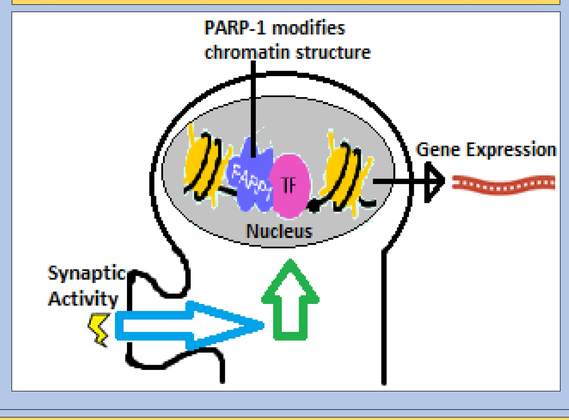
# ABSTRACT

Chromatin remodeling is an integral part of memory encoding, which helps process and store memories in the brain. Previously, the enzyme PARP-1 (PolyADP-ribose-polymerase 1) and its product PAR (PolyADP-ribose) have been implicated in the opening of DNA to transcription factors during chromatin remodeling to allow for gene expression necessary for memory encoding. I predicted a shift to less condensed chromatin in the trained neurons to account for the memory encoding activity. I also hypothesized that during learning, there would be an increase in PARP-1 and PAR expression related with chromatin relaxation necessary for the memory encoding model. I created a novel NetLogo-based computer program to quantify the expression of chromatin structure, PARP-1 and PAR within the nuclei of CA1 region hippocampal neurons of trained and untrained mice neurons based on their intensity values and distribution. Thresholds were placed on the pixels to analyze PAR and PARP-1 intensity distribution in different degrees of relaxed chromatin. The new program showed significant shifts to more relaxed degrees of chromatin structure and increases in the expression of PARP-1 in areas of more relaxed chromatin in trained neurons, indicating an active recruitment of PARP-1 to areas where chromatin is opened and suggesting that learning associated epigenetic changes occur during memory encoding. The program was also able to isolate learning associated changes in PARP-1 expression back to the neurons from which they came. These neurons are believed to be a part of the memory encoding network. This NetLogo-based computer program provides a computational analysis which uses PARP-1 and PAR as novel visual markers for detecting learning associated changes in neurons of trained mice.

# BACKGROUND

**Regulation of Gene Expression is Necessary for Memory Encoding**



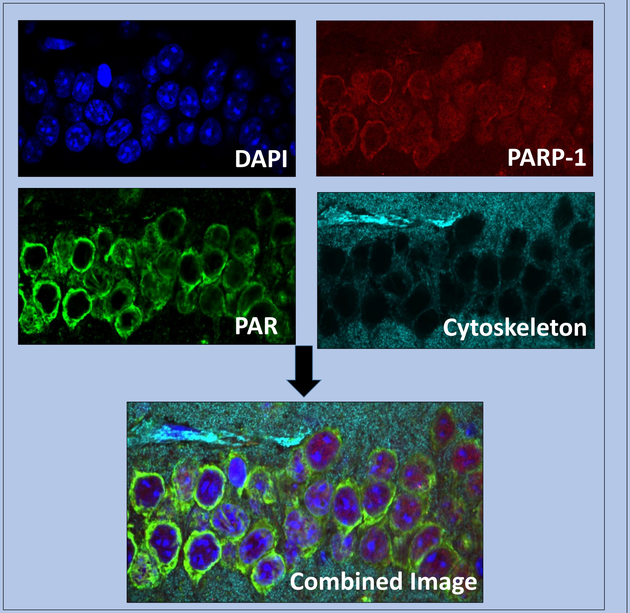

This figure shows that synaptic activity, in the form of a chemical or electrical signal, triggers an increase in PARP-1 recruitment in order to modify chromatin and regulate gene expression, necessary for consolidation of memory after learning.

The protein PARP-1 (PolyADP-ribose-polymerase 1) and its product PAR (PolyADP-ribose) are shown to catalyze PolyADP-Ribosylation, a modification of histones which relaxes chromatin and allows for transcription factors to bind to DNA and regulate gene expression during memory formation.

