High-dose remifentanil prevents development of thermal hyperalgesia in a neuropathic pain model

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Editor’s key points
• Chronic pain after nerve injury is common.
• Spinal nerve transection in rats can mimic this pain.
• The preventative effect of perioperative remifentanil was studied in nerve-transected rats.
• High- but not low-dose remifentanil prevented thermal hyperalgesia.

Background. Intraoperative nerve lesions can lead to chronic postoperative pain. There are conflicting data as to whether or not anaesthetics administered intraoperatively are beneficial. We investigated if remifentanil administered at the time of nerve injury was able to attenuate neuropathic hypersensitivity.

Methods. Rats were anaesthetized with isoflurane, endotracheally intubated, and a tail vein catheter was inserted. Rats received an i.v. infusion of either saline or low- or high-dose remifentanil (2 or 20 μg kg⁻¹ min⁻¹, respectively) for 20 min. During this time, rats received a spinal nerve L5 transection to induce neuropathic pain or a sham procedure. Behavioural tests to assess mechanical and cold allodynia and heat hyperalgesia were performed on postoperative days 1, 3, 7, 14, 21, and 28.

Results. Sham-operated animals exhibited no hypersensitivity regardless of the intraoperative remifentanil dose. In rats which received spinal nerve L5 transection, mechanical and cold allodynia developed with no significant differences between treatment groups. However, thermal hyperalgesia was reduced in rats given high-dose remifentanil: mean (standard deviation) area under the curve 426 (53) compared with 363 (34) and 342 (24) in saline or low-dose remifentanil treated rats, respectively (P<0.05).

Conclusions. High-dose remifentanil administered at the time of transection of the spinal nerve at L5 prevents subsequent thermal hyperalgesia.

Keywords: hyperalgesia; nerve, damage (postoperative); pain, neuropathic; pharmacology, opioids; remifentanil

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Chronic pain after surgery is often the result of intraoperative nerve injury. Pain states due to nerve injury are categorized as neuropathies, frequently manifested by hyperalgesia, allodynia, or both. Although the underlying pathophysiology of neuropathic pain often involves peripheral and central sensitization, spontaneous electrical discharge from injured primary sensory afferents may also be a key component.

Preoperative pain after common surgical procedures is frequent. For example, more than 50% of patients after thoracotomies and up to one-third of patients after inguinal hernia repair complain of neuropathic pain. Preoperative pain is a risk factor for developing chronic pain after surgery, but few clinical studies describe the benefit of perioperative interventions to reduce the neuropathy after surgery. Ong and colleagues reported that the choice of spinal, epidural, or general anaesthesia had no effect on pain 14 months after lower limb amputation.

Animal models of neuropathic pain such as chronic constriction injury or spinal nerve ligation lead to hypersensitivity. However, the intensity and duration of such hypersensitivity varies across laboratories, despite the use of standardized nerve injury, standardized behavioural tests, and the same rat strain. Reports of prospective animal studies investigating the effects of administering anaesthetics or analgesics interoperatively on the subsequent extent and time-course of neuropathic pain have been inconsistent. No reduction in neuropathic pain in the spinal nerve ligation model after intraoperative systemic administration of morphine was found, but anti-hyperalgesic effects of a single intraoperative morphine bolus up to 26 days after chronic constriction injury of the sciatic nerve was reported.

A shortcoming of both studies is that morphine is not usually used to maintain anaesthesia in a modern clinical setting, since more potent opioids are available.

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We therefore hypothesized that the ultra-short-acting and highly potent opioid, remifentanil, which is commonly administered intraoperatively as the analgesic component of clinical anaesthesia regimens, would reduce the hypersensitivity that is normally observed after spinal nerve transection.

**Methods**

**Animals**

After approval of the experimental protocol by the local animal ethics committee, 66 male Sprague–Dawley rats (200–300 g, Charles River, Sulzfeld, Germany) were housed in a 12 h light:12 h dark environment and provided food and water ad libitum. Animal handling was performed in adherence to the German national animal welfare policies and reported according to the ARRIVE guidelines.19

**Anaesthesia**

Animals were placed in a Plexiglas chamber and anaesthesia was induced using 2.5% isoflurane. After induction, rats were endotracheally intubated with a 16 G i.v. catheter and mechanically ventilated (Harvard Apparatus Rodent Ventilator Model 683, tidal volume 10 ml kg⁻¹ body weight, respiratory rate 60 bpm). Anaesthesia was maintained with 2.5% isoflurane in 50% O₂ and 50% air. A 24 G tail vein catheter was inserted and after randomization using the sealed envelope technique, one of the three anaesthetic supplements was infused: (i) normal saline, (ii) remifentanil 2μg kg⁻¹ min⁻¹ (low dose),20–22 or (iii) remifentanil 20μg kg⁻¹ min⁻¹ (high dose).23 24 Remifentanil was purchased from GlaxoSmithKline, Germany (Ultiva®). The infusion was maintained for exactly 20 min, in each animal.

