

# Assessment of TNF in the Brain and Neurogenesis

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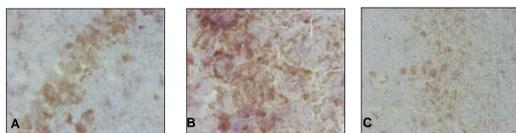
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## Abstract

Chronic pain is an abnormal condition of sufficient duration and intensity to negatively affect a person's level of functioning and quality of life. Neuropathic pain, a type of chronic pain resulting from nerve injury, is very difficult to treat. Clinically, pain relievers (analgesics) are generally effective for nociceptive pain, but are not very effective for neuropathic pain. Tricyclic antidepressant drugs that are normally prescribed for treatment of depression are often prescribed to manage neuropathic pain disorders such as diabetic pain. The question I intend to answer is whether there is an association between increased hippocampal TNF levels and a neuronal process in this brain region, neurogenesis (growth and development of neurons). My hypothesis is that during diabetic neuropathic pain, increased TNF in the brain leads to decreased neurogenesis. This will be tested by staining hippocampal sections, prepared from rat models of neuropathic pain, for TNF and for markers identifying newly developed neurons.

## Background

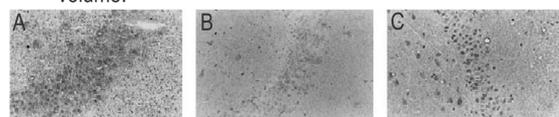
- A common complication of diabetes is peripheral neuropathy, which is damage that occurs to peripheral nerves due to prolonged high blood glucose levels (hyperglycemia).
- Streptozotocin, an anti-neoplastic antibiotic that is toxic to insulin-producing  $\beta$ -islet cells of the pancreas was used to induce diabetic neuropathy, a model of neuropathic pain.
- Tumor necrosis factor-alpha (TNF) is a cytokine (signaling molecule) that is chiefly produced and released by activated macrophages (innate immune cells) involved in a systemic pro-inflammatory response. [1,2]
- TNF is increased in the brain during neuropathic pain, specifically in the hippocampus (Fig.1). [1, 3,6]
- TNF is known to induce apoptosis (programmed cell death) and necrosis (cell death). [4]



**Figure 1-** Immunoreactivity for TNF in coronal hippocampal sections. Shown in each panel is a representative coronal section of hippocampus of four independent animals from: (a) Sham-operated (day-8 post-surgery), (b) Day-8 post-CCI, and (c) Day-8 post-CCI rats with concomitant amitriptyline treatment (10 mg/kg, i.p., twice daily). Scale bar = 50  $\mu$ m.

- Hippocampus volume decreases with chronic pain. [2,5]
- Blocking increased TNF alleviates symptoms of neuropathic pain. [1, 6]

- Neurogenesis, the birth and growth of neurons occurs in the dentate gyrus of the hippocampus. [4]
- Antidepressants increase neurogenesis, decrease TNF expression (Fig. 2), and thus increase hippocampus volume.



**Figure 2-** Immunoperoxidase stained rat hippocampal brain slices. (A) Control, positive staining for TNF $\alpha$  in apical cell bodies and processes throughout the section. (B) Acute (1 day) desipramine administration, lack of TNF $\alpha$  staining in a brain section taken from a similar region of the brain as in (A). (C) Chronic (14 days) desipramine administration, intermediate TNF $\alpha$  staining evident in some of the neuronal cell bodies as compared to that stained under control conditions (A). Note: Same magnification (25X) and light intensity (70%) used for all pictures.

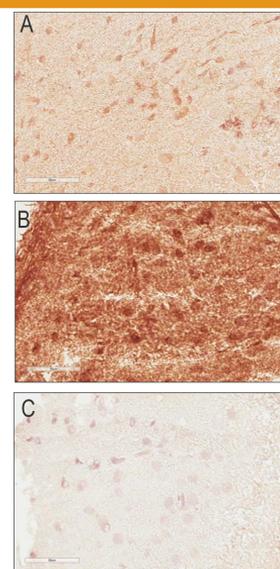
## Purpose

To test the hypothesis that during diabetic neuropathic pain, an increase in TNF in the brain will lead to decreased neurogenesis. Our ultimate goal is to correlate staining for TNF and neurogenesis, with the prediction that increased TNF is associated with decreased neurogenesis.