# METHODS

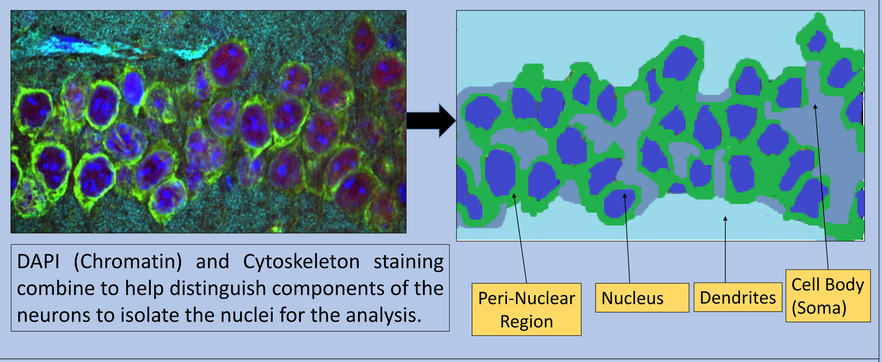
One group of mice were trained in a spatial task, while the other group was kept as a control group of untrained mice. Images of the CA1 regions of the Hippocampus of the brains of these mice were taken. The CA1 region of the Hippocampus is associated with encoding of spatial information. These slices were originally being used for a different experiment. Immunohistochemical methods and confocal microscopy were used to capture images of untrained and trained neurons from these mice.

## Confocal Images of CA1 Hippocampal Neurons




Immunohistochemical staining was used to fluorescently tag markers for Chromatin (Blue), PARP-1 (Red), PAR (Green), and Cytoskeleton (Light Blue). The combined images are inputs of the program and are quantified and analyzed by the program.

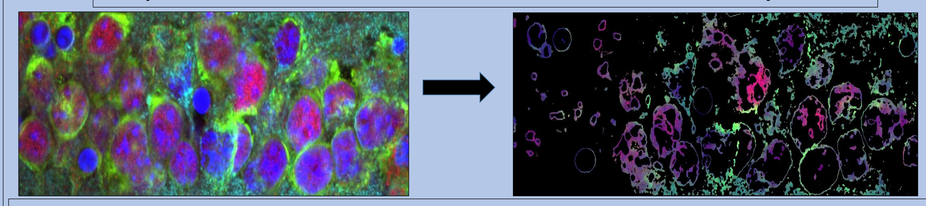
## 1: Using Fluorescent Tags to Distinguish Structural Components of Neurons




DAPI (Chromatin) and Cytoskeleton staining combine to help distinguish components of the neurons to isolate the nuclei for the analysis.

## 2: Imposing Chromatin Based Intensity Thresholds

Imposition of Threshold 3: 102-152 Arbitrary Units



Here the third threshold is imposed, placing a black mask over all pixels without a Chromatin (Blue) value between 102-152 AU. We see the corresponding Chromatin (Blue), PARP-1 (Red) and PAR (Green) values of the remaining pixels and can compare with untrained with trained and with other thresholds.

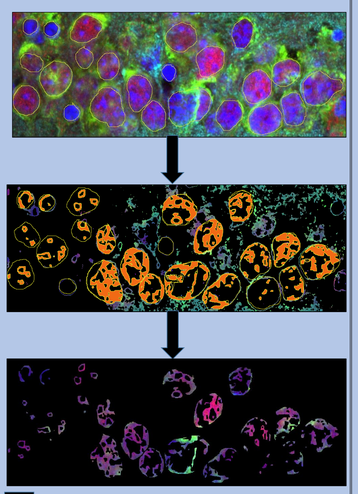
All pixels are given an individual Red, Green, and Blue value for intensity between 0-255 arbitrary units (AU). I divided the pixels into 5 arbitrary intensity thresholds based on the intensity values of the chromatin (degree of relaxation of chromatin), in order to compare PAR and PARP-1 expression in untrained and trained neuronal nuclei in different degrees of relaxed chromatin: 0-50 AU (the most relaxed and least intense chromatin), 51-101 AU, 102-152 AU, 153-203 AU, and 204-255 AU (the most condensed and intense chromatin).

