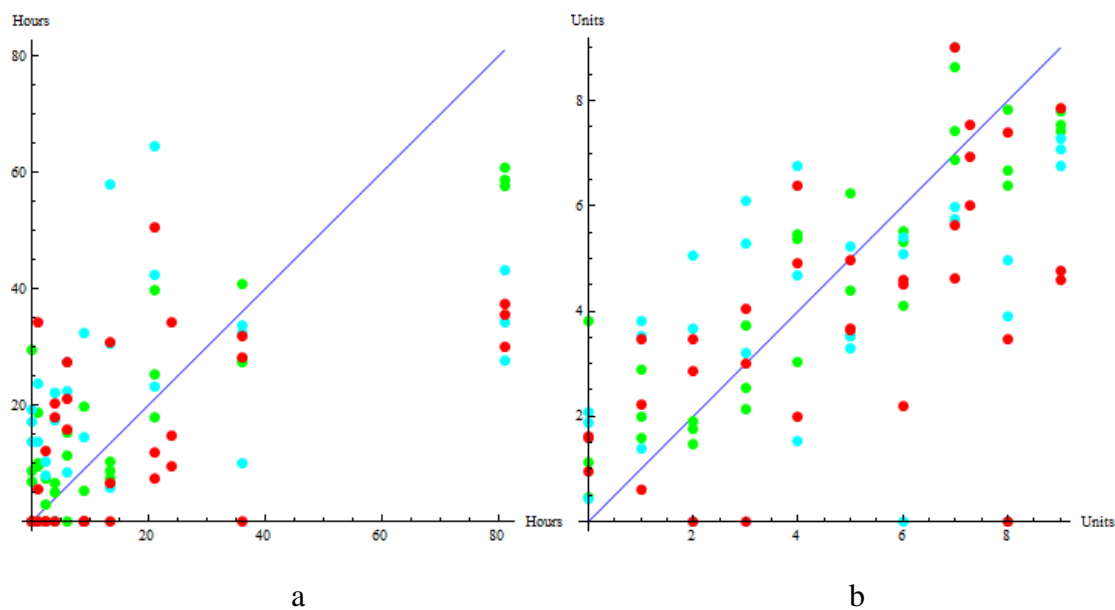


Fig. 5. Results of estimating ischemic times using the second series of images. (a) shows fit to ischemic times as in fig. 3, (b) shows fit to ischemic index u defined for ischemic time t by $u = 10t/(9+t)$.

5. Conclusions

This study appears to be the first attempt to quantitatively estimate ischemic times in brain tissue by algorithmic analysis of EM images. The method had only modest success and may not be suitable for practical applications. Alternatively, it may be that substantially better (if more costly) results would be obtained by averaging signatures over many whole images per exposure rather than over clippings from one, as was done here. Further study is called for, particularly in view of the unexplained, apparent early success followed by less satisfactory results when a more careful experimental methodology could be used. Optimistically, it is possible that some of

the difficulty in estimating ischemic times could be an indication that modest ischemia, up to a few hours, causes relatively little change in the brain's ultrastructure. Such a conclusion is far from demonstrated, however. Another source of difficulty could be the variability of tissue samples, making it more difficult for the algorithm to focus on features that should hold for broad classes of images. (This difficulty might be alleviated by more extensive averaging, as noted above.) It is worth saying that studies of ischemia would profit from the ability to observe the progressive changes in a single tissue sample rather than having to consider separate samples for each exposure point, as here. It is also worth noting that the method could have applications beyond that considered here, for example, in assessing other forms of material damage through image analysis.

References:

1. http://en.wikipedia.org/wiki/Tikhonov_regularization, accessed 11 July 2011.
2. Sergey V. Sheleg et. al., "Stability and autolysis of cortical neurons in post mortem adult rat brains," *Int J Clin Exp Pathol* (2008) 1, 291-299.

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