American College of Neuropsychopharmacology

www.nature.com/npp

ARTICLE

Check for updates

Fentanyl-induced hyperalgesia and analgesic tolerance in male rats: common underlying mechanisms and prevention by a polyamine deficient diet

Emilie Laboureyras[1,2], Meric Ben Boujema[1,2], Annie Mauborgne[3], John Simmers[1,2], Michel Pohl[4] and Guy Simonnet[1,2]✉

Opioids are a mainstay of pain management but can induce unwanted effects, including analgesic tolerance and paradoxical hyperalgesia, either of which leads to increased pain. Clinically, however, the relationship between these two phenomena remains elusive. By evaluating changes in mechanical nociceptive threshold in male rats, we found that in contrast to a purely analgesic control response to a single subcutaneous administration of fentanyl (25 µg/kg), in rats subjected to inflammatory pain 2 weeks previously (Day_0), the same test dose (D_{13}) induced a bi-phasic response: initial decreased analgesia (tolerance) followed by hyperalgesia lasting several hours. Both the tolerance and hyperalgesia were further enhanced in rats that had additionally received fentanyl on D_0. The dose-response profiles (5 fg to 50 µg/kg) of pain- and opioid-experienced rats were very different from pain/drug-naive rats. At ultra-low fentanyl doses (<5 ng/kg and <500 ng/kg for naïve control and pain/drug-experienced rats, respectively), solely hyperalgesia was observed in all cases. At higher doses, which now produced analgesia alone in naive rats, reduced analgesia (tolerance) coupled with hyperalgesia occurred in pain/fentanyl-experienced rats, with both phases increasing with dose. Transcriptomic and pharmacological data revealed that an overactivation of the spinal *N*-methyl-$_D$-aspartate receptor-inducible NO synthase cascade plays a critical role in both acute tolerance and hyperalgesia, and together with the finding that the magnitudes of analgesia and associated hyperalgesia are negatively correlated, is indicative of closely related phenomena. Finally, a polyamine deficient diet prevented inducible NO synthase transcript upregulation, restored fentanyl's analgesic efficacy and suppressed the emergence of hyperalgesia.

Neuropsychopharmacology (2022) 47:599–608; https://doi.org/10.1038/s41386-021-01200-5

INTRODUCTION

Opioids are the analgesic of choice for the relief of moderate-to-severe clinical pain. Such treatment typically requires continuous pain relief over lengthy periods, usually necessitating an opioid dose increase as the response to the drug declines [1–3]. Clinically, however, it is difficult to determine whether the increased dose requirement, which differs widely between individuals, is due to cause of pain enhancement, an increase in drug tolerance, opioid-induced hyperalgesia—i.e., pain sensitization caused by a pre-exposure to the drug itself—or a combination of all three conditions [4–9]. Although numerous mechanisms underlying analgesic tolerance and hyperalgesia after chronic opioid treatment have been proposed, [9–11] the long-lasting changes in opioid effectiveness after a tissue injury, such as from surgery requiring acute peri/postoperative opioid administration, are not well understood.

To better understand these complex phenomena, preclinical animal models mimicking clinical situations using opioids [12–14] are potentially useful for examining the different factors accounting for diminishing opioid analgesic efficacy. The administration of a single high opioid dose to healthy, pain-free rats, induces hyperalgesia in a dose-dependent manner over a period of several days following initial analgesia [5, 6]. This apparently paradoxical opioid effect [4, 8] is mainly dependent on an increased activation of neuronal *N*-methyl-$_D$-aspartate receptor (NMDAR) and related signaling systems [5, 7, 15–18]. In humans, opioid-induced hyperalgesia has also been observed in healthy volunteers administered with remifentanil [19] and in heroin addicts [20].

In animal pain studies, a single dose administration of the opioid fentanyl in association with a surgical lesion or inflammation, not only produces an immediate analgesia, but also acts in a dose-dependent manner to amplify and prolong subsequent hyperalgesia [17, 21–23]. Moreover, although such pain-experienced animals recover apparent normal pain sensitivity after healing, pain remission does not simply involve a return to a pre-injury homeostatic state, but is associated with a long-lasting silent form of insidious pain vulnerability referred to as "latent pain sensitization" [23, 24], a form of pain memory [25]. Correspondingly, hyperalgesia observed in response to a second tissue injury was found to be strongly enhanced in rats that previously received fentanyl after a first lesion, even when the second injury occurs several weeks after apparent complete pain recovery [9, 11, 23, 26]. In both animals and humans, there is growing evidence that the use of opioids prolongs pain states after an

[1]Univ. de Bordeaux, INCIA, 33076 Bordeaux, France. [2]CNRS, INCIA, UMR 5287, 33076 Bordeaux, France. [3]Univ. Pierre et Marie Curie, INSERM UMRS 975, 75013 Paris, France. [4]Univ. Paris Descartes, INSERM UMR 894, 75014 Paris, France. ✉email: ga.simonnet@gmail.com

Received: 1 June 2021 Revised: 20 September 2021 Accepted: 24 September 2021
Published online: 7 October 2021

acute tissue injury and potentially worsens pre-existing pain [10, 12–14, 27–31]. Therefore, the ubiquitous use of opioids for acute analgesic treatment may also be responsible for the long-term activation of pronociceptive systems leading to pain hypersensitivity, which in turn could be an important contributor to the development of opioid analgesic tolerance and hyperalgesia.

The main goal of this study was thus to evaluate changes in the acute effectiveness of fentanyl in male rats with a previous history of acute pain treated by a single opioid administration, an antecedent condition which is frequently encountered in clinical surgical situations. We also assessed the underlying role of spinal NMDAR-related activity and tested a putative nutrient treatment for inhibiting the development of drug tolerance and hyperalgesia.

MATERIALS AND METHODS

Animals

Experiments were performed using adult Sprague-Dawley rats (Charles River Laboratories, L'Arbresle, France) weighing 200–225 g before experimentation. Although gender differences in pain sensitivity have become increasingly recognized [32], the experiments reported here were conducted exclusively on male rats, as in our and most other previous studies. The rats were housed four animals per cage under a 12 h light/dark cycle (illumination at 7h00) and a room temperature of 23 ± 2 °C, with food and water made constantly available. Animal care and all experiments were conducted in accordance with the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP) and after approval by the local Ethics Committee (CEEA50, Project B-33-063-6) of the University of Bordeaux and the "Ministère de l'Agriculture et de l'Alimentation".

Drugs

Fentanyl citrate, naltrexone, L-Canavanine (an inducible nitric oxide synthase (iNOS) inhibitor), carrageenan λ (Sigma-Aldrich, Saint-Quentin Fallavier, France) and BN2572 (a non-competitive NMDAR antagonist [33, 34]; also see Supplementary Materials and Methods) were dissolved in physiological saline (0.9%). Fentanyl, naltrexone (1 mg/kg) and BN2572 (0.3 mg/kg) were administered subcutaneously (s.c.; 1 mL/kg body weight) whereas L-Canavanine (50 mg/kg) was administered intraperitoneally (i.p.; 1 mL/kg body weight). Control animals received an equal volume of saline injections.

Polyamine deficient diet

A synthetic diet lacking in polyamines was kindly supplied by Pr. J.P. Moulinoux (Université de Rennes 1, France) and was prepared with ingredients purchased from Sigma-Aldrich. (St Louis, MO, USA) as previously described [35]. Polyamine determinations were performed by atmospheric pressure chemical ionization mass spectrometry [36]. Our polyamine deficient diet contained <10 µg/kg polyamines, whereas the commercial standard rodent chow (SAFE, Augy, France) fed to all control rats contained 54 mg/kg putrescine, 27 mg/kg spermidine and 7 mg/kg spermine.

Experimental animal groups

After arrival at the laboratory, rats were randomly assigned to four different experimental groups, each comprising 8–10 animals (also see Supplementary Materials and Methods for further details on general experimental procedures):

1. A naive group of rats that had not been previously subjected to either opioid administration or pain exposure (Control group).
2. A group that had received fentanyl administration (four injections of 100 µg/kg fentanyl; see below) on D_0, 13 days prior to a single fentanyl (generally 25 µg/kg) test injection (Fenta group).
3. A group that had been subjected to inflammatory pain (by injection of 1% carrageenan into a hindpaw; see Supplementary Materials and Methods) 13 days prior to the single fentanyl test injection (Inf group).
4. A group that had been subjected to both inflammatory pain and fentanyl exposure (4 × 100 µg/kg injections), 13 days prior to the fentanyl test injection (Inf-Fenta group).

With rats of the Fenta and Inf-Fenta groups, to mimic a treatment with high opioid doses as currently used for surgery in humans, fentanyl (100 µg/kg, s.c.) was injected four times at 15 min intervals on D_0, thus resulting in a total fentanyl dose of 400 µg/kg. When associated with inflammation induction (Inf-Fenta group), the first fentanyl injection was performed 5 min before the carrageenan injection.

