



A Quantitative Assay of Immediate Early Gene Expression in the Mouse Hippocampus

Isha Mehrotra, Kelly Martyniuk, Pei-Ann Lin, Brenda Bloodgood

The Center for Neural Circuits and Behavior, University of California San Diego, 9500 Gilman Drive, La Jolla, California

Abstract

When a neuron is depolarized, calcium influx can result in the expression of immediate-early genes (IEGs). Npas4, c-Fos, and Arc are three Inducible transcription factors (ITFs) within this class of genes and are expressed as a result of certain levels of brain activity. Interestingly, while Npas4 is expressed specifically in response to calcium influx due to neural activity, c-Fos and Arc are also expressed in response to other stimuli such as neurotrophins and growth factors. The expression of IEGs can result in wide-spread changes in cells and circuits and result in changes in synaptic plasticity. For example, Bloodgood et al. found that Npas4 regulates inhibitory connectivity onto CA1 pyramidal cells in the mouse hippocampus. In these experiments, Npas4 was induced via naturalistic behavior by allowing mice to explore an enriched environment (EE) full of interactive toys. In contrast, mice exhibit very little Npas4 expression in the hippocampus when kept in Standard Housing (SH), an environment in which only basic needs are provided. This current study investigates the expression profiles of Npas4, Arc, and c-Fos in different subdomains of CA1 in response to exploration of an enriched environment. We found neurons that express one or multiple IEGs, suggesting that different expression thresholds might exist for Npas4, Arc, and c-Fos. Future work needs to be done to establish what specific activity patterns induce expression of the various IEGs, which will ultimately contribute to a better understanding of how activity shapes a neuron's role within a circuit.

Introduction

- Environmental stimuli provide inputs for processing, thus inducing brain activity.
- The activity induced Npas4 gene regulates inhibitory connectivity onto excitatory CA1 pyramidal cells in the hippocampus (Fig. 1), and therefore regulates the balance between excitatory and inhibitory brain activity (Fig. 2).
- Cognitive neurological disorders such as epilepsy, schizophrenia, and autism are linked to an imbalance in excitation and inhibition.
- When the membrane potential of a neuron reaches ~ -20 mV during an action potential, calcium influx results (Fig. 3).
- Lin et al. showed that Npas4 is induced more in EE than in SH. Feng et al. showed that c-Fos is also more induced in EE. Bloodgood et al. then showed that after 4 days in EE, inhibitory inputs onto the apical dendrite of CA1 pyramidal cells decrease, while inhibitory inputs onto the soma increase. In contrast, after 4 days in SH, no such changes are observed.
- The current study compares Npas4, c-Fos, and Arc expression in mice kept in EE and SH.
- Immunohistochemistry (IHC) and confocal microscopy was used to visualize protein expression, and the number of neurons expressing each IEG was quantified using ImageJ.