**Surgery**

After inserting the tail vein catheter and starting the drug infusion, the incision site was shaved with clippers, and a skin incision was made from the left vertebral transverse process of L4 to the left iliac crest. The underlying muscles were gently retracted, giving access to the L5 and L6 vertebral transverse processes. This lateral approach has the benefit, when compared with the dorsal approach, that the L6 transverse process does not need to be removed, which sometimes causes bleeding. The L5 spinal nerve was identified, lifted slightly, and transected with Noyes curved iris scissors. The wound was irrigated with saline, inspected for haemostasis, and closed in two layers with 4-0 vicryl sutures. In sham-operated animals, an identical exposure was performed, but care was taken to avoid trauma to the nerves and no transection was performed. Animals with inability to flex the left hind limb after operation, indicating damage to the L4 nerve, were removed from the study, since this precluded testing.25 Randomization to the nerve injury or sham operation groups was performed using the sealed envelope technique (n = 11 per group). After the surgery, animals were allowed to recover from anaesthesia in an observation chamber under a warming light. Oxygenation was assessed by monitoring skin colour. Body temperature was measured and animals were warmed using a heating lamp when the body temperature was below 36.5°C. The heating lamp was turned off when the body temperature was higher than 37.0°C. Awakening times, that is, time from placement in recovery cage until first purposeful movement, were documented.

**Behavioural tests**

Inverted ventilated Plexiglas cages upon an elevated aluminium screen surface with 1 cm² mesh openings were used as testing cages. All animals were habituated to the testing scenario on three consecutive days before baseline testing. Habituation was assumed when the animals started to eat a pellet of the standard laboratory diet in the testing cage.26 The same testing cage was used for all three and testing was performed in a quiet, temperature-controlled room between 9 a.m. and 12 p.m. by the same experimenter, who was blinded with respect to treatment groups.

Baseline testing was performed on three consecutive days before surgery and 1, 3, 7, 14, 21, and 28 days after surgery.

**Mechanical stimulus**

Unrestrained rats were tested for mechanical allodynia with custom-built 2 and 12 g von Frey filaments applied to the ipsilateral hindpaw.27 The tips of both filaments consisted of small plastic cylinders with identical diameter. Rats were subjected to three sets of 10 stimulations with each filament, with at least 5 min between each set of stimulations, and mechanical allodynia was measured as the number of paw withdrawals from the 30 tests. Mechanical allodynia was defined as a significantly increased response to this normally non-noxious stimulus to one or both von Frey filaments in nerve injured compared with sham-operated animals.

**Cold stimulus**

Paw sensitivity to cold was assessed as a brisk foot withdrawal in response to acetone application.28 An acetone drop was formed at the end of a piece of 20 G polyethylene tubing attached to a syringe. The acetone drop was then applied to the plantar surface of the foot without touching the paw with the tubing. Acetone was applied three times to each paw with a 90 s interval between drops. The cumulative time the animal spent shaking, biting, or licking the paw indicated cold allodynia. Cold allodynia was defined as a significantly increased response to acetone application in nerve injured compared with sham-operated animals.

**Heat stimulus**

Paw withdrawal latencies to a thermal stimulation applied by the Ugo Basile Plantar Testing apparatus (Stoelting Co., WoodDale, IL, USA) were measured. The device was modified so that a small mirror attached in a 45° angle close to the opening of the radiant heat source enabled the experimenter to focus the light beam on the centre of the left hind paw without hitting the mesh grid. Thermal response latencies were measured three times with an inter-stimulus interval of at least 90 s, and then averaged. A cut-off latency of 20
s was used to prevent tissue damage. Heat hyperalgesia was defined as significantly decreased response latency to the radiant heat beam in nerve injured compared with sham-operated animals. At the end, animals were killed by CO₂ asphyxiation. Rats were placed in an air-filled chamber and CO₂ was infused at a slow filling rate until the rats became unconscious. The filling rate was then increased.

Statistics
Data are presented as mean with standard deviation in parentheses unless indicated otherwise.

