Nitrous oxide or halothane, or both, fail to suppress c-fos expression in rat spinal cord dorsal horn neurones after subcutaneous formalin

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Summary
In rats injected s.c. with formalin, behavioural correlates of the amount and pattern of Fos-like immunoreactivity (Fos-LI) (molecular responses to pain) were studied to test if early phase treatment with 75% nitrous oxide or 2% halothane, or both, suppressed subsequent spinal sensitization. Rats were allocated to four treatment groups: (1) 100% oxygen (control, n = 15), (2) 75% nitrous oxide (0.5 MAC, n = 12), (3) 2% halothane (1 MAC, n = 12), and (4) 75% nitrous oxide with 2% halothane (1.5 MAC, n = 18) for 20 min. Each rat then received a s.c. injection of 1% formalin 50 μl into the left hindpaw and anaesthesia was maintained for another 5 min (early phase). A fifth group of rats receiving fentanyl 100 μg kg⁻¹ (n = 12) 10 min before formalin injection were studied simultaneously as a positive control. Rats in all groups were killed 60 min after formalin injection and maximal counts of Fos-LI labelled neurones in the dorsal horn of the rat spinal cord were compared according to laminar distribution. Formalin-induced hyperalgesia during the early phase was suppressed completely by fentanyl, 75% nitrous oxide, or 2% halothane, or both. The late phase response was attenuated by all four anaesthetic regimens within 20 min after injection, whereas behavioural scores for the nitrous oxide, halothane, or both, groups were nearly identical to the control 20 min later. Fentanyl suppressed the late phase response until 30 min after formalin injection but failed to reduce it thereafter. The numbers of Fos-LI labelled neurones for groups given nitrous oxide, or halothane, or both, were identical to the control, whereas numbers for fentanyl were 47.2% less (P < 0.01). The decrease occurred predominantly in the neck of the dorsal horn (44.9% of control, P < 0.01) and also in the nucleus proprius and superficial laminae (54.4% and 56.2% of control, P < 0.05). In summary, we found that nitrous oxide, or halothane, or both, did not suppress subsequent spinal sensitization to noxious stimulation. This result supports the previous hypothesis that inhalation anaesthesia lacks pre-emptive analgesic action. Inhalation anaesthetic agents, unlike fentanyl, suppress the early and late phase response because of anaesthetic but not analgesic effects. Thus, we suggest that measuring the genetic product of c-fos proto-oncogene is a useful adjunct to pharmacological tests whenever behavioural hyperalgesia is questionable or unobtainable. (Br. J. Anaesth. 1996; 76: 99–105)

Key words

Inhalation anaesthetics are considered poor analgesics despite potent anaesthetic actions in producing surgical immobility. Wall hypothesized that lightly anaesthetizing the spinal cord fails to block the afferent signals set off during surgery, and that other treatments such as regional anaesthesia before skin incision are required to produce significant pre-emptive analgesia during inhalation anaesthesia [1]. The concept that prevention of intense nociceptor activation can suppress the subsequent hyperalgesic state has had a major impact on the practice of postoperative analgesia [2]. Although pre-emptive analgesia is well documented in animal models, the clinical evidence is less convincing [3]. A critical difference between humans and animal models is the method of pain assessment. In animal models, the sensitivity and specificity of behavioural responses as an index of noiception largely remain to be determined.

In contrast with previous hypotheses, recent studies based on formalin-induced behavioural hyperalgesia have demonstrated mild to moderate pre-emptive analgesia after volatile agents (i.e. halothane and isoflurane) [4, 5]. Paradoxically, this response was not found in rats receiving nitrous oxide with halothane, although their combined anaesthetic potency is greater than that of each agent alone. In a parallel study, nitrous oxide, but not halothane, induced dose-dependent pre-emptive analgesia that was partially reversed by naloxone and halothane [6]. The results of these studies vary

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markedly despite identical variables being used. As discrepancies may occur as a result of general attenuation of behaviour because of the non-algesic effect of anaesthetics but not their analgesic effect [7], a further index is required to examine the analgesic effect of inhalation anaesthetics.