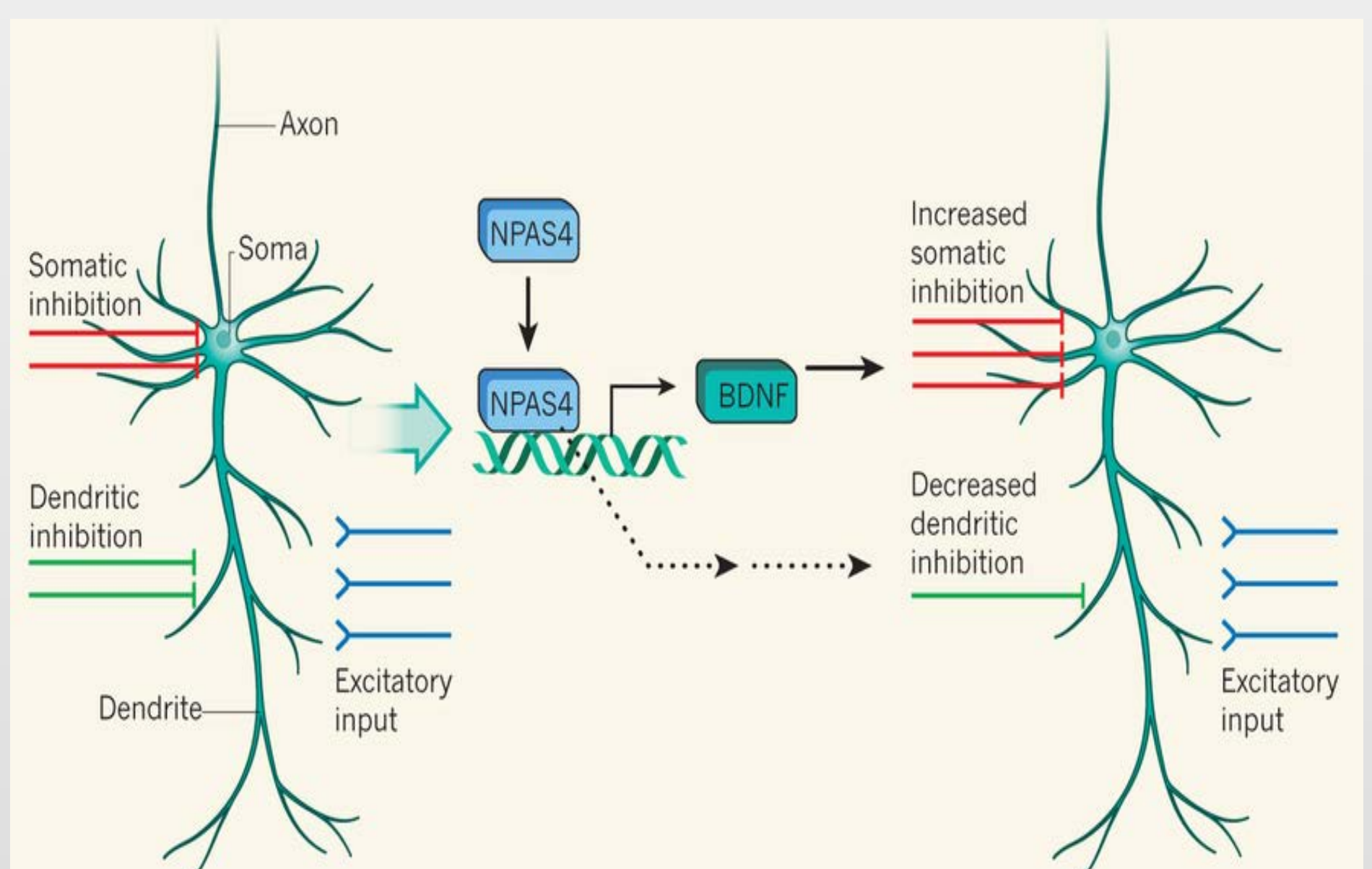


Figure 1 | Modulation of inhibition by Npas4. Behaviorally stimulated increase in brain activity induces Npas4 expression, which results in more inhibition onto the soma and less inhibition onto the proximal apical dendrites of CA1 pyramidal cells.³

