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# The Effect of CRHR1 Blockade on Levels of Tumor Necrosis Factor-Alpha in the Hippocampus Following Global Cerebral Ischemia in Male Rats

Thien-Phuoc Nguyen, Kelsey Leanne Ross, Patricia B. de la Tremblaye & H el ene Plamondon Ph.D  
Behavioral Neuroscience Group, School of Psychology, University of Ottawa

## Introduction

Cerebral ischemia triggers inflammation around the ischemic area that starts within hours and persists for days. The damaged brain tissue activates microglia which releases cytokines such as Tumor Necrosis Factor Alpha (TNF ). Pathological levels of TNF  increase inflammation in the ischemic areas which ultimately results in increased cell death. Corticotropin-Releasing Hormone (CRH), a key neuromodulator of the stress system, has been shown to influence this inflammatory response through its type 1 receptor (CRHR1), causing further neural death.

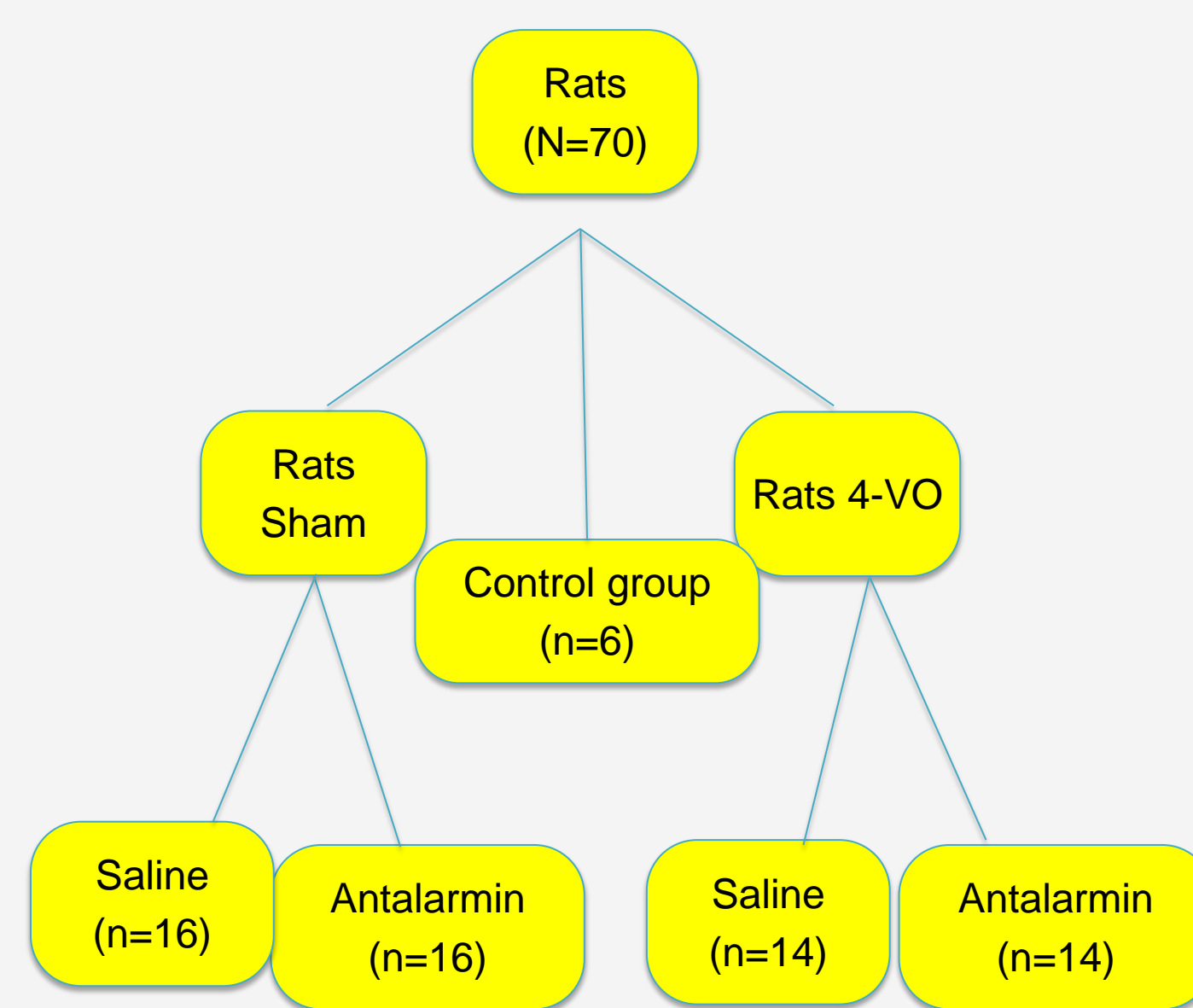
## Objectives

The current study aims to examine the effects of pre-ischemic administration of Antalarmin, a CRHR1 antagonist, to reduce reactive microglia and to restrict the production of TNF . This study will demonstrate whether CRHR1 blockade reduces brain injury through inhibition of pro-inflammatory markers, specifically TNF  and microglia.