Unlike most physiological indices of pain, c-fos expression in the brain is least affected by various i.v. and inhalation anaesthetics [8–10]. The genetic products of c-fos expression are highly associated with neuronal sensitization in the central nervous system and with behavioural hyperalgesia [11]. The number of Fos-like immunoreactivity (Fos-LI) labelled neurones has been used widely to quantify the antinociceptive action of various analgesic agents [12, 13]. Various patterns of suppression for sub-populations of spinal nociceptive neurones may provide further understanding on how the antinociceptive process is involved [14]. By providing both qualitative and quantitative indices for pain, Fos-LI has been accepted as a morphological marker to assess the effectiveness of analgesia [15, 16]. In the present study we used Fos-LI as a biochemical correlate of behavioural indices to test if pre-stimulus inhalation anaesthesia with nitrous oxide or halothane, or both, differentially suppressed the subsequent spinal sensitization in the rat formalin model. To study the possible mechanism of the behavioural response, fentanyl was used as a positive control for analgesia.

Materials and methods

The studies were approved by the Institutional Animal Care Subcommittee of the National Taiwan University Hospital. Male Sprague–Dawley rats, weighing 300–350 g, were maintained in a 12-h light–dark cycle and allowed free access to food and water. Experiments were performed between 10:00 and 16:00 in a randomized order. Rats were allocated randomly to one of five groups: (1) control (100 % oxygen, n = 15), (2) 75 % nitrous oxide (0.5 MAC, n = 12), (3) 2 % halothane (1 MAC, n = 12), (4) 75 % nitrous oxide with 2 % halothane (1.5 MAC, n = 18), and (5) fentanyl 100 μg kg⁻¹ in 100 % oxygen (n = 12). Two phases of response were defined: early (0–5 min) and late (5–60 min) [17]. The inspired concentrations of nitrous oxide and halothane (75 % and 2 %) were chosen to provide approximately 0.5 and 1 MAC of anaesthesia, respectively. These doses were calculated on the basis of MAC, 148–150 % for nitrous oxide and 0.95–1.11 % for halothane [18], and an estimated ratio of end-tidal-to-inspired concentration of halothane of 0.5–0.6 in spontaneously breathing rats after 20 min [19]. Anaesthesia was induced by placing the animals in a Plexiglas box prefilled and flushed continuously at 4 litre min⁻¹ with one of the anaesthetics. Except for rats in the fentanyl group, which were given fentanyl 100 μg kg⁻¹ i.p. 10 min before formalin, animals were left undisturbed for 20 min and then removed briefly from the box for injection of formalin. To ensure an adequate anaesthetic level on injection, rats receiving halothane and nitrous oxide–halothane were subject to further anaesthesia until the corneal reflex and withdrawal to forceful pinch of the forepaw were absent. Animals were returned to the box immediately after injection and maintained under anaesthesia for another 5 min (early phase). Rats were then transferred to a clear open cage and allowed to recover, and their behaviour was observed by a blinded observer for 60 min after formalin injection (late phase). During the recovery phase from inhalation anaesthesia, the forepaw was pinched every 30 s to monitor the level of anaesthesia until spontaneous movement occurred. All gas concentrations were monitored continuously and maintained at the desired level with an attached gas analyser (RGM 5250 Ohmeda, Madison).

FORMALIN TEST

Diluted (1 %) formalin was prepared freshly from 37 % formaldehyde with 0.9 % normal saline before use; 50 μl was injected s.c. into the plantar surface of the left hindpaw using a 26-gauge needle. With a computer program designed by one of the authors (B.C.S.), the amount of time spent in each of four behavioural categories, 0–3, was recorded and calculated every minute, as described by Dubuisson and Dennis [20]. In brief, the categories were: 0 = the injected paw is not favoured (i.e. foot flat on floor with toes splayed); 1 = the injected paw has little or no weight on it with no toe splaying; 2 = the injected paw is elevated and the heel is not in contact with any surface; 3 = the injected paw is licked, bitten or shaken. The scores were then averaged at 5-min intervals. To investigate further the temporal pattern of behavioural responses, the scores were summed for six time intervals: 5–60, 10–60, 20–60, 30–60, 40–50 and 50–60 min, respectively. The summed score was used to facilitate comparison of behaviour with the amount of Fos-LI produced over 0–60 min after injection.

IMMUNOHISTOCHEMISTRY

All animals were killed 60 min after formalin injection. Animals were anaesthetized deeply with an overdose of sodium pentobarbitone and perfused transcardially with saline followed by 4 % paraformaldehyde in phosphate buffer 0.1 mol litre⁻¹ (pH = 7.4). The spinal cord at the lumbar enlargement was removed, post-fixed for 4 h, and cryo-protected overnight in 30 % sucrose. Frozen sections were cut in a cryostat (30 μm) and collected as free-floating sections. Sections were incubated with primary rabbit polyclonal anti-Fos antisera (Ab-2, Oncogene), diluted 1 : 750 with PBS 0.1 mol litre⁻¹, containing 3 % normal goat serum (NGS) and 0.3 % Triton X-100 for 48 h at 4 °C. After being washed in PBS, they were incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) diluted 1:200 in PBS, then incubated with biotinylated second antibody (goat anti-rabbit antisera; Vector) dilute...
No suppression of c-fos by inhalation anaesthesia