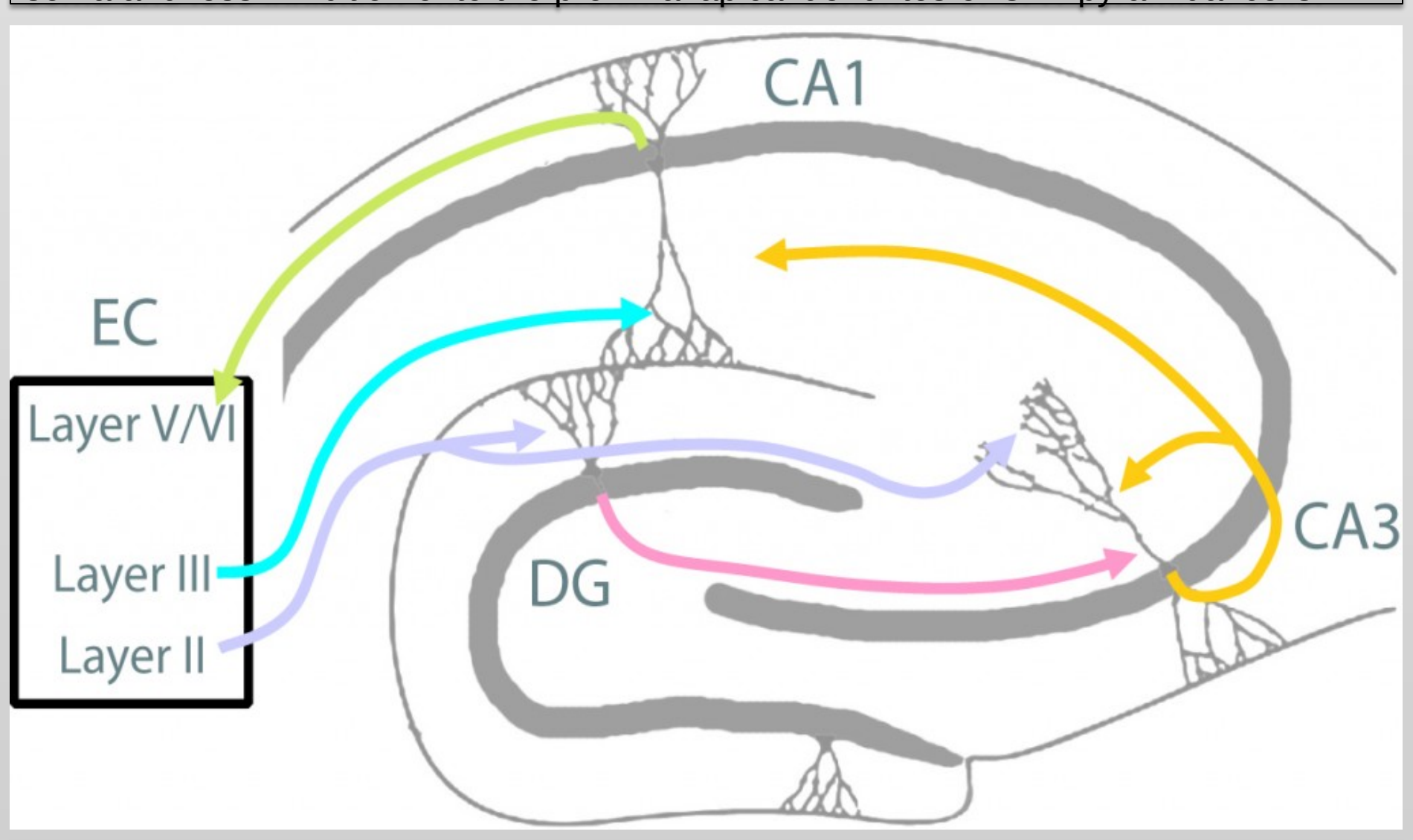


Figure 2 | Schematic of hippocampal subregions and circuitry.⁴

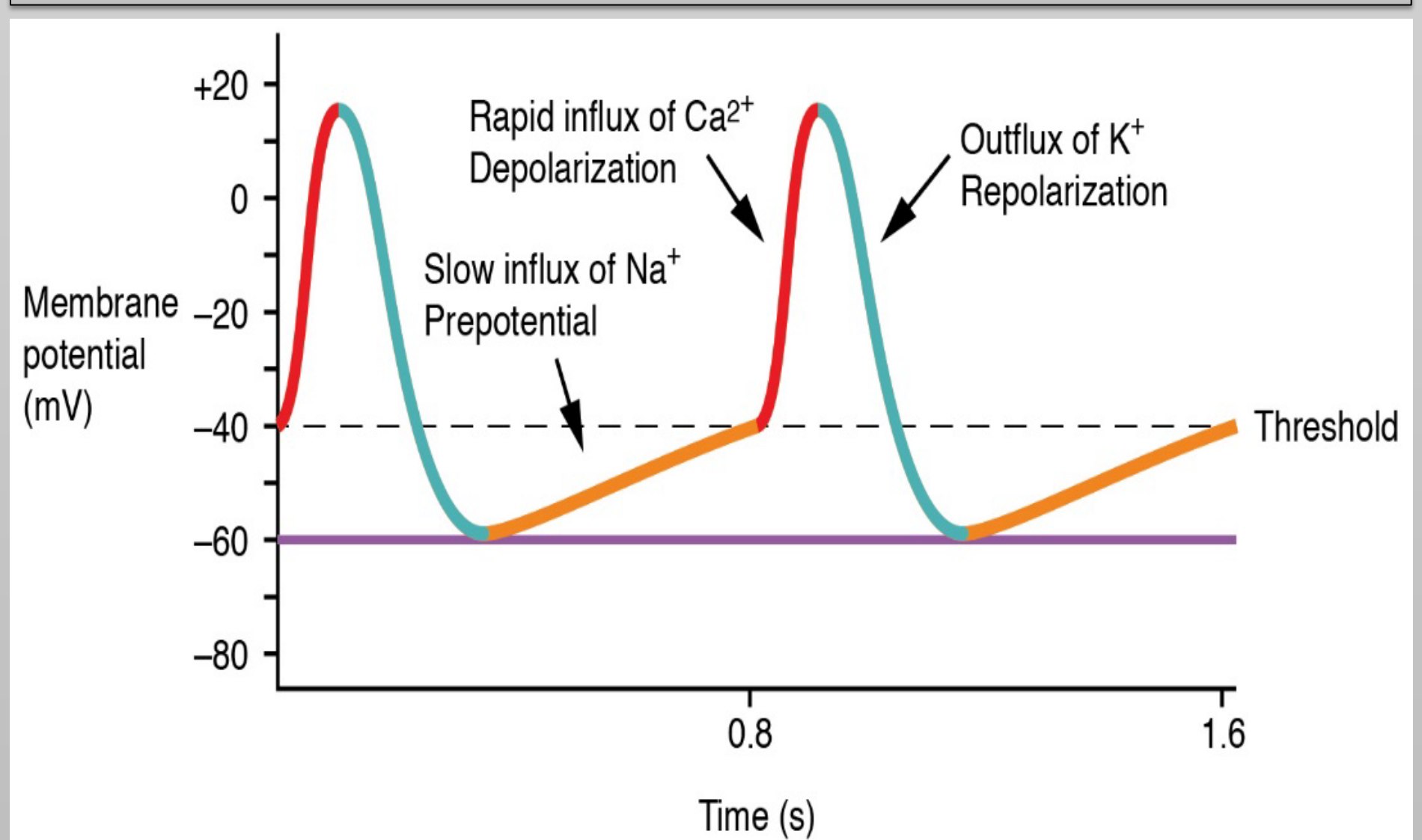


Figure 3 | Calcium influx during an action potential.⁵

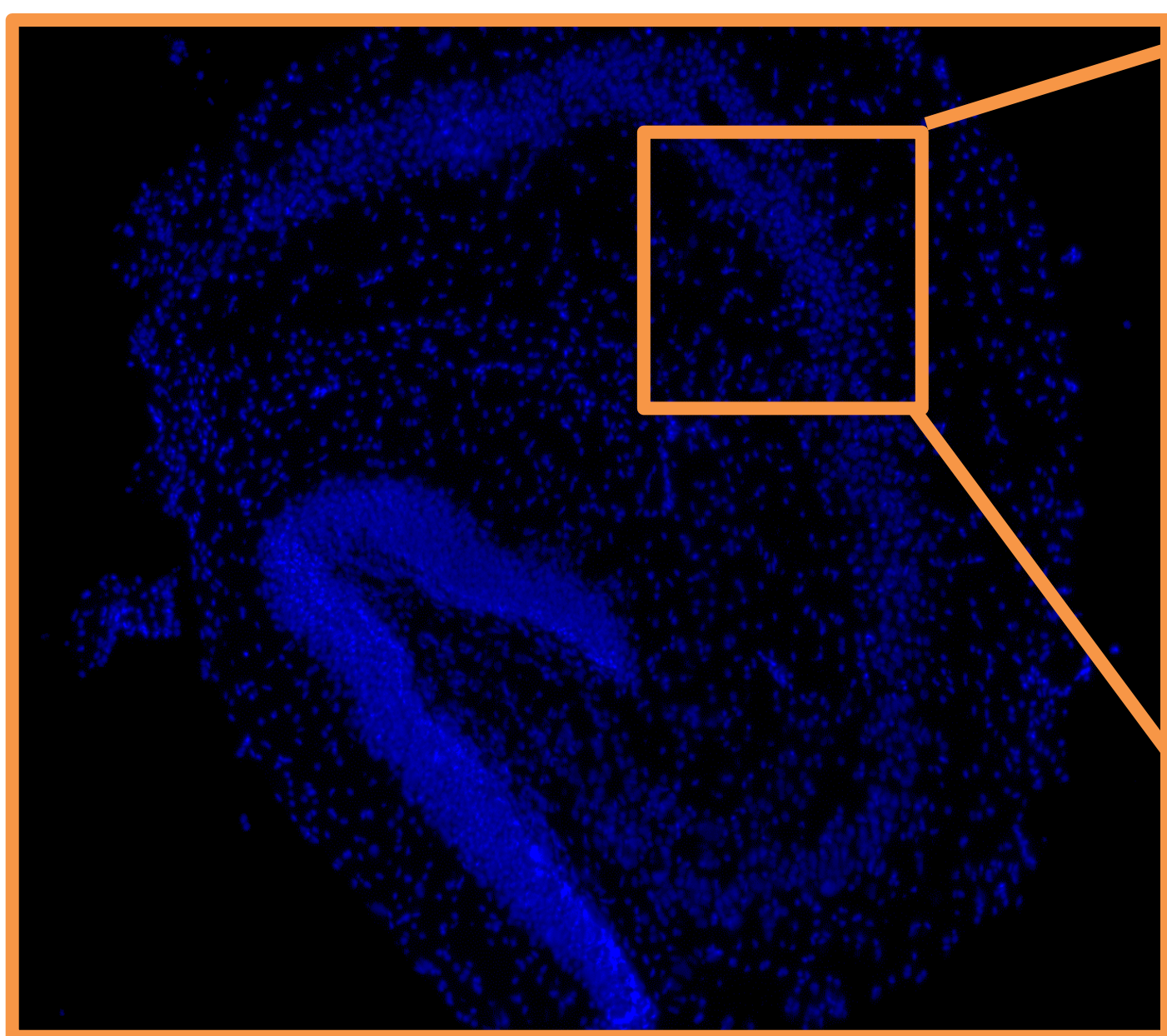


Figure 4 | Hippocampus stained with DAPI and imaged at 5X. Inset: CA1 subregion.

Methodology

Animal Care and Handling:

- Both male and female Npas4 cKO lab mice were used in this study.
- For IEG expression experiments, mice were either moved from SH to a new SH environment or were moved to EE. Four days later, mice were anesthetized and sacrificed for histology.



Hippocampal Dissection and Tissue Preparation:

- Hippocampi from P21 mice were quickly removed and frozen
- A cryostat was used to make 20 μ m transverse slices that were then mounted on slides for immunohistochemistry
- DAPI, a nuclear marker, was used as a mounting medium.

Data Acquisition and Analysis:

- Imaging was performed using a confocal microscope.
- 5X images of whole hippocampal slices were taken, followed by 60X z-stack images of the proximal, medial, and distal portions of CA1
- The number of neurons expressing one or more of our IEGs of interest were counted with ImageJ.

Results

The distal subdomain contained the most neurons that either expressed only Arc, only c-Fos, or both Arc and Npas4 together, while the medial subdomain contained the most neurons that only expressed Npas4. The proximal subdomain contained the most neurons that expressed both c-Fos and Npas4.