## 3: Isolating Pixels Based on Intensity Distribution – Significant Shift in PARP-1 Expression

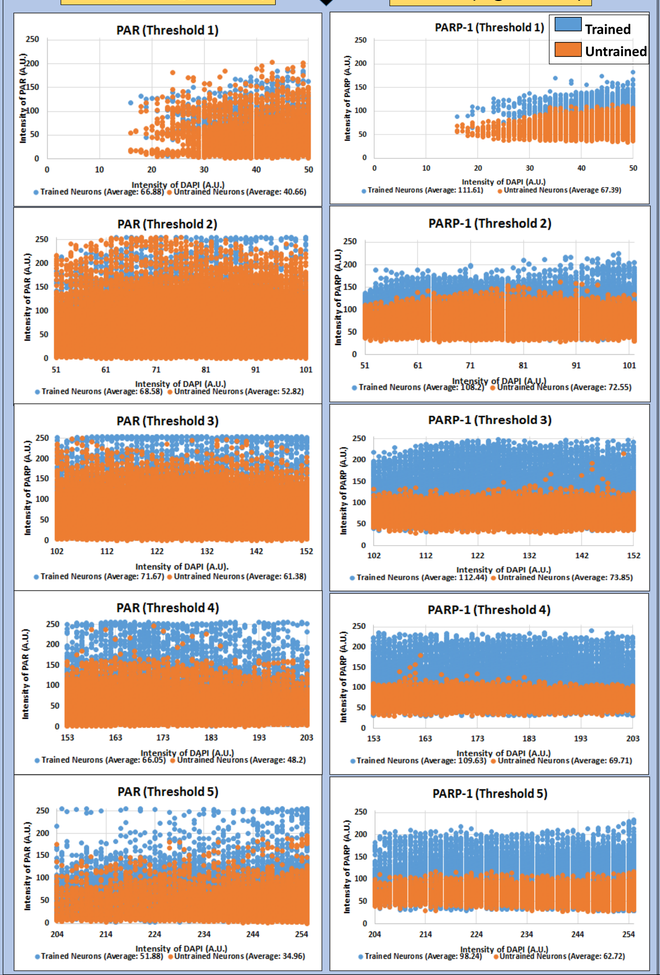
Using the program, regions of interest can be manually bordered (in this study we are looking at the nuclei).

The program isolates pixels in the nuclei which have chromatin intensity values within the indicated threshold, highlighted in orange.

The RGB values of these pixels of interest are collected and exported for analysis.

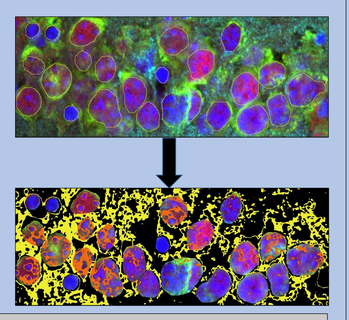


PAR (Not Significant) | PARP-1 (Significant)



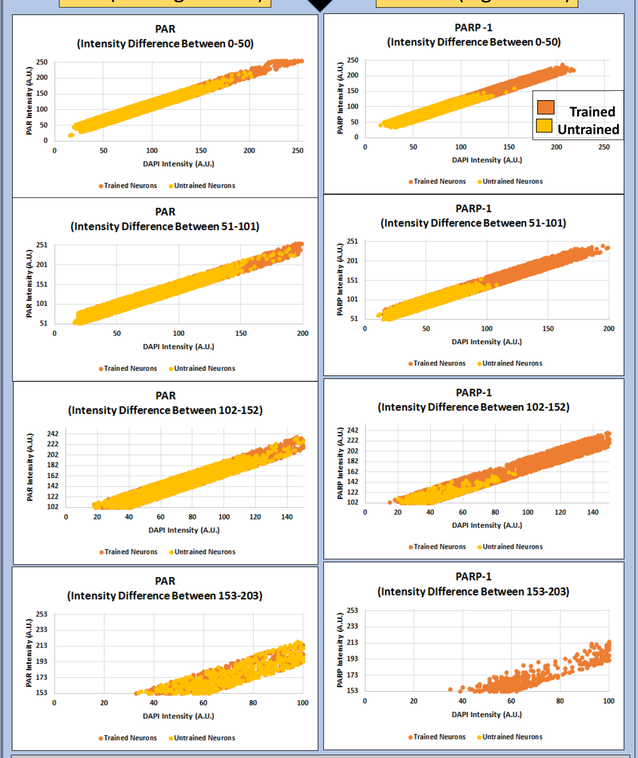

## 4: Isolating Pixels Based on Amount of Intensity Difference – Significant PARP-1 Increase

The program isolates pixels based on the difference between the PARP-1/PAR value and the chromatin value of the same pixel being within the given threshold. Only pixels with a R/G value higher than the B value were considered.



The pixels in yellow exhibit an intensity difference within the threshold, and those in orange are also in the nuclei.