slides, air-dried and protected with a coverslip for light microscopic inspection. Two additional groups of rats, one of which received 75% nitrous oxide with 2% halothane but not formalin injection (n = 2), and other which received neither gas nor formalin (n = 2), were studied as negative controls for non-specific staining caused by either inhalation anaesthetics or animal manipulation. To examine the specificity of the primary antibody, non-specific staining was tested simultaneously for the control rats. Omission of the anti-c-fos antibody was prepared by immunoprecipitation with c-fos peptide (Oncogene) before the primary antibody reaction. Dark staining of the nucleus, as shown in the section treated with antibody alone, was completely absent. This approach ensured high quality of specificity for the antibody in use.

COUNTING OF FOS-LI LABELLED NEURONES

Fos-LI labelled neurones were examined throughout the L3–5 spinal segments and across the laminae. Sections were examined using a dark-field microscope (Axioplan, Zeiss) to determine the segmental level according to the grey matter landmarks, as described by Molander, Xu and Grant [21]. Under dark-field illumination, the dorsal horn of each section was divided further into three regions: (1) the superficial laminae (laminae I/II), (2) the nucleus proprius (laminae III/IV) and (3) the neck (laminae V/VI) [14]. Fos-LI labelled neurones were then examined with bright-field illumination at 100× magnification for each section. All images, in both dark- and bright-field illumination, were printed serially on glossy thermal paper via a connected CCD camera (Videl Lens, Cohu) and video copy processor (P67U, Mitsubishi). Fos-LI labelled neurones, which showed deep staining distinguishable from background, were counted from the printed images with respect to each lamina. To minimize further the individual bias for inspection, all images were duplicated and counted separately by two experienced laboratory assistants throughout the study. When the difference between duplicated counting was greater than 10% of each lamina, a third copy was made and inspected by one of the authors (W.Z.S.) Counts that were questionable in either quantity or quality were excluded. At least 10–15 sections were scanned for each segment, and three sections with the greatest number were selected. The observers who scored formalin-induced behavioural hyperalgesia and investigators who counted Fos-LI labelled neurones were all blind to the treatment of each animal.

DATA ANALYSIS

Statistical analysis was performed to compare the different groups of animals, using one-way analysis of variance (ANOVA) for the pain score at each time point and for the total number of Fos-LI labelled neurones, and two-way repeated measure ANOVA (treatment × lamina) for the number of labelled neurones and the laminar region. The Student–Newman–Keuls test was used for multiple comparisons. P < 0.05 was considered statistically significant.

Results

BEHAVIOURAL RESPONSES

S.c. injection of 1% formalin produced a similar biphasic pain response in the control group to that described by Dubuisson and Dennis [20]. A brief phase of intense pain (i.e. licking, biting and flinching of the injected paw) occurred in the first 5 min. Pain then subsided for 10 min, and a later phase
of moderate pain (i.e. elevation of the injected paw) was expressed from 20 to 60 min. Peak pain score occurred 30 min after injection (fig. 1A). In rats receiving fentanyl, or nitrous oxide or halothane, or both, behaviours such as elevation, biting, shaking and flinching of the injected paw were absent during the early phase. Most rats recovered from inhalation anaesthesia, as shown by responding to painful pinch stimulation within 5 min after anaesthesia, and this was followed by a quiescent period, that is a period without any characteristic pain behaviour. Steady gait and investigatory behaviours (e.g. rearing) persisted for 10–15 min and were followed by a short period of shaking and biting of the injected paw. For rats receiving fentanyl 100 g kg$^{-1}$, a variable degree of rigidity was observed on formalin injection and maintained for approximately 30 min thereafter with attenuated behavioural hyperalgesia.

Summations of the pain scores from various time intervals were compared to investigate the overall behavioural response to the formalin test (fig. 1B). At the 5–60-min interval, the score for the nitrous oxide group was identical to that for the control group, while scores for fentanyl (24 %, $P < 0.01$), halothane (18 %, $P < 0.05$), and nitrous oxide–halothane groups (17 %, $P < 0.05$) were significantly lower. Starting from 20 min after injection, summed scores for all gas groups were indistinguishable ($P > 0.05$), indicating that inhalation anaesthesia attenuated pain scores for 20 min after injection. The score for the fentanyl group was 19 % less than the control score ($P < 0.05$) at the 20–60-min interval, whereas no difference for all groups was found at 30–60, 40–60 and 50–60 min. Despite full recovery, many rats fell asleep for the rest of the time. With the injected paw held dorsiflexed tonically, the rats lay on the uninjected side to avoid accidentally touching the ground.