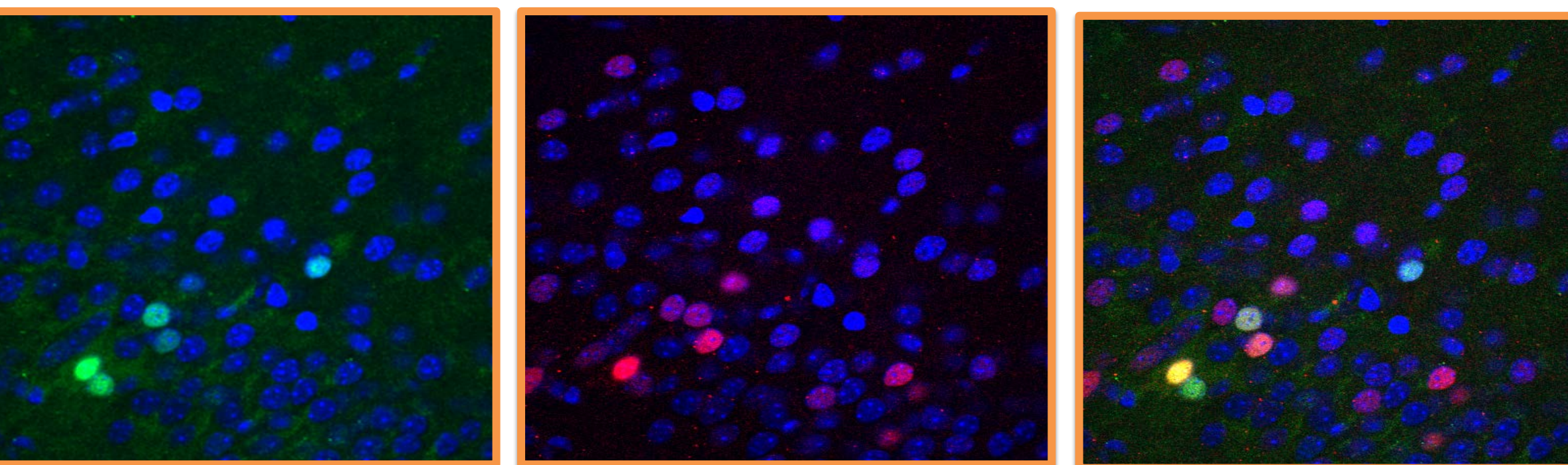


Figure 5 | CA1 Region of Hippocampus Imaged at 60X. Cells expressing DAPI (blue), Npas4 (green), c-Fos (red, top), and Arc (red, bottom) are shown.

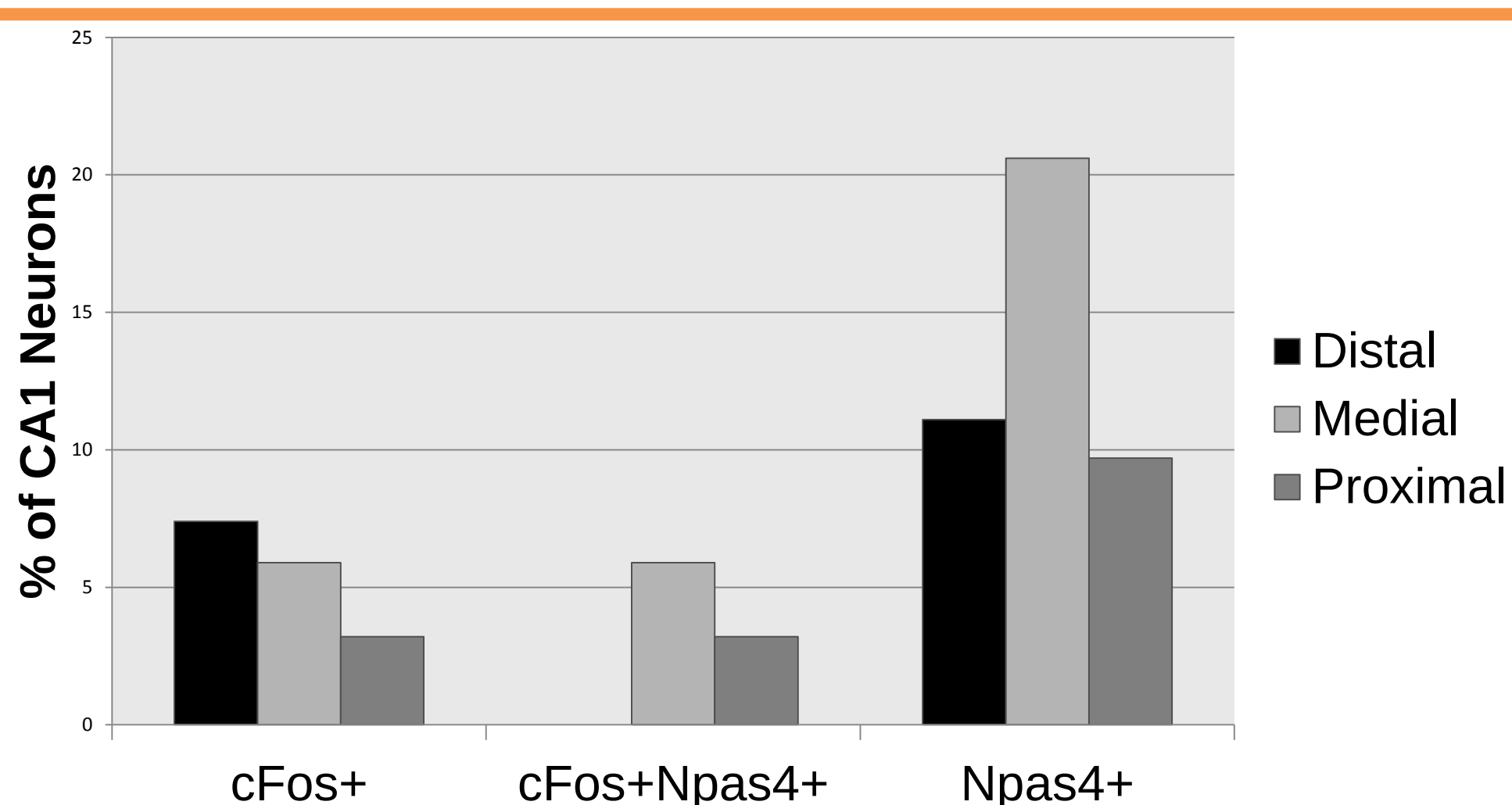
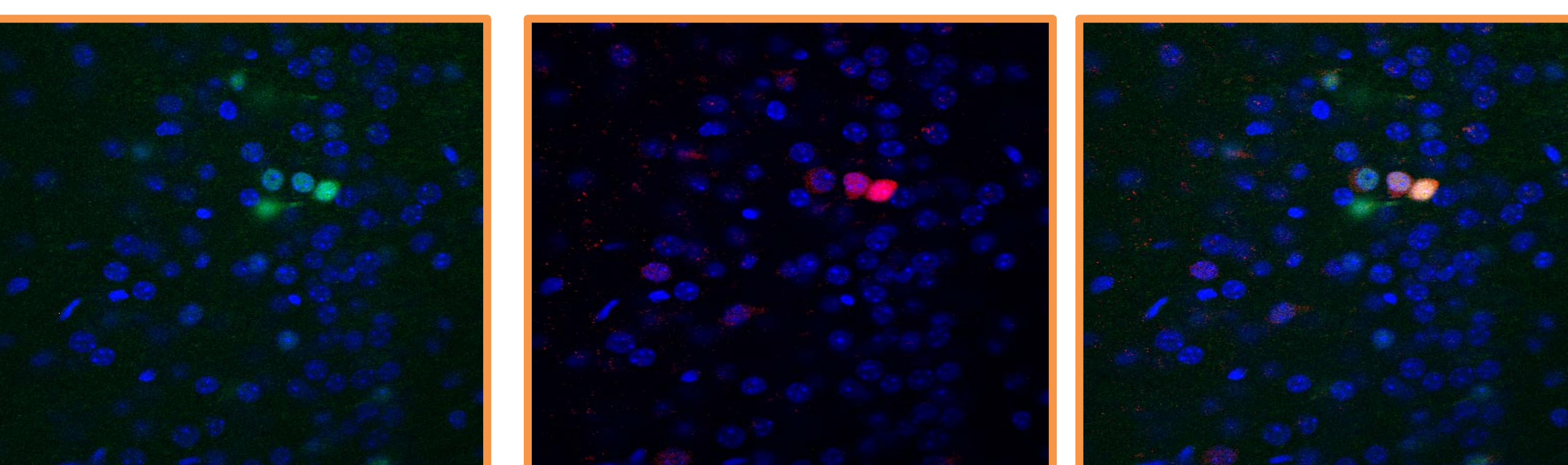