Measurement of nociceptive threshold

Nociceptive thresholds in experimental rats were determined using a Randall-Selitto method as previously described [37, 38], involving a paw pressure vocalization test in which a constantly increasing pressure is applied to the hind paw until the subject squeals. A Basile analgesiometer (Bioseb, Chaville, France; stylus tip diameter, 1 mm) was used with a 600 g cut-off value to prevent tissue damage. In all experiments, nociceptive thresholds were evaluated for both hind paws of each rat at a 30 min interval between measurements. The first nociceptive threshold measured on D_0 (or on D_{13} for the fentanyl test phase) was taken as the baseline reference value (also see Supplementary Materials and Methods).

Fentanyl test injection

In most experiments, rats received a single subcutaneous test injection of 25 µg/kg fentanyl (fentanyl test) on D_{13}, and nociceptive thresholds were measured every 30 min until a return to basal values (up to 4.5–5 h later). In a second series of experiments, a single test injection of fentanyl at different concentrations ranging from 5 fg/kg to 50 µg/kg was administered on D_{13} and nociceptive thresholds were similarly evaluated until a return to baseline values. In a further series on D_{13}, Naltrexone (1 mg/kg), BN2572 (0.3 mg/kg) or L-Canavanine (10 mg/kg) were administered 30 min prior to the fentanyl test injection and subsequent nociceptive threshold evaluation.

Gene expression analyses

Concentrations of mRNA were assessed in four different groups (six rats per group): (1) untreated control rats fed with a standard diet, (2) a group subjected to unilateral left hind paw carrageenan injection, (3), a carrageenan plus fentanyl treated group, and (4) a group having had carrageenan/fentanyl administration associated with a polyamine deficient diet that was initiated 7 days before D_0 and maintained until the end of the experiment. All animals were sacrificed 13 days after carrageenan and/or fentanyl administration and their spinal cords and dorsal root ganglia were rapidly removed. Dorsal left quadrants of the spinal cord were isolated, and all tissue samples were frozen in liquid nitrogen then stored at −80 °C until use. Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis was used to assay mRNA levels of the following genes (also see Supplementary Materials and Methods): cholecystokinin (CCK), dynorphin (DYN), neuropeptide FF, cyclooxygenase type 2 (Cox2), iNOS, neuronal nitric oxide synthase (nNOS), glutamate (NMDA) ionotropic receptor subunits 1, 2B and 3 A, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the ribosomal subunit 18 S. To perform semi-quantitative analyses, GAPDH and RS 18 S were used as reporter genes. Because the relative expression of GAPDH compared with RS 18 S was not significantly different in control vs treated rats, GAPDH mRNA levels were used for normalizing specific mRNA levels in each sample. Data are presented as relative mRNA units compared to control values.

Statistical analyses

Data are presented as means ± SEM. Because of the wide variability in time point intervals for nociceptive threshold measurement (2 h on D_0, 24 h from D_1 to D_{12}, 30 min on D_{13}), data from the 13 day study of each animal were partitioned into three groups (D_0, D_1–D_{12}, D_{13}) for separate statistical analysis. This splitting also enabled localizing analysis to specific study phases, especially the critical D_{13} test period. Two-way repeated measures ANOVAs (one factor repetition), with factors of Time (within) and Group (between), were performed on each timing group with SigmaPlot software. When the ANOVA revealed a significant Time effect ($P < 0.05$), the Student-Newman–Keuls post-hoc test was used to assess the difference at each time point relative to the baseline nociceptive threshold value determined on D_0 or D_{13}. When the ANOVA revealed a significant Group effect and/or Group × Time interaction effect ($P < 0.05$), the Newman–Keuls test was used to assess inter-group differences. For RT-PCR data, the 2-Delta Delta

CT method [39] was used to analyze the relative differences in specific mRNA levels between groups (RQ Study Software 1.2 version; Applied Biosystems). These values were then processed by one-way ANOVA followed by Newman–Keuls post-hoc testing when a significant Group effect ($P < 0.05$) was observed.

Analgesia or hyperalgesia indices, respectively represented by the area below or above the threshold response curve relative to baseline, were calculated for each rat using the trapezoidal method [5] (see Supplementary Materials and Methods), and index means were compared by one-way ANOVA followed by Newman–Keuls post-hoc testing or Students *t* test for analgesia and hyperalgesia indices, respectively. Linear regression analysis, we used Prism software (GraphPad Software, CA, USA).

RESULTS

Effects of opioid and pain experience on acute responsiveness to fentanyl

We first examined the long-lasting influence of opioid exposure or/and inflammatory pain on the pharmacological effects of a subsequent test administration of fentanyl. For the latter, we initially chose a relatively low test dose (25 µg/kg) that alone was sufficient to induce solely analgesia but not the typical bi-phasic response (immediate analgesia followed by hyperalgesia) observed in rats subjected to higher (>200 µg/kg) doses [40]. This uniquely analgesic response on D_{13} can be seen in Fig. 1A (right; see also control profiles in B, C) where a test injection of 25 µg/kg fentanyl produced analgesia (indicated by a transient increase in nociceptive threshold relative to baseline value) in a control group (opioid- and pain-naïve rats, $n = 10$) that had only received saline injections 13 days previously on D_0 of the experiment (Time effect: $F(9,153 = 514.71$, $P < 0.001$, two-way repeated measures ANOVA with Newman–Keuls post-hoc testing revealing $P < 0.05$ at 30 min and 1 h). A similar single phase analgesic response to the same test dose on D_{13} was also observed in rats ($n = 9$) previously subjected to 4 × 100 µg/kg fentanyl administration on D_0 (Fig. 1A, right; Group × Time interaction: $F(9,153) = 0.18$, $P = 0.99$). This lack of significant difference thus indicated an absence of any lasting drug tolerance to such a high dose that itself on D_0 produced an immediate bi-phasic nociceptive threshold profile, i.e., analgesia followed by hyperalgesia for several days, as previously reported [40] (Fig. 1A, left; D_0, Group × Time interaction: $F(3,51) = 282.30$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Fenta vs Control groups at 2 h, 4 h and 6 h; then D_1–D_{12}, Group × Time interaction: $F(11,187) = 26.07$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Fenta vs Control groups from D_1 to D_6).

In a next step, a group of rats ($n = 8$) was subjected to inflammatory pain 13 days prior to testing by a single hind paw injection of the polysaccharide, carrageenan, which induced immediate hyperalgesia on D_0 (Fig. 1B, left; Group × Time interaction: $F(3,42) = 67.81$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf vs Control ($n = 8$) groups at 2 h, 4 h and 6 h) that then lasted several days (D_1–D_{12}, Group × Time interaction: $F(11,154) = 27.89$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf vs Control groups from D_1 to D_5). In the pain-experienced rats, a bi-phasic profile of analgesia immediately followed by hyperalgesia lasting ~2–3 h was now observed in response to the later 25 µg/kg fentanyl test injection on D_{13} (Fig.1B, right; Group × Time interaction: $F(9,126) = 20.04$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf vs Control groups from 30 min to 3h30, except at 1h30 where $P > 0.05$).

In a further group of rats ($n = 10$) previously exposed to both inflammatory pain induction and 4 × 100 µg/kg fentanyl on D_0, a bi-phasic threshold profile was also observed in response to 25 µg/kg fentanyl test injection on D_{13} (Fig. 1C, right; Group × Time interaction: $F(9,162) = 12.60$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta vs Control ($n = 10$) groups from 30 min to 4 h). Note that the fentanyl injections on D_0 evoked immediate analgesia followed by exaggerated hyperalgesia lasting for several days, as previously reported [40] (Fig. 1C, left; D_0, Group × Time interaction: $F(3,54) = 131.03$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta vs Control groups at 2 h, 4 h and 6 h; D_1–D_{12}, Group × Time interaction: $F(11,198) = 15.96$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta vs Control groups from D_1 to D_{11}).