**COUNTING OF FOS-LI LABELLED NEURONES**

Fos-LI labelled neurones were distinguished readily by their dark blue nucleus and scant staining of the cytoplasm. In control rats injected with 1 % formalin, numerous Fos-LI labelled neurones were observed in the superficial laminae and neck of the dorsal horn. The most dense concentration of Fos-LI neurones, 48.3 (6.5) per section, occurred in the superficial lamina (fig. 2). Modest numbers of labelled neurones were observed in the neck of the dorsal horn, 19.4 (6.5) per section. Fewer Fos-LI neurones, 8.9 (3.2) per section, were observed in the nucleus proprius. Fos-LI labelled neurones in the negative control rats were found occasionally (less than 5 per section) suggesting that inhalation anaesthesia per se failed to enhance c-fos expression. Significant differences were evident across the five groups ($P < 0.05$) and across spinal cord laminae ($P < 0.01$). These differences were the result of a pronounced decrease in the fentanyl group. Fentanyl reduced overall numbers of Fos-LI labelled neurones (56 % of control, $P < 0.05$). Suppression of Fos-LI occurred in the superficial lamina (61 % of control, $P < 0.05$), nucleus proprius (46 % of control, $P < 0.05$) and the neck (39 % of control, $P < 0.01$) (fig. 3). None of
the rats receiving inhalation anaesthesia had reduced numbers of Fos-LI labelled neurones ($P > 0.05$).

**Discussion**

We have demonstrated that inhalation anaesthesia with 75% nitrous oxide or 2% halothane, of both, failed to suppress formalin-induced behavioural hyperalgesia in the late phase (20–60 min after injection) and the expression of c-fos proto-oncogene, a molecular index of spinal sensitization to pain. Both behavioural and biochemical evidence demonstrated a lack of pre-emptive analgesic effect of inhalation anaesthesia at 0.5–1.5 MAC. Our results clearly contradict previous studies showing a modest reduction in late phase behavioural hyperalgesia using the same experimental paradigms but with a lower anaesthetic potency (30–75% nitrous oxide, 0.7–1.8% halothane, 1–2.5% isoflurane) [4–6]. The major difference between previous studies and ours is the behavioural index used to quantify formalin-induced hyperalgesia. Abram and Yaksh [4] and Goto, Marota and Crosby [6] counted the occurrence of flinch behaviour, whereas a mean rating score was used in our study. Although flinch is generally believed to be more robust and spontaneous [22], none of these studies compared flinching with mean rating score. Moreover, the stability and reliability of these behavioural measurements have never been proved under general anaesthesia. In the present study, we have shown the limitation of using behavioural observation to detect antinociceptive effect by inhalation anaesthesia. For a similar degree of suppression of behavioural response, fentanyl reduced the number of Fos-LI labelled neurones whereas inhalation anaesthesia did not. Thus, attenuation of formalin-induced behavioural hyperalgesia may occur as a result of analgesic in addition to non-analgesic effects.

The reason why we used a mean rating score was because of variable responses in previous studies that used flinch behaviour. Abram and Yaksh [4] demonstrated a modest reduction in the late phase whereas no decrease was found by Goto, Marota and Crosby [6]. Furthermore, Goto, Marota and Crosby showed that 0.9% halothane suppressed early phase flinch only partially whereas O’Connor and Abram demonstrated that 0.7% halothane completely suppressed it [5]. These results, together with ours, are a clear demonstration of how behavioural observations can vary markedly whether or not a similar variable is used. Mean rating score was thus chosen by us to avoid further confusion. One possible explanation of the discrepancy between previous studies and ours is the occurrence of flinch that may be more susceptible to prolonged amnesia after 25 min of exposure to 0.5–1.5 MAC of anaesthesia. It has been shown that single-variable measures of formalin-induced nociceptive behaviours, such as flinching or licking, are likely to be influenced by pharmacological agents which either stimulate or depress locomotor activity or cause competing stereotypic behaviours [23]. Although no residual anaesthetic effect was present during the late phase, many rats remained asleep despite full recovery from anaesthesia, as shown by painful pinch. It is unlikely that the hypnotic property of inhalation anaesthesia outlasts its anaesthetic property. Hence, potentiation of the normal sleep cycle during observation (10:00–16:00) may explain the cause of prolonged sleep. However, the lingering effect of residual gases on flinch is not supported by Abram and Yaksh [4] who found that continuous exposure to 0.25% isoflurane during the late phase failed to reduce flinch responses. This, again, does not explain the pre-emptive analgesic effect of 75% nitrous oxide (0.5 MAC) and not 0.9% halothane with or without 75% nitrous oxide (0.5–1 MAC), became nitrous oxide is less soluble in blood and so should have less of an effect in rats. Another explanation for our results is the use of a different concentration of formalin; 1% compared with 5% in previous studies. It has been shown that some behavioural responses, for example flinching and licking, are more common after 5% formalin [24]. Thus to use flinch as a dependent measure in a rat model, one must use a high concentration of formalin (5%) to obtain a meaningful level of response. However, this is inconsistent with the recent understanding that low formalin concentrations (0.5–2.5%) have the advantage of increasing the sensitivity of the test [7]. Detection of the effect of weak analgesics is facilitated by eliminating the problem of a ceiling effect, and it is furthermore possible to detect a state of hyperalgesia [17, 25]. We believe 1% formalin is more sensitive than 5% in detecting the weak, if any, analgesic effect of general anaesthesia.