## Methods

### Animals

Seventy Wistar male rats (N= 70) were randomly assigned to one of five groups. Among the experimental groups, rats underwent a global cerebral ischemia (4-VO) (ischemia), a simulated operation



(sham) or no surgery at all (control). Each surgical group was further divided into two pharmacological groups, one of them receiving Antalarmin (2 g/ l) and the other one receiving a saline solution.

### Immunohistochemistry

#### Immunohistochemistry

Thirty days after the occlusion, rats were anesthetized with isoflurane and beheaded. Brains were collected, frozen in dry ice and stored at -80 C before being sectioned at 14 m using a cryostat. Brain sections were processed with primary antibodies: polyclonal rabbit anti IBA1 (1:1000, Wako Chemicals USA, Inc.), polyclonal mouse anti TNF  (1:200, Santa Cruz Biotechnology, Inc.), monoclonal mouse GFAP (dilution of 1:1000; Abcam), and polyclonal rabbit anti BDNF (1:400, Santa Cruz Biotechnology, Inc.), and secondary antibodies: Alexa Fluor 488 donkey anti-mouse IgG (dilution 1:500; Invitrogen), Alexa 594-conjugated donkey anti-rabbit IgG (dilution 1:500; Invitrogen). Immunofluorescence signals were measured with the Olympus DX51 microscope and the numeric images were obtained through Progress Pro 2.7.6 with 20x magnification. Immunoreactivity was quantified with Image J software.