To minimize the problem of multiple comparisons, we calculated the area under the curve of the behavioural response–time curves (AUC) for each individual animal and each performed behavioural test, respectively, according to the following formula:

$$AUC = \frac{1}{2} \sum_{i=0}^{n-1} (t_{i+1} - t_i)(y_i + y_{i+1})$$

where $y_i$ represents the behaviour measured at times $t_i$ ($i=0, \ldots, n$)\(^{29}\). The three baseline measures performed on separate days before induction of the nerve injury were averaged for each animal and used for $t_0=0$. AUCs were then averaged according to drug and operation treatment groups, that is, (i) saline, low-dose remifentanil, or high-dose remifentanil, and (ii) sham operation or nerve injury. After testing for normal distribution using the D’Agostino–Pearson omnibus K2 test, (ii) sham operation or nerve injury. After testing for normal saline, low-dose remifentanil, or high-dose remifentanil, and according to drug and operation treatment groups, that is, (i) saline, low-dose remifentanil, or high-dose remifentanil, and tested for significance using one-way ANOVA with the post hoc Bonferroni comparison of individual means.

Awakening times were averaged according to drug treatment group, that is, saline, low-dose remifentanil, or high-dose remifentanil, and tested for significance using one-way ANOVA with the post hoc Bonferroni analyses.

For all performed statistical tests, the significance level was set to $<0.05$. With a group size of $n=11$, a significance level of $P<0.05$ and a power of $\beta=0.8$, a standardized effect (difference between means divided by standard deviation) of 1.25 is detectable with a simple t-test.

Results
One animal in the saline/sham-operation group was culled 4 days after the surgical procedure because of an infection at the incision site and was excluded from data analysis. Awakening times after surgery were similar in all groups (data not shown). There was no difference in mechanical allodynia, cold allodynia, or heat hyperalgesia compared with baseline measurements in any group (data not shown).

Rats with spinal nerve L5 transection that received saline or low-dose remifentanil intraoperatively exhibited mechanical and cold allodynia and thermal hyperalgesia, which developed between postoperative days 1 and 3. This hypersensitivity remained throughout postoperative day 28 (Fig. 1, left panels).

There was no statistical difference between saline or low-dose remifentanil-treated rats with respect to mechanical and cold allodynia or heat hyperalgesia (Fig. 1, right panels). Rats that received high-dose remifentanil developed mechanical and cold allodynia to a comparable extent as the saline and low-dose remifentanil groups did not develop thermal hyperalgesia during the postoperative observation period of 28 days. The AUC for thermal hyperalgesia in the high-dose remifentanil group was significantly higher ($P<0.05$, two-way ANOVA with the post hoc Bonferroni comparison) than in the saline and low-dose remifentanil groups indicating attenuation/prevention of thermal hyperalgesia (Fig. 1, right panel).

Discussion
This study sought to investigate the effects of the ultra-short-acting and highly potent opioid remifentanil, administered intraoperatively, on spinal nerve transaction induced hypersensitivity in rats.

Even in highly standardized animal models of neuropathic pain, there is considerable variability in the extent and duration of hypersensitivity. One reason for this observation could lie in the fact that anaesthesia for the induction of the nerve injury is not standardized. Bennett and Xie\(^{15}\) used 40 mg kg\(^{-1}\) of intraperitoneal (i.p.) sodium pentobarbital for placing loose ligations around the sciatic nerve and observed thermal hyperalgesia until 70–80 days after placing the sutures. Kingery and colleagues\(^{9}\) used i.p. sodium pentobarbital (50 mg kg\(^{-1}\)) for placing loose ligations around the sciatic nerve and observed heat hyperalgesia up to 50 days after ligation, which was the latest time-point tested. Kim and Chung\(^{16}\) used 0.8% halothane in 50% N\(_2\)O and 50% O\(_2\) for placing a tight ligature around the spinal nerve L5. They observed significant mechanical and cold hypersensitivity up to 98 and 35 days, respectively, and heat hyperalgesia from postoperative day 3 until postoperative week 5. Lancellotta and colleagues\(^{33}\) used halothane for induction (3%) and maintenance (1–1.5%) of anaesthesia to perform spinal nerve L5 ligation and transection. They observed significant mechanical and cold hypersensitivity up to 33 and 54 days, respectively. None of the above studies was designed to show effects of the anaesthetic regimen for the induction of the nerve injury.