A potential advantage of Fos-LI is its usefulness in determining the most appropriate times for comparison. Pain scores for all treatment groups were significantly less than for the control within 20 min after injection (i.e. 5–60 and 10–60 min, respectively). However, scores for all anaesthetics were indistinguishable after 20 min (i.e. 20–60, 30–60, 40–60 and 50–60 min, respectively). As no suppression of Fos-LI was found for rats receiving inhalation anaesthesia, we suggest that the behavioural response within 20 min after anaesthesia should be disregarded. It makes no difference if a brief period (e.g. 50–60 min) or a longer period (e.g. 20–60 min) is used. On the other hand, fentanyl produced suppression of the late phase response within 30 min after injection but no suppression thereafter. Comparison of its antinociceptive effect must therefore include the initial response (e.g. 5–60, 10–60 and 20–60 min). Thus, the behavioural response at the 20–60-min time period, a significantly lower pain score by fentanyl, and an identical score by all anaesthetic gases, are comparable to the molecular index of spinal sensitization. Despite the fact that behavioural responses to formalin test and postoperative pain vary greatly with time, potential bias from selecting an appropriate time interval for comparison is overlooked by most investigators. Various times between 5 and 75 min after injection are chosen, for example, 10–60 min (Abram and Yaksh [4]) and 30–75 min (Goto, Marota and Crosby [6]). No comparative study has been performed to explain how the specific times were chosen. Using Fos-LI as a molecular correlate of behavioural...
hyperalgesia, this is the first study to describe why and how the specific times were chosen.

Despite being used extensively as a quantitative tool for nociception, the physiological role of the c-fos gene product in the spinal cord dorsal horn remains to be determined. c-fos expression in the neureons is produced by either noxious or non-noxious stimulation. Non-noxious stimuli, such as brushing and manipulation of the hindlimb, evoke a moderate increase in Fos-LI in areas of the spinal cord (lamineae III/VI) that receive primarily non-nociceptive afferents [26]. Without nociceptive input, walking on a rotating rod produces a large increase in Fos-LI and c-fos mRNA level, while the pattern of labelled neurones is distinct from that produced by noxious stimulation [14]. Under controlled stimulus conditions, it is well documented that the pattern of Fos-LI distribution is related to nociception [27]. However, non-noxious stimulation (e.g. itching) can also evoke a similar pattern of c-fos expression [28]. Hence, the use of biochemical markers for pain measurement must be strictly evaluated during and immediately after the response as an index for nociception must be accepted as a useful adjunct to behavioural observation when correctly designed.

In summary, both our molecular and behavioural indices demonstrated that pre-emptive analgesia was lacking after inhalation anaesthesia, regardless of gas and anaesthetic potency. Our study failed to confirm previous findings that isoflurane [4], halothane [5] and nitrous oxide [6] produced moderate pre-emptive analgesia, and thus in turn supports the hypothesis that general anaesthesia fails to block the afferent signals set off during intense surgical pain. The differential Fos-LI response between fentanyl and general anaesthesia suggests that suppression of behavioural hyperalgesia may occur as a result of analgesic and non-analgesic effects. Thus behavioural response as an index for nociception must be evaluated carefully during and immediately after general anaesthesia. We suggest c-fos expression is a useful adjunct to pharmacological tests whenever behavioural observation is questionable or unobtainable.

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