# Assessment of c-Fos as a Marker of Spinal Neuronal Activity in a Pain Model of Rheumatoid Arthritis

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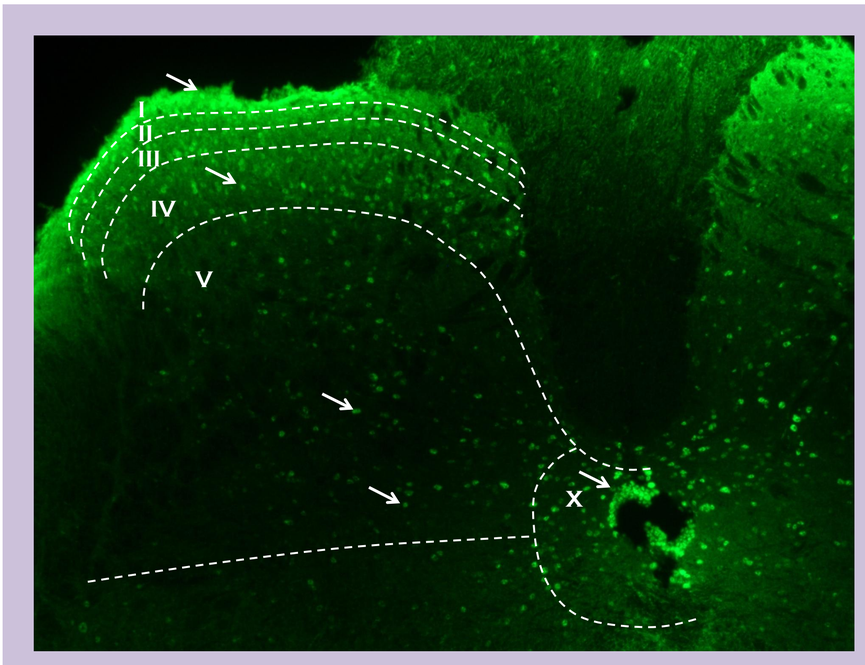



**Figure 1.** Immunohistochemistry of c-Fos expression shows neuronal activity in the lumbar spinal cord of mice. The arrows show active neurons in the dorsal horn (Rexed laminae I-V) and around the central canal (X).

## c-Fos expression in the spinal dorsal horn is elevated subsequent to induction of joint inflammation

The number of c-Fos immunoreactive neurons in the lumbar region of the spinal cord of mice (fig. 1, 3) is significantly higher following induction of Collagen Antibody Induced Arthritis (CAIA), as compared to controls. The increase was most pronounced in the deep dorsal horn (laminae IV-V) on day 9, coinciding with the inflammatory phase of the model (fig. 2, 3). Interestingly, the allodynia persists beyond the inflammatory phase (fig. 2), and though the number of c-Fos positive neurons decreases when the signs of arthritis disappear, the number is still significantly increased as compared to control mice on day 29. In summary, this study indicates that arthritis induces long-term activation of dorsal horn neurons that persists even after the inflammation subsides.

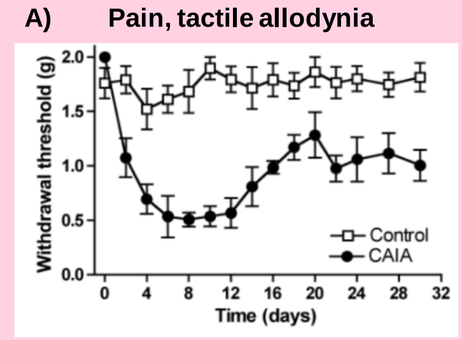



**Figure 2.** Collagen antibody induced arthritis leads to persistent pain in mice **(A)**, even after cessation of inflammation on day 20 **(B)**.