Comparison of the analgesia indices on D_{13} (i.e., the mean area below the individual nociceptive threshold response curves relative to baseline) of the Inf group (Fig. 1B) and Inf-Fenta group (Fig. 1C) compared to naïve Control rats indicated that in both cases, this initial phase was significantly reduced ($F(2,24) = 45.07$, $P < 0.001$, one-way ANOVA with post-hoc revealing $P < 0.001$ for Inf or Inf-Fenta vs Control groups and $P < 0.001$ for Inf-Fenta vs Inf groups). The analgesic index of the Inf group was reduced by 34.3% compared to the Control group, whereas the decrease in

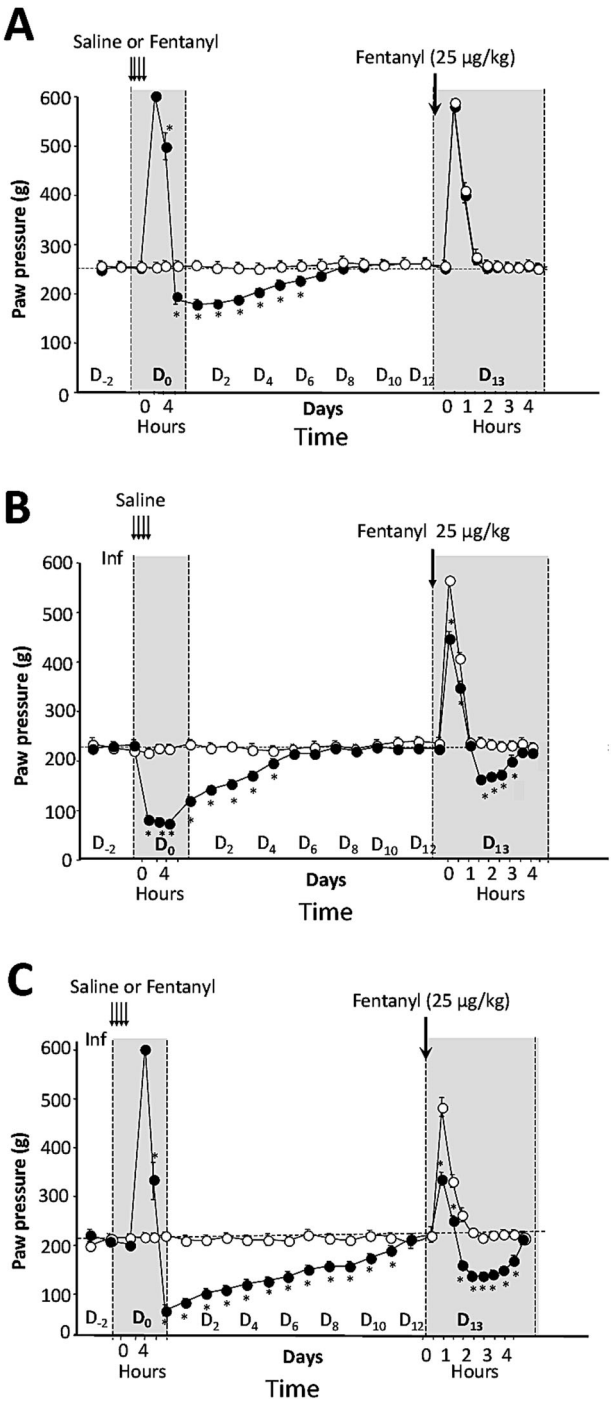

Fig. 1 The effects of a single subcutaneous (sc) injection of fentanyl (25 µg/kg) on basal nociceptive threshold is dependent on previous pain and/or opioid experience. Nociceptive threshold values were determined once daily from $D-_2$, every 2 h throughout D_0, then once daily from D_0 through to D_{12}. On D_{13}, thresholds were measured every 30 min for 4–5 h following the fentanyl test injection. Data are expressed as means ± SEM. * Newman–Keuls post-hoc test, $P < 0.05$ for time-point comparisons with control group. **A** Opioid- and pain-naive animals that had received sc injections of saline (○ Control group, see also **B**, **C**) or fentanyl (100 µg/kg injected four times at 15 min intervals; total dose 400 µg/kg; ● Fenta group) on day zero (D_0), 13 days prior to the 25 µg/kg fentanyl test injection. **B** Rats subjected to inflammatory pain induction (by single paw carrageenan injection) on D_0 (○ Control group, ● Inf group) before the fentanyl test on D_{13}. **C** Rats exposed to both inflammatory pain and fentanyl (100 µg/kg injected 4 times at 15 min intervals; total dose 400 µg/kg on D_0 (○ Control group, ● Inf-Fenta group) before the fentanyl test on D_{13}.

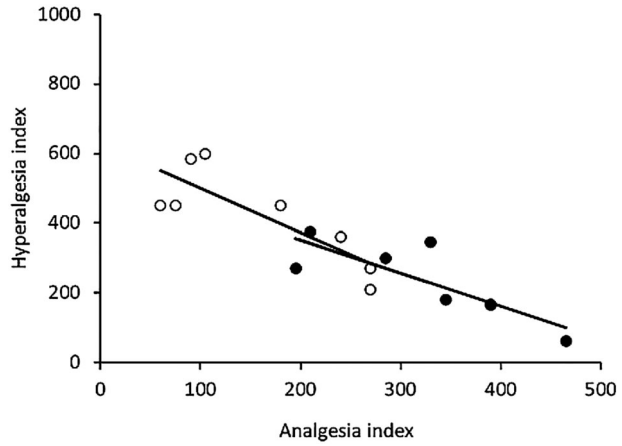

Fig. 2 Negative relationship between bi-phasic analgesic and hyperalgesic effects of fentanyl. Linear regression analysis of the responsiveness to a fentanyl (25 µg/kg) test injection on D_{13} in pain experienced (● Inf group) and pain plus fentanyl-experienced rats (○ Inf-Fenta group) as shown in Fig. 1B, C. Each plot represents the areas measured above (analgesia index) and below baseline (hyperalgesia index) of individual nociceptive threshold response curves (see Materials and Methods).

the analgesic phase of the Inf-Fenta group vs Control was even more pronounced (−70.7%), being 55.4% less compared to Inf group rats previously exposed to inflammatory pain only. In contrast, moreover, the hyperalgesic phase induced by the fentanyl test injection was strongly enhanced (+91.6%) in the combined opioid and inflammation experienced rats compared to the equivalent phase observed in inflammation experienced rats without fentanyl treatment 13 days previously (*t*-test, $t_{16} = 2.72$, $P = 0.01$).

Linear regression analysis of the data from the Inf and Inf-Fenta groups revealed a significant negative correlation between the analgesia and hyperalgesia indices on D_{13} (Fig. 2): in both pain alone and pain-fentanyl exposed rats, the lower the initial analgesic response to the 25 µg/kg fentanyl test injection, the higher was the magnitude of subsequent hyperalgesia. Regression values calculated for the two experimental groups were: $y = -0.953x + 543$, $R^2 = 0.68$ ($F(1,6) = 12.69$, $P = 0.012$) and $y = -1.262x + 625$, $R^2 = 0.67$ ($F(1,6) = 12.42$, $P = 0.012$) for the Inf and Inf-Fenta groups, respectively. Importantly, moreover, the lack of a significant difference between the slopes ($F(1,12 = 0.48$, $P = 0.50$) and Y intercepts ($F(1,13) = 0.02$, $P = 0.88$) of the individual group scatterplots indicated that statistically, they constitute the negative linear regression of a single population ($y = -1.102x + 596$). Altogether, these data indicate that pain experience has a profound impact on the subsequent acute effect of fentanyl, leading to the transition from a purely analgesic to a dual component analgesic/hyperalgesic response in which analgesic potency is diminished. Importantly, moreover, the analgesic potency is even further diminished and the hyperalgesic phase augmented by an additional pre-exposure to fentanyl.

Fentanyl dose-response relationships in rats with or without pain and opioid experience

To more fully characterize the variable acute responses described above, we next compared the pharmacological effects of varying the fentanyl test doses over a range from 50 µg/kg to ultra-low (5 fg/kg) concentrations in naive control rats and rats previously exposed to both pain and opioid administration, as indeed is often the historical context in clinical surgery. As evidenced by the representative examples shown in Fig. 3A–C, with individual test doses on D_{13} of 5 pg, 5 ng and 25 µg/kg, respectively, the profiles of fentanyl's effects on nociceptive threshold not only depended on dose but varied strikingly according to previous pain and opioid experiences (Fig. 3A, 5fg/kg: Group × Time interaction: $F(9,117) = 14.28$, $P < 0.001$, two-way ANOVA, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta ($n = 7$) vs Control ($n = 8$) groups from 30 min to 2h30; Fig. 3B, 5 ng/kg: Group × Time interaction: $F(9,117) = 29.04$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta ($n = 7$) vs Control ($n = 8$) groups from 30 min to 4 h; Fig. 3C, 25 µg/kg: Group × Time interaction: $F(9,126) = 33.87$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta ($n = 8$) vs Control ($n = 8$) groups at 30 min, $P > 0.05$ at 1 h and 1h30, and $P < 0.05$ from 2 h to 4 h).

Predictably, dose-dependent analgesic responses were observed in the control pain- and opioid-naïve group (Fig. 3D, unfilled bars at right, $n = 7$–10 rats per tested dose), declining progressively as the test dose was decreased from 50 µg/kg, eventually reaching an observable threshold at a dose of ~5 ng/kg (Fig. 3B, D). Surprisingly, however, with even lower fentanyl concentrations (from 500 pg down to 50 fg/kg), naïve rats switched from exclusively analgesic to hyperalgesic responsiveness, with the latter increasing to attain a maximal amplitude at test doses of 5 pg/kg (Fig. 3A, D, unfilled bars at left). Furthermore, this hyperalgesia observed at ultra-low fentanyl doses was completely blocked by a pre-fentanyl test administration of either the opioid receptor antagonist naltrexone or the NMDAR antagonist BN2572 indicating the specific involvement of both these receptor types in the paradoxical hyperalgesia's induction (see Supplementary Material, Fig. 1).