PAR (Not Significant) | PARP-1 (Significant)

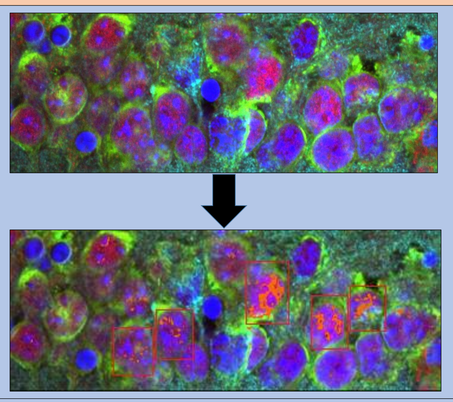



No pixels had PARP-1/PAR values which were 204-255 AU higher than the corresponding chromatin value.

The Intensity Distribution analysis shows a significant increase in PARP-1 expression in trained neurons, most significant in threshold 3 (chromatin intensities between 102-152 AU). PAR activity is insignificant, mostly mimicking baseline expression from the untrained neurons.

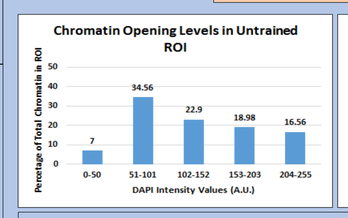
The Intensity Difference analysis shows more pixels in trained neurons exhibiting a larger difference between PARP-1 intensity and chromatin intensity. The amount of pixels exhibiting varied levels of intensity differences between PAR and chromatin remains similar in both types of neurons.

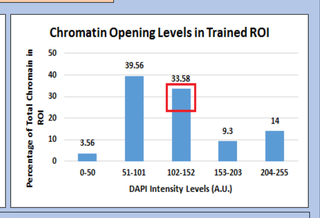
# TRACING CHANGES TO MEMORY ENCODING NEURONS



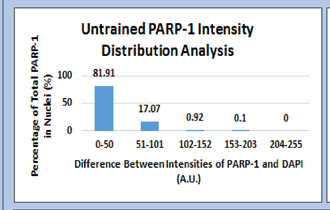
The program traced learning associated PARP-1 expression from threshold 3 (102-152 AU), where there was the greatest increase in PARP-1 intensity and the greatest increase in amount of pixels with large differences between PARP-1 and chromatin (relaxed chromatin and intense PARP-1). These neurons are the 20-25% believed to be involved in memory encoding. The other neurons mimic baseline activity, accounting for the overlap in expression.

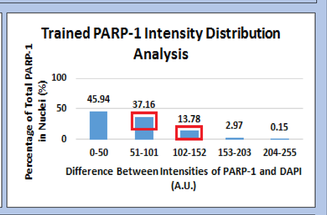
# ANALYSIS



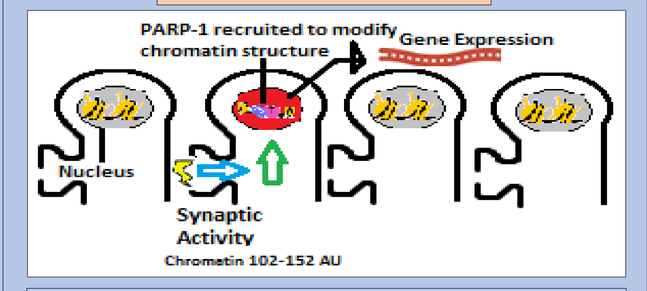

Trained neurons show a significant shift from chromatin in the most condensed thresholds 4 and 5 (153-255 AU) to a degree of relaxation in threshold 3 (102-152 AU) where we believe memory encoding activity occurs.




In trained neurons, there is a significant shift to more intense PARP-1 expression in areas of relaxed chromatin, resulting in more pixels exhibiting a larger difference between corresponding PARP-1 and chromatin values. This would indicate the increased recruitment of PARP-1 to areas of chromatin remodeling post-learning.

# CONCLUSIONS




The program detected learning-associated changes in the 102-152 AU range for trained neurons. This allows us to propose a model in which a subset of neurons are recruited for memory encoding and PARP-1 is only recruited to these neurons for the regulation of gene expression. The chromatin in these memory encoding neurons would relax to an intensity between 102-152 AU, an indicator of learning-associated changes. PARP-1 is a novel marker for learning-associated changes. The program can also be used to detect abnormal protein expression in neurological disorders and to locate neurons which should be studied in order to understand the neurophysiological effects of memory encoding in order to reverse the loss of cognitive function in neurological disorders.

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