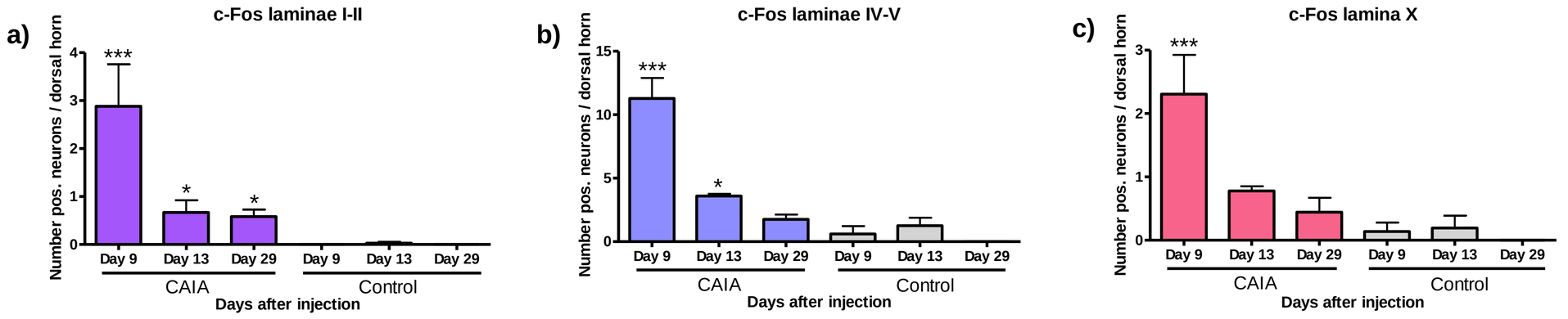



**Figure 3.** Graphs **a**, **b** and **c** show the number of active pain neurons (c-Fos expressed) on day 9, 13 and 29 after CAIA induction (joint inflammation). There are some in laminae **I-II (a)**, a majority in IV-V **(b)**, and some in lamina X **(c)**. *P<0.05, **P<0.01, ***P<0.001

## Background

Pain in chronic inflammatory diseases, such as rheumatoid arthritis (RA), is a major clinical problem. Joint inflammatory factors trigger peripheral pain nerves locally (i.e. Aδ- and C-fibers), which in turn transmit noxious stimuli as afferents entering the dorsal horn of the spinal cord [1]. Neurotransmitters, i.e. substance P and glutamate, are constantly released for a long period of time. Such constant stimuli provoke the spinal neurons, in turn developing hypersensitivity. Thus, RA patients become more susceptible to neuropathic pain. However, the peripheral pathology does not correspond to the amount of pain experienced by the patient. Even after anti-inflammatory treatment and reduced inflammatory symptoms, chronic pain persists (fig. 2). Previous work has shown that increased reactivity and prolonged activation of spinal sensory neurons (spinal sensitization) is an important component of chronic pain. Thus, pain transduction and processing at the level of the spinal cord must be considered. Analgesic treatment of pain associated with chronic inflammatory diseases, including RA, is frequently insufficient or associated with side effects [2]. Therefore, it is of higher importance to increase our knowledge about how chronic inflammatory pain is generated and maintained in order to identify new targets for pharmacological treatments of chronic pain.

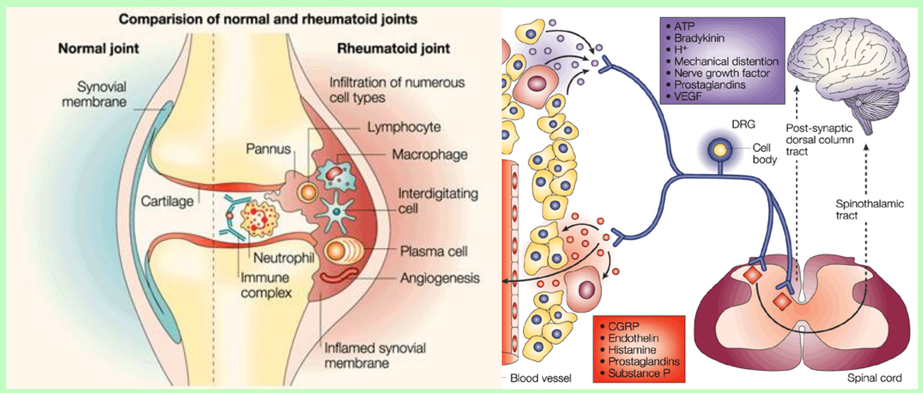



**Figure 4.** Inflammation of rheumatoid arthritis causes neuronal hypersensitivity. Afferent pain fibers enter the dorsal horn of the spinal cord. Constant neuronal activity in the central nervous system induces chronic pain.