The fentanyl dose-response profile was very different in rats previously exposed to inflammatory pain and fentanyl in combination. As seen in Fig. 1C, a bi-phasic effect was consistently observed for the highest fentanyl doses (Fig. 3C, D, filled bars at right), with the amplitudes of both phases diminishing progressively as the test dose decreased from 50 µg (Fig. 3D). However, the threshold concentration for any evident analgesic effect now occurred at doses between 500 ng and 5 µg/kg/kg, and the dose threshold at which a purely hyperalgesic effect emerged was also strongly shifted toward the right (500 ng/kg vs 500 pg/kg) compared to that found in naive rats (Fig. 3D). Consequently, whereas 5 ng/kg fentanyl had no effect in naive rats, an exclusively hyperalgesic response was already maximal with the same dose in pain and fentanyl-experienced animals (Fig. 3B, D), but this response had already waned at 5 pg/kg where hyperalgesia expressed by naïve rats was maximal (Fig. 3A, D). Together these results confirm that fentanyl's pharmacologic effect in terms of thresholds and the development of analgesia vs hyperalgesia is strongly influenced by previous pain and opioid experience and is not merely dictated by immediate dose.

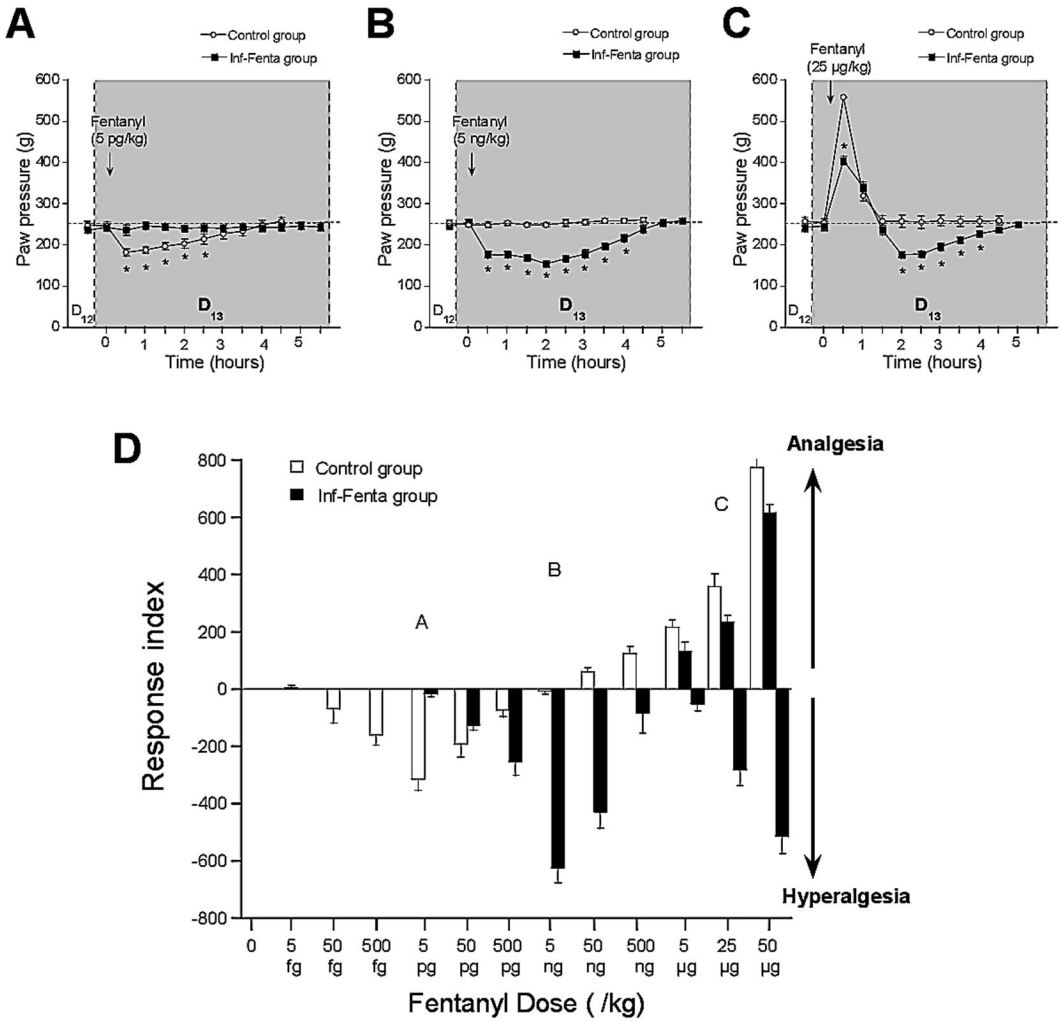

Fig. 3 Acute effects of variable test fentanyl dosages (5 fg to 50 µg/kg) on nociceptive threshold are dependent on prior pain and opioid exposure. A–C Sample plots comparing the mean effects of 5 pg/kg (**A**), 5 ng/kg (**B**) and 25 µg/kg (**C**) sc test fentanyl on nociceptive thresholds in opioid and pain naive rats (○ Control groups) that received saline injections on D_0 prior to the fentanyl test on D_{13}, and 3 groups of rats subjected to both inflammatory pain and fentanyl (4 × 100 µg/kg; ● Inf-Fenta groups) on D_0. Nociceptive thresholds were evaluated repetitively at 30 min intervals on D_{13} as described for Fig. 1. Data points are means ± SEM. * Newman–Keuls post-hoc test, $P < 0.05$ for between group (Group × Time interaction) comparisons in each case. **D** Complete data set showing the mean areas above (analgesia) and/or below (hyperalgesia) baseline in individual response curves (as illustrated in **A–C** and indicated in **D**) induced by different fentanyl test dosages in the naive control (unfilled histogram bars) and inflammation plus opioid pre-exposed (filled bars) groups. Data are expressed as means ± SEM ($n = 7$–10 rats per dose). Note the switch from purely analgesic to hyperalgesic responsiveness at ultra-low fentanyl test doses in control rats, and the significant rightward shift both in analgesic threshold and the emergence of hyperalgesia alone as test doses decreased to lower levels in the Inf-Fenta group.

Involvement of the NMDAR-iNOS cascade in tolerance and associated hyperalgesia

To assess the role of NMDA receptor activation in the acute tolerance to fentanyl's analgesic effects and related hyperalgesia induction, we next examined mRNA levels of several known targets of the NMDAR system in the dorsal horn of the spinal cord. For this, transcriptomic analysis was used to compare mRNA levels in naïve control rats with those of animals previously exposed to inflammatory pain alone or to both pain and fentanyl. Significantly, of the range of mRNA markers evaluated in the spinal cord (see Materials and Methods, and Supplementary Material, Table 1), only those identifying mRNAs of the iNOS enzyme and the endogenous opioid DYN were at higher levels at D_{13} in pain- and opioid-experienced rats than in control animals (iNOS: $F(3,17) = 8.04$, $P = 0.001$, one-way ANOVA, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta ($n = 5$) vs Control ($n = 6$) groups, see Fig. 4A inset; DYN: $F(3,20) = 5.97$, $P = 0.004$, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta ($n = 6$) vs Control ($n = 6$) groups and see Supplementary Material, Table 1 for statistical data for 7 other mRNA levels that remained unchanged). However, no significant alteration in either iNOS or DYN mRNAs was evident in rats previously subjected to inflammatory pain only (see Supplementary Material, Table 1). These initial findings were therefore consistent with the idea that changes specifically in spinal NMDAR-iNOS cascade regulation play a critical role in the development of the bi-phasic effects of acute fentanyl in pain and opioid-experienced rats.