Figure 11 | Quantification of CA1 neurons expressing either Npas4, c-Fos, or both.

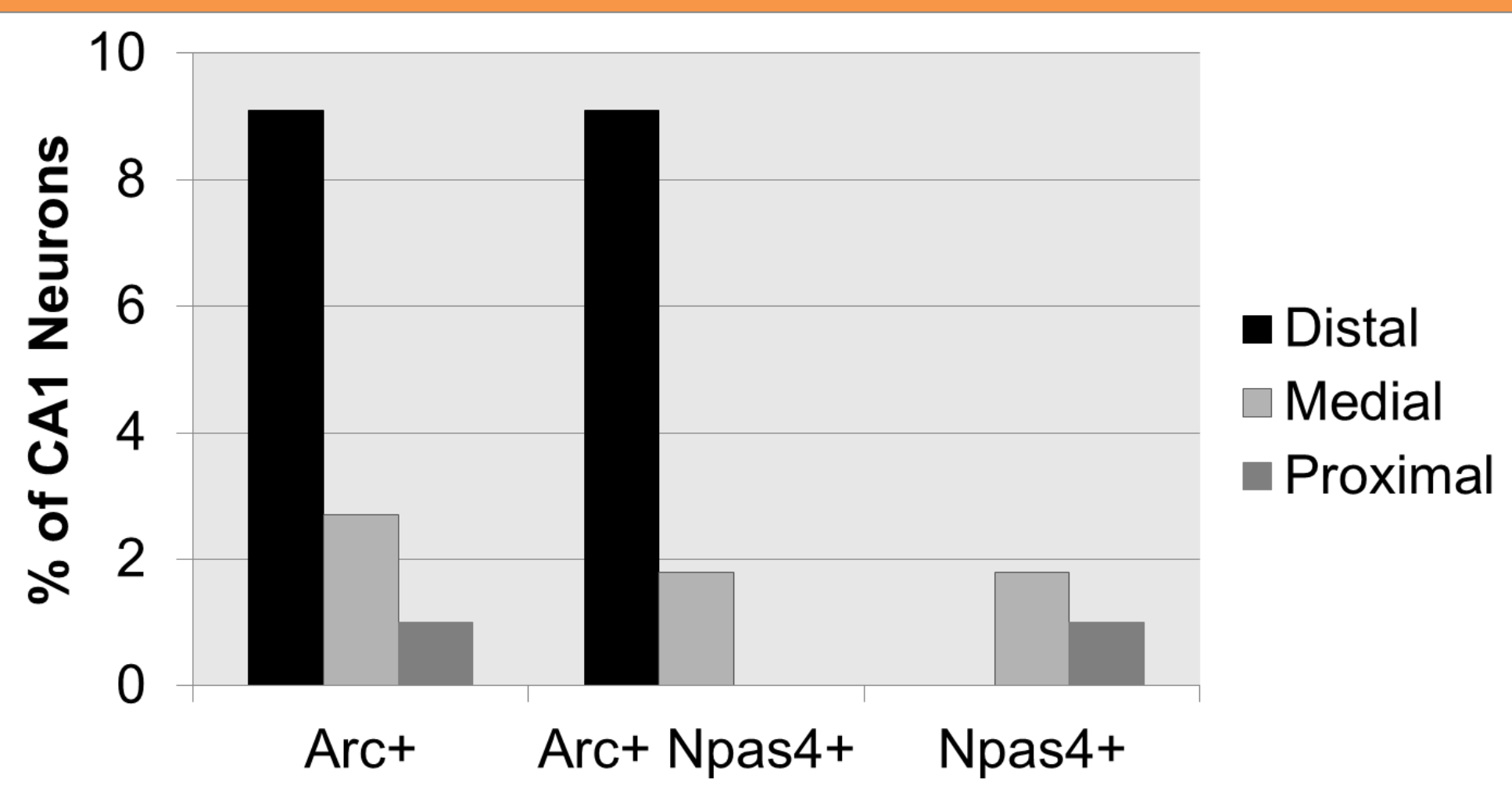


Figure 12 | Quantification of CA1 neurons expressing either Npas4, Arc, or both.

Discussion

The results were inconclusive because housing conditions were not well-controlled. The building in which all mice are kept was under internal construction, giving the SH mice more environmental inputs than intended. SH and EE tissue were indistinguishable in terms of Npas4 expression, which was inconsistent with results described by Bloodgood et al. However, our results still successfully indicated which cells expressed the IEGs Npas4, c-Fos, and Arc. This project confirms that Npas4, c-Fos, and Arc are activity-dependent, and our data suggests that distinct portions of CA1 may process inputs differently through gene expression, consistent with previous studies showing that the functional role of CA1 during behavior is known to differ along the proximal-distal axis (Deshmukh and Knierim, 2011). While our experiments need to be repeated in more well-controlled conditions, the current study has validated a robust method for comparison of several IEGs within the same tissue.

References

- Lin Y, Bloodgood BL, Hauser JL, Lapan AD, Koon AC, Kim T-K, Hu L-S, Malik AN, and Greenberg ME. (2008) Activity-dependent regulation of inhibitory synapse development by Npas4. *Nature* 455: 1198-204.
- Feng X. The Short Term Effect of Enriched Environment on C-Fos Protein Expression. University of Wyoming, 2011. Web. 30 July 2015.
- Sylwestrak E and Scheiffele P. (2013) Subcellular-specific regulation of neuronal activity. *Nature*. Neural Circuits of the Hippocampus. Digital image. Neuralcircuits.uwm.edu. N.p., n.d. Web. 30 July 2015.
- The prepotential. Digital image. Wikipedia. N.p., n.d. Web. 30 July 2015.
- "Epilepsy, Autism, Schizophrenia: Master Switch That 'Balances The Brain' Found." *ScienceDaily*, 25 Sept. 2008. Web. 30 July 2015.
- Kawashima T, Okuno H, and Bito H. (2014) "A New Era for Functional Labeling of Neurons: Activity-dependent Promoters Have Come of Age." *Frontiers in Neural Circuits*.