## Results

### TNF  expression in the CA1

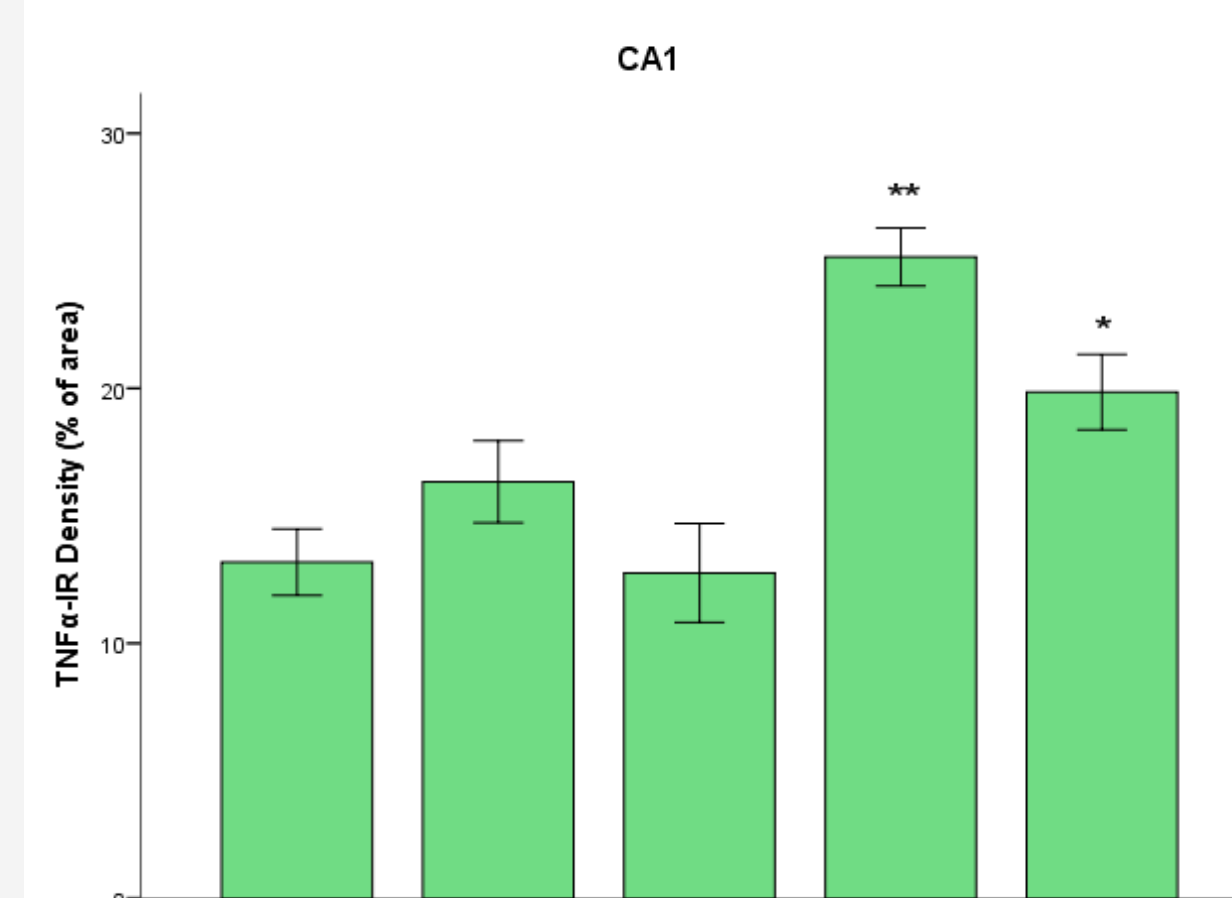
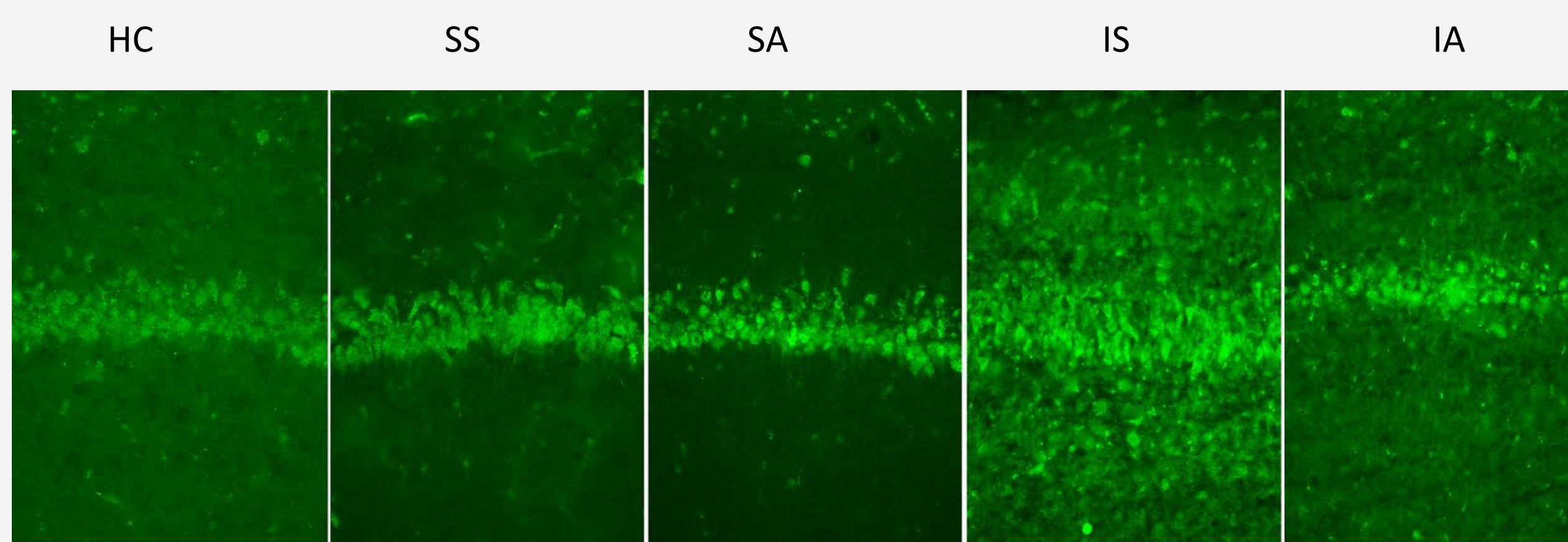