Further strong support for this conclusion derived from pharmacological experiments where, immediately prior to fentanyl testing (25 µg/kg) on D_{13}, pain- and fentanyl-experienced animals were administered with either L-canavanine, a specific inducible NOS inhibitor (Fig. 4A), or the NMDAR antagonist BN2572 (Fig. 4B). In both pharmacological blockade conditions, the initial analgesic phase of the opioid's acute action was substantially enhanced, approaching levels found in control animals without prior pain or opioid exposure. In addition, in both cases, the subsequent hyperalgesic phase of the fentanyl test response on D_{13} was

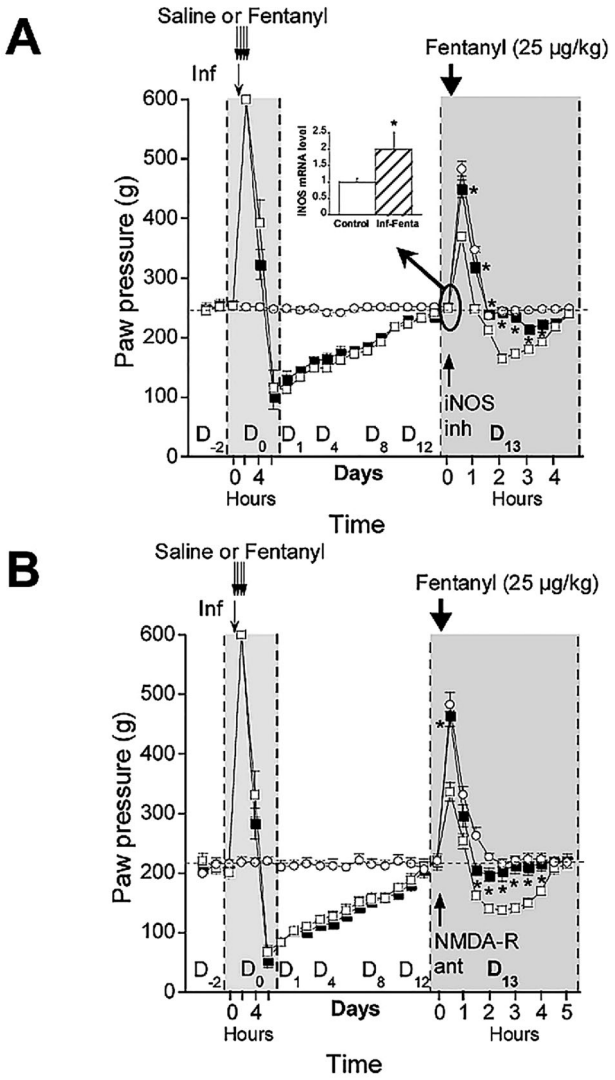

Fig. 4 Involvement of a persistent NMDAR-iNOS cascade over-activation in the acute bi-phasic response to fentanyl in pain and opioid-experienced rats. On D_{13}, nociceptive thresholds were measured every 30 min for 4–5 h following the fentanyl test injection. Data are expressed as means ± SEM. * Newman–Keuls test, $P < 0.05$ for between Inf-Fenta group comparisons. **A** Nociceptive threshold profiles of three groups of rats: opioid and pain naive rats (○ Control group) and two Inf-Fenta groups (□, ■) exposed to both inflammatory pain and fentanyl (4 × 100 µg/kg, sc) on D_0 prior to fentanyl (25 µg/kg; sc) testing on D_{13}. 30 min before the fentanyl test injection, the Inf-Fenta groups additionally received either single injections of saline (□) or the iNOS inhibitor L-canavanine (■; 50 mg/kg, ip). Inset shows increase in mean spinal dorsal horn iNOS mRNA levels (see Materials and Methods, and Supplementary Material, Table 1) in a different set of pain and opioid-experienced rats (hatched bars) vs control rats (unfilled bar) on D_{13}. **B** Equivalent nociceptive threshold data from a further experimental series in which, two Inf-Fenta groups received either single injections of saline (□) or the NMDAR antagonist BN2572 (■; 0.3 mg/kg, sc) 30 min before the fentanyl test injection on D_{13}. ○, pain and opioid naïve Control group. Both iNOS inhibition (**A**) and NMD**A** receptor blockade (**B**) restored the analgesic response to the test fentanyl and decreased the subsequent hyperalgesia.

significantly reduced compared to that of pain- and opioid-experienced rats without inhibitor or antagonist pre-treatment (Fig. 4A, right: Group × Time interaction: $F(18,225) = 23.91$, $P < 0.001$, two-way ANOVA, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta ($n = 8$) vs Control ($n = 10$) groups from 30 min to 4 h, $P < 0.05$ for Inf-Fenta + L-canavanine treated ($n = 10$) vs Inf-Fenta groups from 30 min to 3h30, but $P > 0.05$ for Inf-Fenta + L-canavanine treated vs Control groups from 1h30 to 2h30; Fig. 4B, right: Group × Time interaction, $F(18,225) = 7.51$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for Inf-Fenta ($n = 10$) vs Control ($n = 10$) groups from 30 min to 4 h, $P < 0.05$ for Inf-Fenta + BN2572 treated ($n = 8$) vs Inf-Fenta groups from 30 min to 4 h, but $P > 0.05$ for Inf-Fenta + BN2572 vs Control groups at 30 min and from 2h30 to 4h30. Finally, it is noteworthy that these reversal effects were not at least partly due to direct influences of the iNOS inhibitor or NMDAR antagonist themselves, since neither blocker caused changes in basal nociceptive threshold or fentanyl's acute analgesic effect in naïve rats (see Supplementary Material, Fig. 2).

A polyamine deficient diet down-regulates NMDAR-iNOS activity and restores fentanyl's analgesic capability in pain and fentanyl-experienced rats

We previously reported that rats subjected to a diet devoid of organic polyamines prevents long-lasting hyperalgesia induced by tissue injury, probably via a specific action on NMDAR function [35]. In a final step, therefore, we assessed the impact of a polyamine deficient diet in the context of pain and opioid history as addressed in the present study. To this end, 3 groups of rats were compared: two control groups, one naïve ($n = 10$) and the other subjected to inflammatory pain and fentanyl administration on D_0 ($n = 10$), both of which received standard nutrient before and throughout experimentation. A third group of rats ($n = 8$) were restricted to a polyamine deficient diet commencing seven days prior to tissue injury and fentanyl conditioning on D_0, then continuing until the end of the experiment (Fig. 5). The modified diet strongly reduced post-inflammatory hyperalgesia in the polyamine deficient group compared to that expressed by the Inf-fenta group of rats fed with a normal diet (Fig. 5, D_1–D_{12}; Group × Time interaction: $F(22,275) = 8.63$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ between the two Inf-Fenta groups from D_1 to D_{10}) as previously reported [35]. Subsequently, in pain and fentanyl-experienced rats fed with a polyamine deficient diet, a single fentanyl test injection (25 µg/kg) administered on D_{13} now gave rise to an enhanced analgesic response without an associated hyperalgesic phase as observed under a normal diet, and which had virtually disappeared (Fig. 5, right; Group × Time interaction: $F(18,225) = 7.41$, $P < 0.001$, with post-hoc testing revealing $P < 0.05$ for normal diet Inf-Fenta ($n = 10$) vs Control ($n = 10$) groups from 30 min to 4 h, $P < 0.05$ for polyamine deficient Inf-Fenta ($n = 8$) vs normal diet Inf-Fenta groups from 30 min to 4 h, but $P > 0.05$ for polyamine deficient Inf-Fenta vs Control groups from 30 min to 4h30, except at 1h30 where $P < 0.05$). Importantly moreover, these data indicate that the purely analgesic response occurring in polyamine-deprived animals displayed a fentanyl potency profile that was virtually identical to that of naïve control rats without any previous pain or opioid experience.

Consistent with this finding, in a different set of animal groups under the same three experimental conditions, transcriptomic analysis on D_{13} revealed a blockade of iNOS and DYN transcript overexpression in the spinal cord of polyamine deficient rats, with dorsal horn mRNA remaining at an expression level found in naïve control rats (Fig. 5, inset and Supplementary Material, Table 1; iNOS: $F(3,17) = 8.04$, $P = 0.001$, one-way ANOVA, with post-hoc testing revealing $P < 0.05$ for normal diet Inf-Fenta ($n = 6$) vs polyamine deficient Inf-Fenta groups ($n = 5$), whereas $P > 0.05$ for the latter group vs Control group; DYN: $F(3,20) = 5.97$, $P = 0.004$, with post-hoc testing revealing $P < 0.05$ for normal diet Inf-Fenta vs polyamine deficient Inf-Fenta groups, whereas $P > 0.05$ for the latter group vs Control group. Thus, further to our previous data [35, 41] the long-term absence of polyamines from the diet of pain and drug-experienced rats prevented an overactivation of the