Prospective studies investigating the effects of intraoperatively administered opioid analgesics on development, extent or time-course of hypersensitivity after nerve injury reported inconsistent data: Kontinen and colleagues\(^{31}\) investigated the effects of systemic morphine given either subcutaneously (10 mg kg\(^{-1}\)) before tight ligation of the L5 and L6 spinal nerves or immediately after surgery using slow-release pellets (75, 150, or 375 mg). The authors found no attenuation of neuropathic pain-like symptoms by any treatment and concluded that neither premedication nor postoperative pain management with
systemic morphine effective in preventing postoperative neuropathic pain. It is noteworthy, however, that the rats in this study developed both mechanical and cold allodynia but no thermal hyperalgesia. Thermal hyperalgesia is usually one of the hyperalgesic behaviours that can consistently be observed after spinal nerve ligation and...
therefore the lack of thermal hyperalgesia might have been attributed to the perioperative morphine administration. Consistent with this observation are our findings of robust thermal hyperalgesia in the saline and low-dose remifentanil groups, but no occurrence of thermal hyperalgesia in the high-dose remifentanil group.

Smith and colleagues\textsuperscript{28} investigated the effects of morphine and other drugs on chronic constriction injury of the sciatic nerve in rats. Animals received 5 mg kg\textsuperscript{-1} morphine s.c. 30 min before loose ligation and another dose 6 h after surgery. Although only mechanical allodynia was measured, pre-treatment with morphine reduced the magnitude of hypersensitivity for at least 26 days after nerve injury.

Drugs, doses, and administration routes used in previous studies often do not relate to modern clinical opioid analgesic regimens. Remifentanil is \textasciitilde60-fold more potent than morphine\textsuperscript{33} and its use at relevant doses usually necessitates mechanical ventilation due to respiratory depression. However, such circumstances are not usually reflected in animal experiments. Thus, relative ‘underdosing’ of opioid analgesics may lead to an inability to show beneficial effects of opioids on neuropathic pain after nerve injury.

At a first glance, our results contradict the growing evidence for opioid-induced hyperalgesia (OIH).\textsuperscript{35} However, acute OIH after perioperative opioid administration is not unequivocal; there have been conflicting reports.\textsuperscript{35,36} OIH can usually be observed immediately after cessation of opioid administration, that is, in the recovery room, whereas hypersensitivity after nerve injury gradually develops over the subsequent 1–3 postoperative days. Cabanero and colleagues\textsuperscript{37} studied the pro-nociceptive effects of remifentanil in a postsurgical model. They made a plantar incision in mice receiving sevoflurane with either saline or remifentanil and a subcutaneous administration route. Most importantly, however, they chose a different species, a much smaller remifentanil dose (~1.3 $\mu$g kg\textsuperscript{-1} min\textsuperscript{-1}), and a subcutaneous administration route. They made a plantar incision in mice receiving sevoflurane with either saline or remifentanil and observed mechanical allodynia and thermal hyperalgesia. However, they used a different species, a much smaller remifentanil dose (~1.3 $\mu$g kg\textsuperscript{-1} min\textsuperscript{-1}), and a subcutaneous administration route. Most importantly, however, they chose a postsurgical as opposed to a neuropathic pain model. Again, no intubation or mechanical ventilation was used in this study, which might indicate a relatively underdosing of remifentanil resulting in the failure to achieve protective effects.

Therefore, we postulate that OIH and neuropathic hypersensitivity after nerve injury are based on two distinct pathomechanisms. While our study was not designed to investigate potential mechanisms of the protective effects of remifentanil, we strongly suggest that future studies should be designed to account for possible differences between these two pain states.

In conclusion, our results indicate that perioperatively administered high-dose remifentanil prevents the development of thermal hyperalgesia normally observed after L5 spinal nerve transection in rats. Furthermore, we suggest that pain-related animal studies investigating postsurgical effects and potential benefits of intraoperatively administered drugs should be more comparable with typical human surgical anaesthetic regimens.

Declaration of interest
None declared.

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References
5 Perkins FM, Kehlet H. Chronic pain as an outcome of surgery. Anesthesiology 2000; 93: 1123–33
11 Bach S, Noreng MF, Tjellden NU. Phantom limb pain in amputees during the first 12 months following limb amputation, after pre-operative lumbar epidural blockade. Pain 1988; 33: 297–301
14 Ong BY, Arneja A, Ong EW. Effects of anesthesia on pain after lower-limb amputation. J Clin Anesth 2006; 18: 600–4
15 Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33: 87–107

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24 Taylor BK, Peterson MA, Basbaum AI. Early nociceptive events influence the temporal profile, but not the magnitude, of the tonic response to subcutaneous formalin: effects with remifentanil. J Pharmacol Exp Ther 1997; 280: 876–83.


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