Figure 1. Effects of Antalarmin on TNF  level in CA1 30 days post Ischemia. (A) Representative photomicrographs of TNF  (Green) and Hoescht (blue) immunopositive labeling within the CA1 at 20X magnification. (B) Histogram shows the mean percent area optical densities in the CA1, for each of the groups: HC (Home Cage), SS (Sham Saline), SA (Sham Antalarmin), IS (Ischemic Saline), and IA (Ischemic Antalarmin). Data are expressed as mean   SEM. \*P < 0.05; \*\*P < 0.01.

### TNF  expression in the CA3

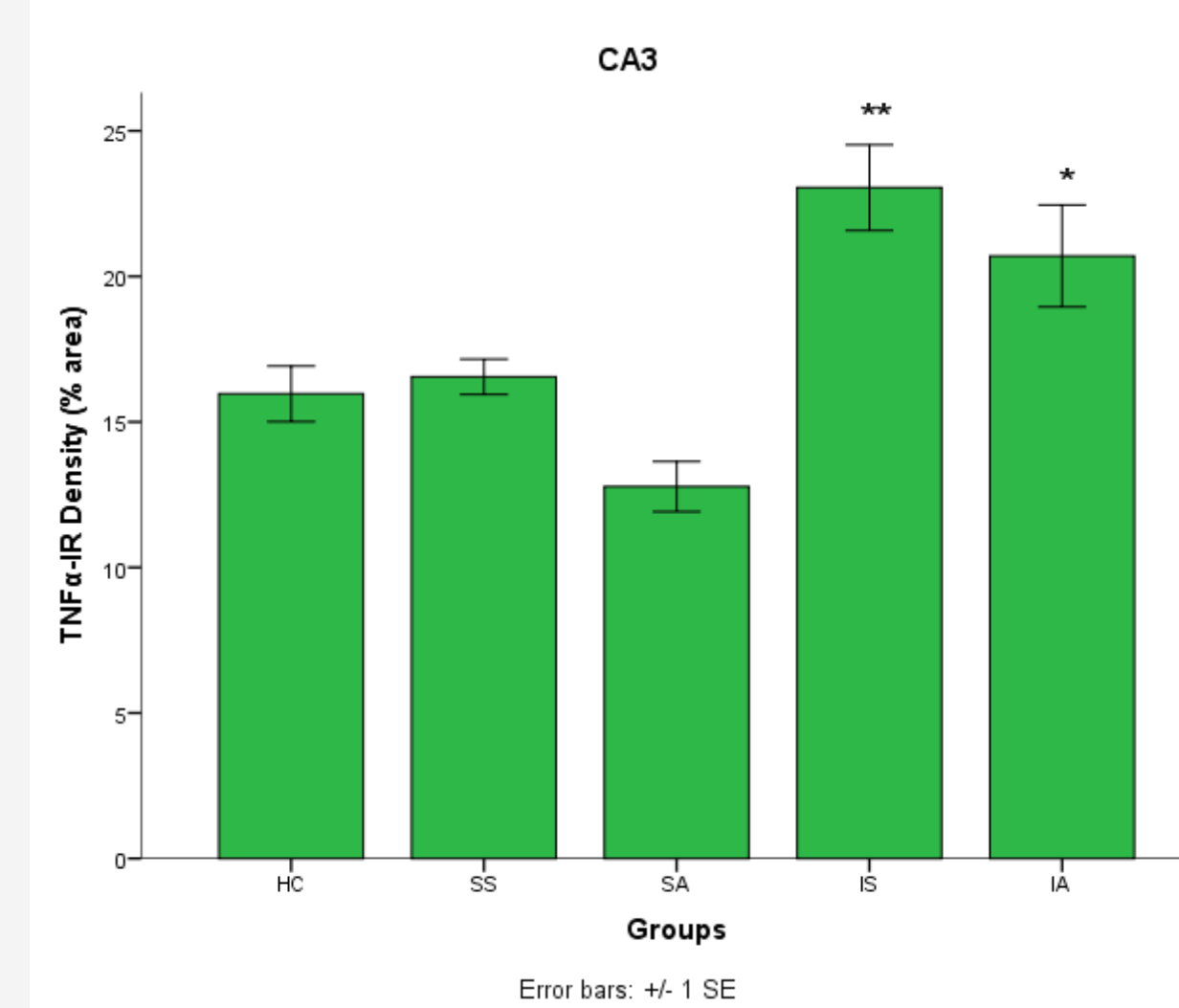