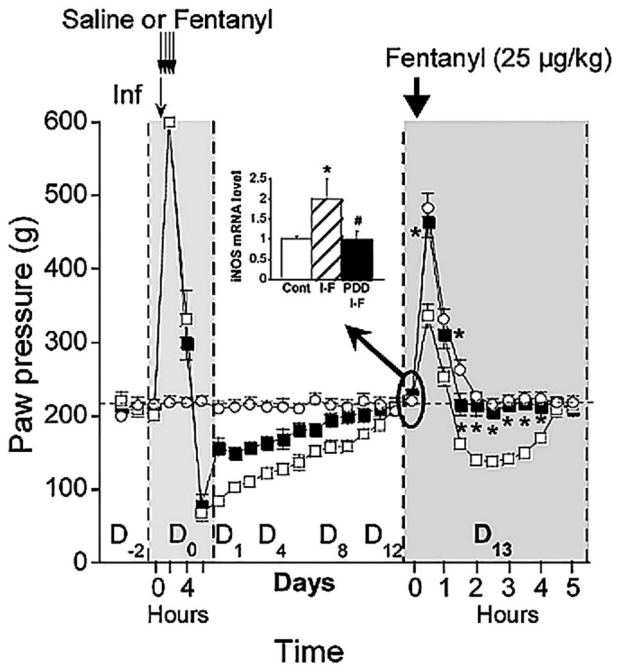

Fig. 5 Maintenance of acute analgesic efficacy and suppression of hyperalgesia by a polyamine deficient diet. Shown are nociceptive threshold profiles of two groups of rats fed with a normal diet (Control group ○ and an Inf-Fenta group □,) and a second pain and opioid-experienced group fed with a polyamine deficient diet (Inf-Fenta group+PDD ■) which was instigated 7 days before D_0 and maintained until the end of the experiment. Nociceptive thresholds were measured on D_{13}, every 30 min for 4–5 h following the fentanyl (25 µg/kg) test injection. Data are expressed as means ± SEM. * Newman–Keuls test, $P < 0.05$ for between Inf-Fenta group comparisons. Note that the polyamine deficient diet had no effect on basal nociceptive thresholds in Inf-Fenta rats as indicated by similar threshold values from D_{-2} to D_0 (at left), but reduced hyperalgesia from D_1 to D_{10} as previously reported [37]. The inset indicates spinal dorsal horn iNOS mRNA levels (means ± SEM) on D_{13} in a different set of naïve control (unfilled bar), Inf-Fent rats with a normal diet (hatched bar) and an Inf-Fent rats with a polyamine deficient diet (PDD-I-F, filled bar) groups. * Newman–Keuls post-hoc test, $P < 0.05$; Inf-Fenta normal diet group vs Control group; $^{\#}P < 0.05$ between the two Inf-Fenta groups. A polyamine deficient diet blocked iNOS mRNA upregulation observed in rats fed with a normal diet after pain and opioid exposure and prevented both analgesic tolerance and hyperalgesia. Note that iNOS mRNA levels in Inf-Fenta rats fed with a polyamine deficient diet vs Control rats fed with a normal diet were not significantly different ($P > 0.05$, see Supplementary Materials and Methods, Table 1).

NMDAR/iNOS cascade with a commensurate suppression of acute fentanyl-induced hyperalgesia and the re-establishment of analgesic efficacy.

DISCUSSION

An important concept in pain plasticity and central sensitization is hyperalgesic priming that contributes to chronic pain states after an acute tissue injury and can persist beyond pain resolution in a long-lasting silent state of pain vulnerability [23, 25, 26, 42–44] referred to as latent pain sensitization [23, 24]. Animal studies have shown that latent pain sensitization triggered by injury-derived nociceptive inputs to the CNS requires NMDAR-mediated activation of adenylate cyclase and is rapidly masked by a sustained upregulation of the endogenous opioid system via an induction of µ-opioid receptor constitutive activity (MOR_{CA}), thereby accelerating pain recovery after acute injury [14, 22–24]. Accordingly, in seemingly pain recovered rodents, administration of a centrally acting MOR antagonist [22–24] or an inverse agonist [24], unmasks latent pain sensitization and leads to pain reinstatement. Our results show that such long-lasting sensitization impacts on the effectiveness of subsequent analgesic opioid treatment. Although animal model evidence has suggested that prior pain may prevent the development of opioid analgesic tolerance [45], our present findings indicate that a previous tissue injury not only promotes such tolerance but can also induce hyperalgesia in response to further opioid (fentanyl) administration, even after an apparent complete recovery from the initial injury. Moreover, our experiments revealed that an exposure to fentanyl at the time of a tissue injury enhanced both tolerance and associated hyperalgesia observed 2 weeks later with further fentanyl administration. Therefore, despite the opioid initially producing analgesia, the underlying single MOR stimulation might act as a catalyst that facilitates long-lasting pain sensitization induced by the acute tissue injury [9, 10, 23, 46], and as a direct consequence, promotes subsequent resistance (tolerance) to the opioid's analgesic effectiveness.

Tolerance and hyperalgesia vs dose and pain/opioid history

Our new observations on the effects of low to ultra-low fentanyl doses illustrate the complexity of opioid actions. Whereas in pain- and opioid-naïve rats a low dose (5 ng/kg) of fentanyl was completely ineffective, in pain- and fentanyl-experienced rats, the same dose unexpectedly provoked an exclusive and substantial hyperalgesia. However, at even lower doses, a uniquely hyperalgesic response that progressively increased then decreased was now also observed in naïve rats, but over a dose range (5 ng/kg to 50 fg/kg) where the hyperalgesia expressed alone in pain- and fentanyl-experienced rats had already subsided. Interestingly, low doses of morphine (1–10 µg/kg) [47], and buprenorphine (0.1 µg/kg) [48, 49] have also been reported to elicit hyperalgesia and not analgesia in naïve rats. In addition, we previously found that endogenous opioid release associated with non-nociceptive environmental stress induces analgesia in naïve rats, but induces solely hyperalgesia in pain- and fentanyl-experienced rats [37]. Thus, low exogenous opioid dose-induced hyperalgesia appears to be a generalized physiological effect mimicking that of naturally-occurring opioids. Altogether these findings support the novel conclusion that although opioids are well known potent analgesics with higher (subtoxic) doses, the primary effect of both exogenous and endogenous opioids could be pain facilitation rather than inhibition.

In our experiments, furthermore, when analgesia began to appear with higher fentanyl doses, the effectiveness and nature of responses again varied according to each animal's experimental history. In naïve rats, the emergence of an analgesic effect, which was dose-dependent, was only observed with moderate fentanyl doses (from 50 ng to 50 µg/kg). In contrast, 50 ng/kg fentanyl continued to evoke hyperalgesia alone in pain- and fentanyl-experienced rats, this effect declining with a further dose increase until 5 µg/kg where a transition to bi-phasic and dose-dependent analgesia/hyperalgesia occurred. Significantly, an equivalent switch, from analgesia to bi-phasic analgesia/hyperalgesia, occurs in naïve rats with fentanyl doses above the 50 µg/kg maximum used in the present study [40]. Since pro- and antinociceptive systems are simultaneously activated by elevating fentanyl doses [5, 8, 9], the response is ultimately determined by an escalation of two competing systems that in opposing one another, leads to apparent analgesic tolerance and/or hyperalgesia. The longer-lasting hyperalgesic component of bi-phasic responses could arise from a sustained activation of pronociceptive systems by residual low/ultra-low concentrations of fentanyl that remain after its administration. Altogether, these considerations, which have important clinical implications, indicate that the outcome of an acute opioid administration, here fentanyl, is not solely dependent

on its immediate concentration, but also reflects a subject's previous history of pain and drug exposure.

Role of NMDAR-iNOS cascade in latent pain sensitization and associated opioid tolerance

Our data showed that blockade of increasing iNOS activity in the spinal cord dorsal horn of rats 2 weeks after pain and fentanyl exposure by the specific inhibitor L-Canavanine both restored the opioid's analgesic effectiveness and suppressed hyperalgesia. Similar bimodal reversal effects were obtained with an NMDAR antagonist, BN2572. Therefore, although previous studies have proposed that tolerance and opioid-induced hyperalgesia arise from distinct and dissociable processes [18, 50–52], these findings, together with the strong negative correlation found between analgesia and hyperalgesia indices further support the conclusion that they are closely related phenomena [15, 53] mediated, at least partly, by common NMDAR-iNOS-dependent mechanisms in the spinal cord. Interestingly, a clinical study reported that experimental hyperalgesia induced by hydromorphone also correlates negatively with its clinical analgesic effect in patients with chronic lumbar radicular pain [54].

A surprising finding was the lack of a statistically significant change in iNOS mRNA levels in rats previously subjected to inflammatory pain only, since an induction of spinal iNOS gene expression has been reported in the early phase of inflammatory pain in mice [55] in association with the development of central pain hypersensitivity [56]. Moreover, the deletion of the iNOS gene attenuates both incision- and remifentanil-induced pronociceptive effects in a murine model of postoperative pain [41] and NO is also known to participate in the development of opioid tolerance [57, 58]. A plausible explanation of our finding, therefore, is that MOR stimulation amplifies transient changes in NMDAR/iNOS cascade sensitivity initially triggered by an acute phase of inflammatory pain, thereby prolonging the plasticity that underlies subsequent tolerance and associated hyperalgesia. Consistent with this proposal, opioid-induced long-term potentiation in the spinal cord has been found to result from an increase in glutamate release from a primary afferent subpopulation expressing TRPV1 and MORs [59], and glutamate's activation of presynaptic NMDARs contributes to opioid-induced hyperalgesia [60] and tolerance [61]. Our finding that DYN mRNA levels were also enhanced by pain and opioid exposure supports this hypothesis, since the upregulation of spinal DYN induced by morphine administration in rats has been found to produce hyperalgesia and tolerance by promoting the release of excitatory amino acids [62]. Therefore, without excluding the possibility of other contributing mechanisms at both spinal and supra-spinal levels [11], the spinal NMDAR-iNOS system, because of its strategic location at the first order synapse of pain pathways, is a plausible target for hyperalgesia and tolerance facilitation.

