Editorial

Remembrance of times past: the significance of c-fos in pain

“Everlasting layers of ideas, images, feelings have fallen upon your brain… Each succession has seemed to bury all that went before. And yet in reality not one has been extinguished…”

Thomas de Quincey

Advances in neurobiology have provided us with the tools to identify the molecular events that underlie functional and structural change within the nervous system. Examining the molecular responses to nerve stimulation and injury have led not only to an increased understanding of the biological basis of acute and chronic pain states, but also suggest a molecular memory for painful stimulation. One particular molecular marker, c-fos, has been the focus of much research after the key observation that painful, but not innocuous, peripheral stimulation rapidly causes the expression of c-fos gene expressions within the spinal cord [1]. The article by Sun, Shyu and Shieh [2] in this issue of the journal demonstrates that apparently adequate anaesthesia fails to prevent expression of an immediate early gene “c-fos” within the spinal cord after painful peripheral stimulation. In this editorial, clinical consequences of this observation are discussed in relation to the fundamental role of c-fos in linking short-term stimuli to long-term structural change within the nervous system.

Immediate early genes such as v-fos (viral-fos) were first shown to be capable of inducing cell proliferation and were subsequently known as proto-oncogenes. When the same gene was then described in cells it was termed c-fos (cellular-fos). More than 100 immediate early genes are now known and have fundamental roles in cell proliferation, differentiation and programmed cell death [3]. The basic property shared by all is that they are capable of being induced (i.e. their messenger RNA is produced) without prior protein synthesis, typically within minutes after stimulation. This observation indicates that the cellular machinery required to effect gene transcription is in a state of readiness and contrasts with the “late response genes” which do require further de novo protein synthesis before their transcription can be altered. Immediate early genes were shown to form part of the signal transduction cascade from the cell surface to the nucleus leading to long-term changes in structure and function (i.e. plastic changes) and hence were given the title of “third messengers” [4] (fig. 1). Studies using Fos showed that its appearance could be used to mark those cells that had been stimulated and activated [5]. Indeed Fos expression has been used to map pathways of activation after various types of peripheral stimulation and generally shows good correlation with known anatomical pathways, for example Fos activation in the spinal cord and brain after noxious stimulation matches known routes which carry pain sensation [6]. As Fos activation can be seen only if sufficient stimulation has occurred in a cell, then it is obvious that Fos activation can be used not only to map metabolic activity in cells but also the effectiveness of therapies to suppress the activity of cells, for example to examine the efficacy of drugs such as those that may prevent secondary brain injury, antiepileptics and analgesics. One advantage of Fos expression over standard neurophysiological techniques is that the responses of large numbers of cells can be assessed as one may examine a whole section of brain or spinal cord for Fos protein or mRNA.

In this issue of the journal, Sun, Shyu and Shieh [2] show that 2% halothane with or without nitrous oxide and the μ agonist, fentanyl, reduced pain-related behaviour in response to painful stimulation but fentanyl alone reduced Fos expression in the spinal cord. The study raises a number of points.

(1) Using Fos expression as an index, volatile agents and nitrous oxide are not adequate analgesics for painful stimulation. However, one could argue that as long as the subject is anaesthetized with the volatile agent and not apparently responding, does it matter that peripheral stimulation reaches the spinal cord and the brain and causes Fos expression? To answer this point we need to know if there is any evidence that using different types of anaesthetic regimens can have long-term consequences for the individual.

(2) Even the large dose of fentanyl only reduced Fos expression by up to 50% in this study, that is significant Fos expression still occurred; if Fos expression were associated with deleterious physiological effects, should we be using other techniques or agents that are more effective at suppressing Fos expression than opioids?

Regarding the first point, there is increasing evidence that pain intensity and duration in acute and chronic pain models can be reduced and perhaps prevented if painful stimulation is impeded from reaching the cord either by the use of prior local anaesthesia or systemic analgesics but not by volatile agents alone [7–9]. In one study, different types of analgesic regimens were tested for their ability to
Studies have shown that the state of anaesthesia at the time of nerve injury. Other regimens were effective at producing an apparently satisfactory outcome compared with isoflurane alone or morphine pretreatment (see fig. 1) [27–32]. Even very high-dose opioids are not as efficacious as other analgesics in preventing Fos expression and subsequent pain [2, 10, 22, 23]. As clinical studies examining pre-emptive analgesia often use opioids, it may not be that surprising that the pre-emptive analgesia effect is often small or not significant. Another reason may be that again, as suggested previously [21], the duration of the injury outlasts the effective analgesia, for example local anaesthetic block may have worn off while the discharge from the sensitized primary afferent fibres continues minimizing the beneficial effect of the original procedure (see [24–26]).

On one level, Fos can be used simply as a marker of nociception but recent studies have made physiological consequences of Fos expression clearer. Fos may act on many genes to cause their expression, including that of preprodynorphin, neuropeptide Y (NPY) and nerve growth factor [4]. Both dynorphin and NPY have analgesic properties and so Fos may not only signal the magnitude of the insult reaching the spinal cord but also mediate some of its adaptive responses to it. In fact, one can now envisage a cascade of molecular events in the spinal cord after peripheral stimulation leading eventually to permanent structural changes within the nervous system (see fig. 1) [27–32].

Figure 1  Schematic and partially speculative diagram outlining the cascade of molecular events which may follow peripheral nerve injury or stimulation, which attempts to place in context the role of c-fos in this cascade. Some of the changes only occur in certain situations, for example VIP and cholecystokinin are, in general, more effective than opioids, it may not be that surprising that the pre-emptive analgesia effect is often small or not significant. Another reason may be that again, as suggested previously [21], the duration of the injury outlasts the effective analgesia, for example local anaesthetic block may have worn off while the discharge from the sensitized primary afferent fibres continues minimizing the beneficial effect of the original procedure (see [24–26]).

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In summary, the findings of Sun, Shyu and Shieh and other studies are as follows [2, 9–12, 22].

(1) An anaesthetic regimen based solely on oxygen–nitrous oxide and a volatile agent is not sufficient analgesia for painful stimulation despite the fact that during the procedure there may be no obvious subjective responses. In fact, as far as the spinal cord is concerned, it is totally unprotected against these painful insults which may have consequences for the perception of acute postoperative pain and the development of chronic pain states.

(2) Although most anaesthesiologists do provide some analgesia at some stage of the operation, pre-emptive local anaesthetic block or analgesic administration which is then continued into the postoperative period is likely to be most beneficial.

(3) The differential effects of various analgesics on Fos expression suggest that the use of other analgesics (such as clonidine, an a2 agonist, and ketamine, an NMDA antagonist) should be considered, certainly as adjuncts to morphine.

Suggestions that providing operating conditions in which the transmission of peripheral stimulation into the central nervous system is impeded are not new [33, 34]. However, as we have suggested previously, studies using Fos expression in the spinal cord may provide a more rational basis in designing novel pre-emptive anaesthetic regimens.

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References
30. Munglani R, Christadoulou E, Smith G, Harrison S, Elliot PJ, Birch PJ, Hunt SP. Persistent changes in neuronal