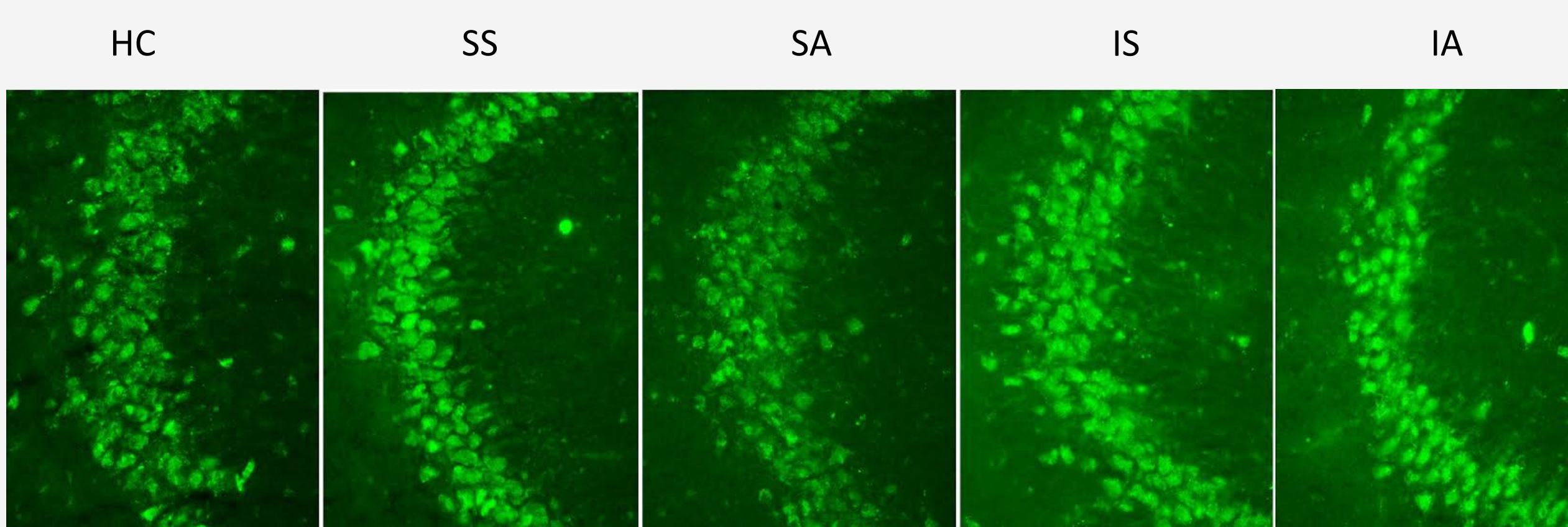


Figure 2. Effects of Antalarmin on TNF  level in CA3, 30 days post Ischemia (A) Representative photomicrographs of TNF  (Green) and Hoescht (blue) immunopositive labeling within the CA3 at 20X magnification. (B) Histogram shows the mean percent area optical densities in the CA3, for each of the groups: HC (Home Cage), SS (Sham Saline), SA (Sham Antalarmin), IS (Ischemic Saline), and IA (Ischemic Antalarmin). Data are expressed as mean   SEM. \*P < 0.05; \*\*P < 0.01.