The restorative impact of a polyamine deficient diet

Our finding that the development of acute tolerance and associated hyperalgesia is repressed if an overactivation of the NMDA-iNOS system is prevented, is also potentially important for pain management. Since the clinical use of iNOS inhibitors or NMDAR antagonists is limited by their adverse secondary effects [63–65], an alternative strategy would be to negatively modulate NMDAR function without blocking basal receptor/channel activity [35]. One functional hallmark of NMDARs is their ability to be allosterically regulated by a variety of small extracellular ligands, especially positive modulators such as polyamines [66, 67], which attenuate proton-mediated inhibition of NR1-NR2B receptor channel functioning [68]. Since diet is a major source of polyamines [69], we hypothesized that dietary polyamine suppression offers the therapeutic potential to prevent neuroplastic changes associated with an overactivation of the NMDAR-iNOS cascade. We previously reported that a polyamine deficient diet prevents long-lasting hyperalgesia induced by tissue injury by inhibiting tyrosine phosphorylation enhancement in the NR2B subunit of spinal NMDARs [35]. Here, we found that a long-term polyamine deficient diet prevented increased spinal iNOS mRNA expression in pain- and fentanyl-experienced rats. Coincidentally, and without exerting an antinociceptive effect per se [35], this modified diet resulted in a restoration of the analgesic effectiveness of a subsequent fentanyl administration and a suppression of associated hyperalgesia. This therefore indicates that dietary polyamine suppression can prevent latent pain sensitization by negatively modulating the NMDAR-iNOS system at least partly at the level of the first synapse in the spinal dorsal horn. Importantly, in contrast to NMDAR antagonist or iNOS inhibitor treatments, the implementation of a polyamine deficient diet over several weeks in rats [35, 70] or up to 2 years in humans [71] is without any noticeable adverse effects. In addition to being inexpensive, such a dietary intervention is easily manageable by patients themselves in limiting to foodstuffs preferably enabling a 20-fold reduction (<100 nmoles/g) in polyamine intake) [72]. Clinical trials are thus now required to evaluate whether a dietary polyamine suppression could serve as an effective adjuvant strategy for improving opioid pain management after tissue injury.

More generally, and in variance with the traditional dose-centered unified approach, a subject's history of pain, opioid exposure and even nutrition should be considered, if modern medicine's individualized management of pain relief is to be improved. Since a polyamine reduced diet also prevents increased anxiety-like behavior associated with opioid withdrawal in rats [70], such a nutritional modification offers a potential innovative therapy for reducing the "pharmakon-like" effects of opioids, especially concerning chronic postsurgical pain affecting millions of patients worldwide [18, 46, 73], neuropsychological disorders and even overdose-related outcomes [74].

REFERENCES

1. Dowell D, Haegerich TM, Chou R. CDC Guideline for Prescribing Opioids for Chronic Pain—United States, 2016. JAMA. 2016;315:1624–45.
2. Kalso E, Edwards JE, Moore RA, McQuay HJ. Opioids in chronic non-cancer pain: systematic review of efficacy and safety. Pain. 2004;112:372–80.
3. Relieving Pain in America. A Blueprint for Transforming Prevention, Care, Education, and Research. Institute of Medicine (US) Committee on Advancing Pain Research, Care, and Education. Washington (DC): National Academies Press (US) (2011).
4. Angst MS, Clark JD. Opioid-induced hyperalgesia: a qualitative review. Anesthesiology. 2006;104:570–87.
5. Célèrier E, Rivat C, Jun Y, Laulin JP, Larcher A, Reynier P, et al. Long-lasting hyperalgesia induced by fentanyl in rats: preventive effect of ketamine. Anesthesiology. 2000;92:465–72.
6. Laulin JP, Larcher A, Célèrier E, Le Moal M, Simonnet G. Long-lasting increased pain sensitivity in rat following exposure to heroin for the first time. Eur J Neurosci. 1998;10:782–85.
7. Mao J, Price DD, Mayer DJ. Thermal hyperalgesia in association with the development of morphine tolerance in rats: roles of excitatory amino acid receptors and protein kinase C. J Neurosci. 1994;14:2301–12.
8. Simonnet G, Rivat C. Opioid-induced hyperalgesia: abnormal or normal pain? NeuroReport. 2003;14:1–7.
9. Simonnet G, Laboureyras E. Hyperalgesia modulation and chronic pain after surgery. In: Postoperative Pain Science and Clinical Practice, IASP. Edited by Wilder-Smith O, Arendt-Nielsen L, Yarnitsky D, Vissers KCP Philadelphia: Wolters Kluwer; 2014. p 175–90.
10. Colvin LA, Bull F, Hales TG. Perioperative opioid analgesia — when is enough too much? A review of opioid-induced tolerance and hyperalgesia. Lancet. 2019;393:1558–68.
11. Rivat C, Ballantyne J. The dark side of opioids in pain management: basic science explains clinical observation. Pain Rep. 2016;1:1–9.
12. Guignard B, Bossard AE, Sessler DI, Lerault C, Alfonsi P, Fletcher D, et al. Acute opioid tolerance: intraoperative remifentanil increases postoperative pain and morphine requirement. Anesthesiology. 2000;93:409–17.
13. Lavand'homme P, Steyaert A. Opioid-free anesthesia opioid side effects: tolerance and hyperalgesia. Best Pract Res Clin Anesthesiol. 2017;31:487–98.