Feldmann, M. (2002). Development of anti-TNF therapy for rheumatoid arthritis. Nature Reviews Immunology. 2: 364-371

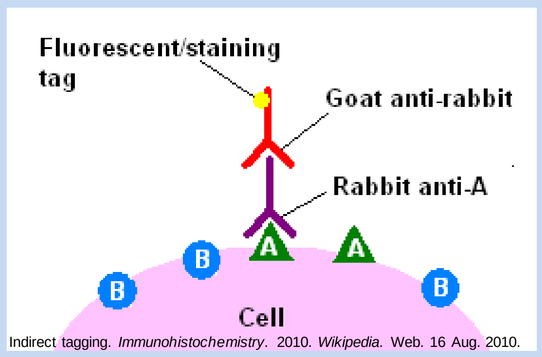
Mantyh, P. W., Clohisy, D. R., Koltzenburg, M., Hunt, S. P. (2002). Molecular mechanisms of cancer pain. Nature Reviews Cancer. 2: 201-209

The study was performed using a CAIA (Collagen Antibody Induced Arthritis) mouse model. Arthritis was induced by intravenous injection of arthritogenic monoclonal antibodies (mAbs) targeted for collagen II (the main component of cartilage in joints) provoking RA symptoms [3]. c-Fos is a factor commonly used as a marker for increased spinal neuroexcitability [4].

**The purpose of this investigation was to examine if there is a change in the number of c-Fos immunoreactive neurons in the dorsal horn of the spinal cord 9, 13 and 29 days after CAIA induction.**

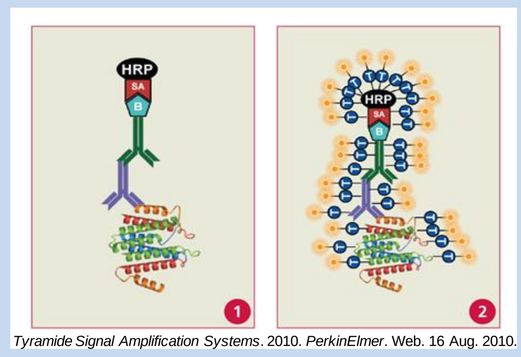
## Method

Spinal cords were harvested from CAIA and age-matched control mice 9, 13 and 29 days after induction of arthritis and processed for immunohistochemistry (fig. 5). The lumbar part of the spinal cords was cut at -20°C (30µm), and the sections were incubated with a goat anti-rabbit antibody against c-Fos (Santa Cruz Biotechnology, 1:1000), and visualized using a goat anti-rabbit IgG antibody conjugated to a fluorescent molecule (Alexa-594, Invitrogen, 1:250). The experimentor was blinded to the study groups, and c-Fos positive neurons were quantified using a fluorescence microscope. The distribution of c-Fos positive cells in the Rexed Laminae were determined using a mouse histology atlas. Tyramide Signal Amplification (TSA) was applied in order to assess if this improved the signal to noise ratio, as the background was high using the conventional protocol (fig. 6).




Indirect tagging. *Immunohistochemistry*. 2010. *Wikipedia*. Web. 16 Aug. 2010.

**Figure 5.** Immunohistochemistry: a rabbit anti-A antibody binds to A (c-Fos). Then a goat anti-rabbit antibody conjugated to a fluorescent tag binds to the first antibody. The result is perceived via fluorescent microscopy.




*Tyramide Signal Amplification Systems*. 2010. *PerkinElmer*. Web. 16 Aug. 2010.

**Figure 6.** TSA: an antibody binds to target protein (c-Fos), and a horse radish peroxidase (HRP) conjugated antibody binds to the latter. HRP catalyzes activation of tyramide (T) conjugated dye (Alexa 488) to generate fluorescent signals.

## References

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