### TNF  expression in the DG

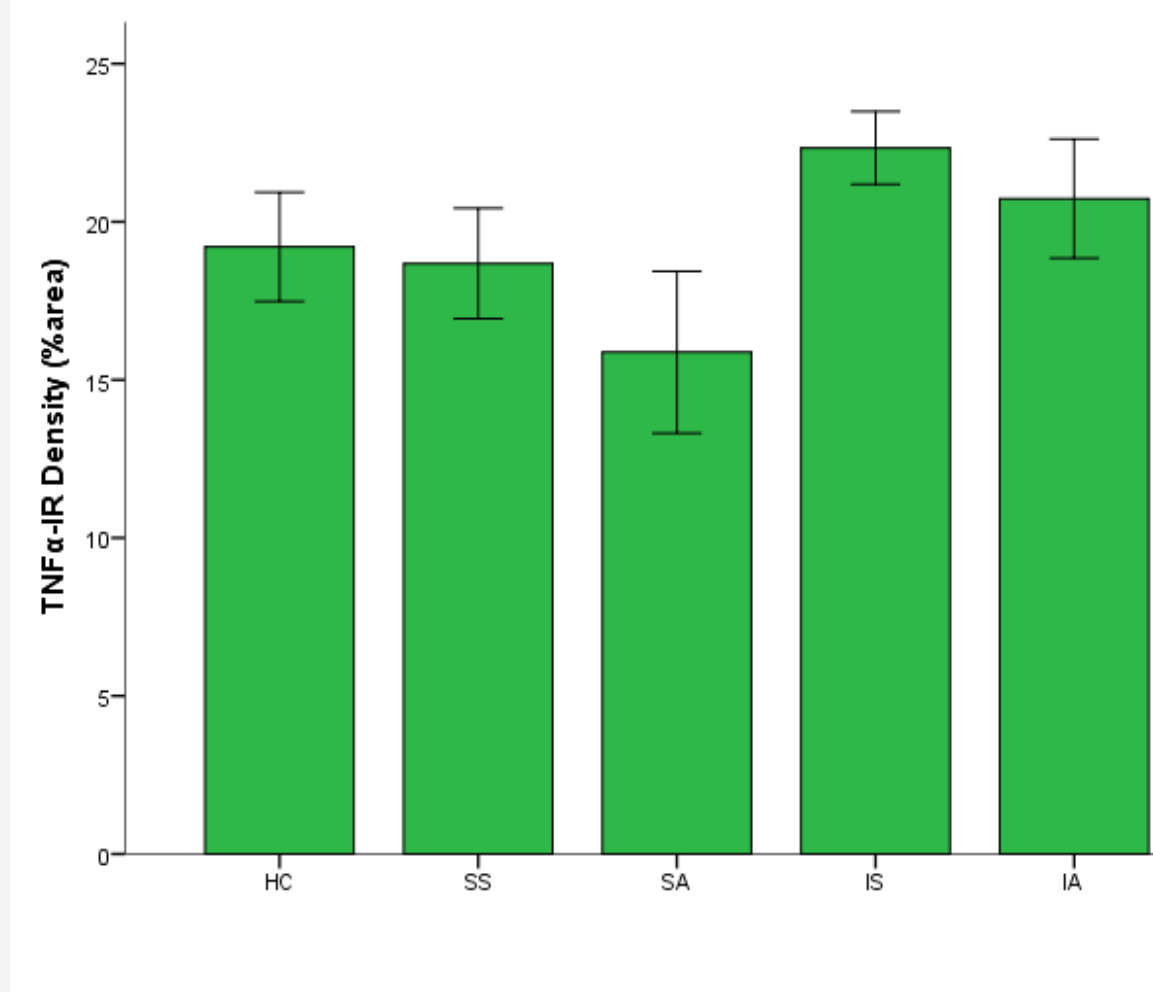
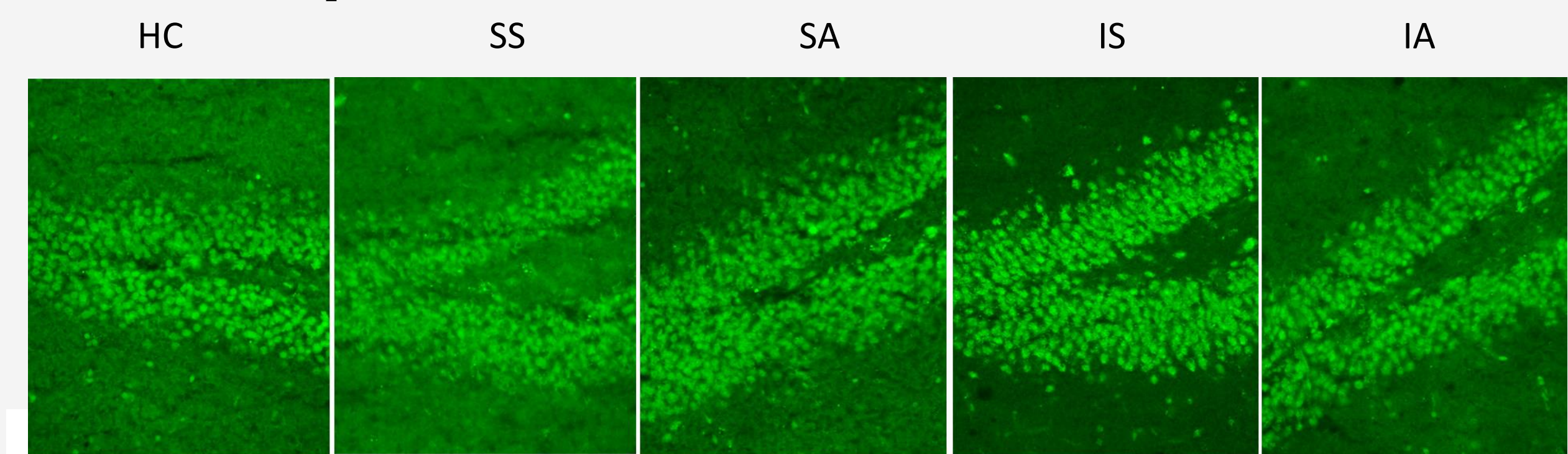