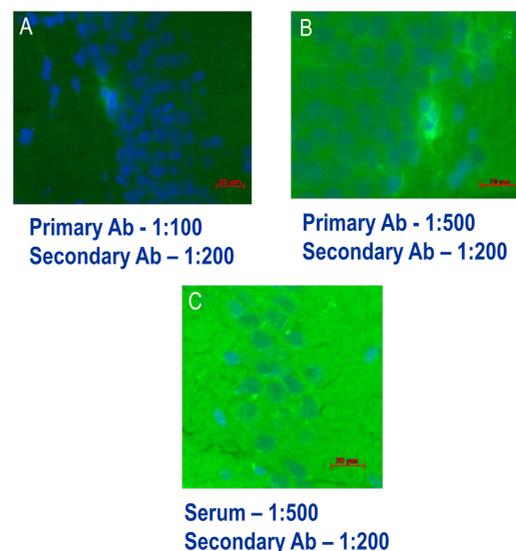
## Methods

In order to test the hypothesis, tissue sections taken from STZ-induced diabetic rats experiencing neuropathic pain and from control rats were labeled for TNF and for markers identifying newly developed neurons. Assessing staining for TNF and newly developed neurons will give us insight into whether there is an association between TNF levels and neurogenesis. We also compared staining to samples taken from rats administered drugs that are known to alleviate pain behavior and improve depressive behavior in animals, antidepressant drugs; this was used as a positive control for Ki-67 staining. Rat brain tissue was sectioned into thin 8-10 micrometer slices via a cryostat and placed onto electrostatically charged slides. The tissue was labeled with specific antibodies for: TNF; doublecortin (DCX), a marker expressed by newly born immature neurons; and Ki-67, a nuclear protein easily detected in newly dividing cells. Immunostaining for TNF and markers for neurogenesis in hippocampal tissue sections from rodents with diabetic neuropathy gave us further insight into the effect of diabetes on the brain, furthering our knowledge on the illness. After staining, digitized pictures of the stained tissue sections were taken by Zeiss LSM 510 Meta NLO Confocal Microscope with attached Zeiss Axioimager Z1 and Axiovert 200M.

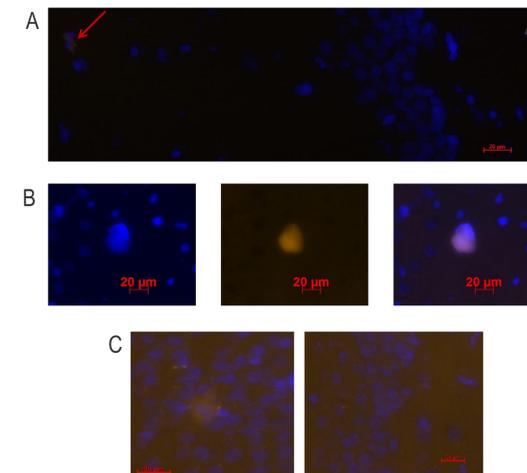
## Data



**Figure 3-** Immunoperoxidase staining for TNF $\alpha$  in coronal hippocampal sections. (A) Saline control rat, (B) Streptozotocin (STZ)-induced diabetic rat, (C) Negative control (normal rabbit serum) stained section from (B). All slides were stained on the same day under the same conditions, except section (C) had the primary antibody substituted with normal serum.



**Figure 4-** Indirect immunofluorescence labeling of hippocampal tissue sections with doublecortin (DCX) antibody. (A) DCX cytoplasmic protein is highly expressed in developing brain; initial antibody titration (DCX at 1:100, 1:200, 1:300, 1:500) and secondary antibody donkey anti-goat IgG-FITC (1:200, 1:400) showed specific labeling (A) and (B), even in the presence of high background autofluorescence (B) and (C).



**Figure 5-** Direct immunofluorescence labeling of coronal hippocampal tissue sections from Sham-operated control rats with Ki-67-TRITC. (A) Ki-67-TRITC at 1:75 labeled dentate gyrus cells. (B) Ki-67-TRITC at 1:50 labeled nuclear protein that is expressed in proliferating cells. Left panel: DAPI (0.1  $\mu$ g/ml) nuclear staining (blue); Middle panel: Ki-67-TRITC signal detection (orange); Right panel: Overlay of DAPI and Ki-67-TRITC labeling. (C) Ki-67-TRITC labeling in hippocampal dentate gyrus from a rat administered the antidepressant desipramine (10 mg/kg) twice daily for 14 days. Left panel: Ki-67-TRITC at 1:50; Right panel: negative control (absence of Ki-67-TRITC antibody).

## Conclusions

- STZ-DN rat shows increased TNF staining, thus increased TNF in the hippocampus; the negative control shows no staining for TNF.
- Too much background autofluorescence occurred with DCX labeling.

## References

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## Future Studies

- Continued staining of hippocampal tissue from both control and diabetic neuropathy rats for comparison.
- Decrease autofluorescence of the DCX labeling.
- Quantitation of positively labeled neurons to determine whether a decrease or increase in neurogenesis occurs during neuropathic pain and its treatment.