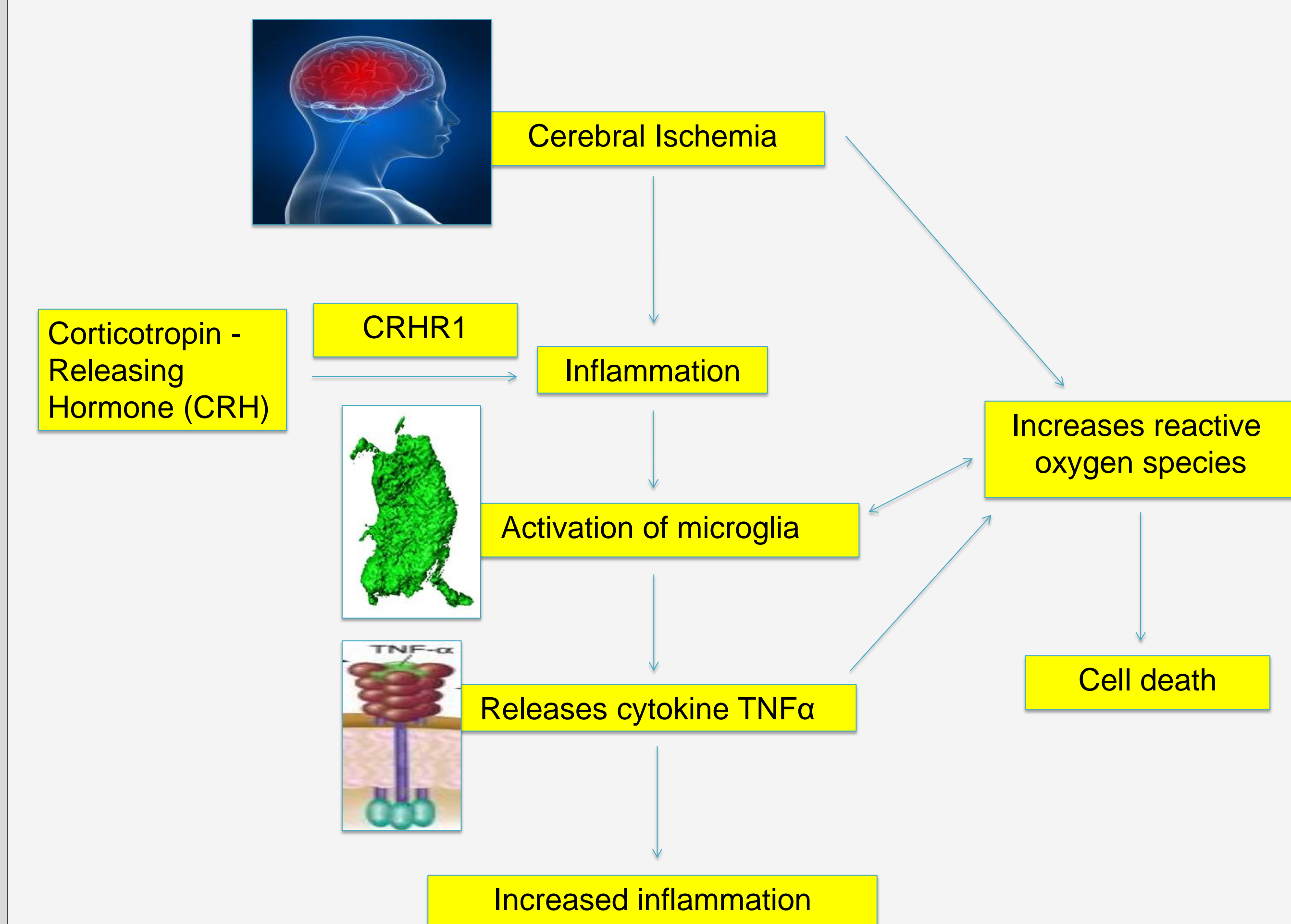
Figure 3. Effects of Antalarmin on TNF  level in CA1, 30 days post Ischemia. (A) Representative photomicrographs of TNF  (Green) and Hoescht (blue) immunopositive labeling within the CA1 at 20X magnification. (B) Histogram shows the mean percent area optical densities in the CA1, for each of the groups: HC (Home Cage), SS (Sham Saline), SA (Sham Antalarmin), IS (Ischemic Saline), and IA (Ischemic Antalarmin). Data are expressed as mean   SEM. \*P < 0.05.

## Discussion

The accumulation of reactive microglia is a key cellular event that occurs following global cerebral ischemia, creating a chronic inflammatory environment that results in increased cell death. The proinflammatory cytokines tumor necrosis factor alpha (TNF ) is involved in microglial activation following cerebral ischemia, playing a role in inflammation-induced neuronal death. In regard to the hippocampus, previous studies have mainly focused on CA1 pyramidal neurons, which are very vulnerable to ischemia, but the DG and CA3 have also been shown to be effected by ischemia. In this study, we explored the neuroprotective effect of pre-ischemic administration of Antalarmin in different areas of the hippocampus 30 days post ischemia.

Our results show that global cerebral ischemia led to an increase in TNF  immunoreactive expression in the CA1, which is most likely due to ischemic cell death in CA1 pyramidal cells. There was a significant effect of the Antalarmin treatment to reduce TNF  levels, but this effect was not strong enough to bring the ischemic-Antalarmin group to the same level as controls.

The effect of ischemia and Antalarmin were more limited in the CA3, and especially in the DG, which are more resistant to ischemic damage. This study provides evidence that Antalarmin is effective in reducing levels of TNF , which was more pronounced in specific areas of the hippocampus, depending on the severity of the ischemic damage.



## References

- Nikonenko, A. G., Radenovic, L., Andjus, P. R., & Skibo, G. G. (2009). Structural features of ischemic damage in the hippocampus. *Anat Rec (Hoboken)*, 292(12), 1914-1921. doi: 10.1002/ar.20969
- Watters, O., & O'Connor, J. (2011). A role for tumor necrosis factor-alpha in ischemia and ischemic preconditioning. *Journal of Neuroinflammation*, 8(1), 87.