14. Weinbroum AA. Postoperative hyperalgesia — A clinically applicable narrative review. Pharm Res. 2017;120:188–205.
15. Célèrier E, Laulin J, Larcher A, Le Moal M, Simonnet G. Evidence for opiate-activated NMDA processes masking opiate analgesia in rats. Brain Res. 1999;847:18–25.
16. Célèrier E, Laulin JP, Corcuff JB, Le Moal M, Simonnet G. Progressive enhancement of delayed hyperalgesia induced by repeated heroin administration: a sensitization process. J Neurosci. 2001;21:4074–80.
17. Richebé P, Rivat C, Laulin JP, Maurette P, Simonnet G. Ketamine improves the management of exaggerated postoperative pain observed in perioperative fentanyl-treated rats. Anesthesiology. 2005;102:421–28.
18. Yu EHY, Tran DHD, Irwin MG. Remifentanil tolerance and hyperalgesia:short-term gain, long-term pain? Anaesthesia. 2016;71:1347–62.
19. Angst MS, Koppert W, Pahl I, Clark DJ, Schmelz M. Short-term infusion of the mu-opioid agonist remifentanil in humans causes hyperalgesia during withdrawal. Pain. 2003;106:49–57.
20. Compton P, Charuvastra VC, Ling W. Pain intolerance in opioid-maintained former opiate addicts: effect of long-acting maintenance agent. Drug Alcohol Depend. 2001;63:139–46.
21. Minville V, Fourcade O, Girolami JP, Tack I. Opioid-induced hyperalgesia in a mice model of orthopaedic pain: preventive effect of ketamine. Br J Anaesth. 2010;104:231–38.
22. Richebé P, Rivat C, Creton C, Laulin JP, Maurette P, Lemaire M, et al. Nitrous oxide revisited. Evidence for potent antihyperalgesic properties. Anesthesiology. 2005;103:845–54.
23. Rivat C, Laulin JP, Corcuff JB, Célèrier E, Pain L, Simonnet G. Fentanyl enhancement of carrageenan-induced long-lasting hyperalgesia in rats: prevention by the N-methyl-D-aspartate receptor antagonist ketamine. Anesthesiology. 2002;96:381–91.
24. Corder G, Donahue RR, Winter MK, Jutras BL, He Y, Hu X, et al. Constitutive μ-opioid receptor activity leads to long-term endogenous analgesia and dependence. Science. 2013;341:1394–99.
25. Kandasamy R, Price TJ. The pharmacology of nociceptor priming. Handb Exp Pharm. 2015;227:15–37.
26. Cabañero D, Campillo A, Célèrier E, Romero A, Puig MM. Pronociceptive effects of remifentanil in a mouse model of postsurgical pain: effect of a second surgery. Anesthesiology. 2009;111:1334–45.
27. Crofford LJ. Adverse effects of chronic opioid therapy for chronic musculoskeletal pain. Nat Rev Rheumatol. 2010;6:191–97.
28. Fletcher D, Martinez V. Opioid-induced hyperalgesia in patients after surgery: a systematic review and a meta-analysis. Br J Anaesth. 2014;112:991–1004.
29. Grace PM, Strand KA, Galer EL, Urban DJ, Wang X, Baratta MV, et al. Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation. Proc Natl Acad Sci USA. 2016;113:3441–50.
30. Grace PM, Galer EL, Strand KE, Corrigan K, Berkelhammer D, Maier SF, et al. Repeated morphine prolongs postoperative pain in male rats. Anesth Analg. 2019;1:161–67.
31. Li W-W, Irvine K-A, Sahbaie P, Guo T-Z, Shi X-Y, Tawfik VL, et al. Morphine exacerbates postfracture nociceptive sensitization, functional impairment, and microglial activation in mice. Anesthesiology. 2019;130:292–308.
32. Martin LJ, Acland EL, Cho C, Gandhi W, Chen D, Corley E, et al. Male-specific conditioned pain hypersensitivity in mice and humans. Curr Biol. 2019;29:192–201.
33. Hirbec H, Teilhac J, Kamenka J, Privat A, Vignon J. Binding properties of [3H] gacyclidine (cis(pip/me)-1-[1-(2-thienyl)-2-methylcyclohexyl]piperidine) enantiomers in the rat central nervous system. Brain Res. 2000;859:177–92.
34. Hoizey G, Kaltenbach ML, Dukic S, Lamiable D, Millart H, D'Arbigny P. Pharmacokinetics of gacyclidine enantiomers in plasma and spinal cord after single enantiomer administration in rats. Int J Pharm. 2001;229:147–53.
35. Rivat C, Richebé P, Laboureyras E, Laulin JP, Havouis R, Noble F, et al. Polyamine deficient diet to relieve pain hypersensitivity. Pain. 2008;137:125–37.
36. Gaboriau F, Havouis R, Moulinoux JP, Delcros JG. Atmospheric pressure chemical ionization-mass spectrometry method to improve the determination of dansylated polyamines. Anal Biochem. 2003;318:212–20.
37. Rivat C, Laboureyras E, Laulin JP, Le Roy C, Richebé P, Simonnet G. Non-nociceptive environmental stress induces hyperalgesia, not analgesia, in pain and opioid-experienced rats. Neuropsychopharmacology. 2007;32:2217–28.
38. Kayser V, Basbaum AI, Guilbaud G. Deafferentation in the rat increases mechanical nociceptive threshold in the innerevated limbs. Brain Res. 1990;508:329–32.
39. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25:402–8.
40. Laulin JP, Maurette P, Corcuff JB, Rivat C, Chauvin M, Simonnet G. The role of ketamine in preventing fentanyl-induced hyperalgesia and subsequent acute morphine tolerance. Anesth Analg. 2002;94:1263–69.
41. Célèrier E, Gonzalez JR, Maldonado R, Cabanero D, Puig M. Opioid-induced hyperalgesia in a murine model of postoperative pain. Anesthesiology. 2006;104:546–55.
42. Laboureyras E, Chateauraynaud J, Richebe P, Simonnet G. Long-Term Pain Vulnerability After Surgery in Rats: Prevention by Nefopam, an Analgesic with Antihyperalgesic Properties. Anesth Analg. 2009;109:623–31.
43. Reichling DB, Levine JD. Critical role of nociceptor plasticity in chronic pain. Trends Neurosci. 2009;32:611–18.
44. Taylor BK, Corder G. Endogenous analgesia, dependence, and latent pain sensitization. Curr Top Behav Neurosci. 2014;20:283–325.
45. Vaccarino AL, Marek P, Kest B, Ben-Eliyahu S, Couret LC Jr., Kao B, et al. Morphine fails to produce tolerance when administered in the presence of formalin pain in rats. Brain Res. 1993;627:287–90.
46. Glare P, Aubrey KR, Myles PS. Transition from acute to chronic pain after surgery. Lancet. 2019;393:1537–46.
47. Sanna MD, Ghelardini C, Galeotti N. Regionally selective activation of ERK and JNK in morphine paradoxical hyperalgesia: a step toward improving opioid pain therapy. Neuropharmacology. 2014;86:67–77.
48. Gerhold KJ, Drdla-Scutting R, Honsek SD, Forsthuber L, Sandkühler J. Pronociceptive and antinociceptive effects of buprenorphine in the spinal cord dorsal horn cover a dose range of four orders of magnitude. J Neurosci. 2015;35:9580–94.
49. Wala EP, Holtman JR. Buprenorphine-induced hyperalgesia in the rat. Eur J Pharm. 2011;651:80–95.
50. Arout CA, Edens E, Petrakis IL, Sofuoglu M. Targeting Opioid-Induced Hyperalgesia in Clinical Treatment: Neurobiological Considerations. CNS Drugs. 2015;29:465–86.
51. Chu LF, Cun T, Ngai LK. Modulation of remifentanil-induced postinfusion hyperalgesia by the beta-blocker propranolol in humans. Pain. 2012;153:974–81.
52. Ferrini F, Trang T, Mattioli TA. Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl(−) homeostasis. Nat Neurosci. 2013;16:183–92.
53. Larcher A, Laulin JP, Célèrier E, Le Moal M, Simonnet G. Acute tolerance associated with a single opiate administration: involvement of N-methyl-D-aspartate-dependent pain facilitatory systems. Neurosci. 1998;84:583–89.
54. Suzan E, Eisenberg E, Treister R, Haddad M, Pud D. A negative correlation between hyperalgesia and analgesia in patients with chronic radicular pain: is hydromorphne therapy a double-edged sword? Pain Phys. 2013;16:65–76.
55. Gühring H, Görig M, Ates M, Coste O, Zeilhofer HU, Pahl A, et al. Suppressed injury-induced rise in spinal prostaglandin E_2 production and reduced thermal hyperalgesia in iNOS-deficient mice. J Neurosci. 2000;20:6714–20.
56. Tang Q, Svensson C, Fitzsimmons B, Webb M, Yaksh TL, Hua X-Y. Inhibition of spinal constitutive NOS-2 by 1400W attenuates tissue injury and inflammation-induced hyperalgesia and spinal p38 activation. Eur J Neurosci. 2007;25:2964–72.
57. Heinzen EL, Pollack GM. Pharmacodynamics of morphine-induced neuronal nitric oxide production and antinociceptive tolerance development. Brain Res. 2004;1023:175–84.
58. Wong CS, Hsu MM, Chou YY, Tao PL, Tung CS. Morphine tolerance increases [3H] MK-801 binding affinity and constitutive neuronal nitric oxide synthase expression in rat spinal cord. Br J Anaesth. 2000;85:587–91.
59. Zhou H-Y, Chen S-R, Chen H, Pan H-L. Opioid-induced long-term potentiation in the spinal cord is a presynaptic event. J Neurosci. 2010;30:4460–66.
60. Vardanyan A, Wang R, Vanderah TW, Ossipov MH, Lai J, Porreca F, et al. TRPV1 receptor in expression of opioid-induced hyperalgesia. J Pain. 2009;10:243–52.
61. Chen Y, Geis C, Sommer C. Activation of TRPV1 contributes to morphine tolerance: involvement of the mitogen-activated protein kinase signaling pathway. J Neurosci. 2008;28:5836–45.
62. Ossipov MH, Lai J, Vanderah TW, Porreca F. Induction of pain facilitation by sustained opioid exposure: relationship to opioid antinociceptive tolerance. Life Sci. 2003;73:783–800.
63. Chizh B, Headley PM, Tzschentke TM. NMDA receptor antagonists as analgesics: focus on the NR2B subtype. Trends Pharm Sci. 2001;22:636–42.
64. Kemp JA, McKernan RM. NMDA receptor pathways as drug targets. Nat Neurosci. 2002;5:1039–42.
65. Thompson TP, Whiter F, Gallop K, Veronese N, Solmi M., Newton P, et al. NMDA receptor antagonists and pain relief: A meta-analysis of experimental trials. Neurology. 2019;92:1652–62.
66. Mony L, Kew JN, Gunthorpe MJ, Paoletti P. Allosteric modulators of NR2B-containing NMDA receptors: molecular mechanisms and therapeutic potential. Br J Pharm. 2009;157:1301–17.
67. Seiler N. Pharmacological properties of the natural polyamines and their depletion by biosynthesis inhibitors as a therapeutic approach. Prog Drug Res. 1991;37:107–59.
68. Zhu S, Paoletti P. Allosteric modulators of NMDA: multiple sites and mechanisms. Curr Opin Pharmacol. 2015;20:14–23.

69. Bardocz S. The role of dietary polyamines. Eur J Clin Nutr. 1993;47:683–90.
70. Le Roy C, Laboureyras E, Laulin JP, Simonnet G. A polyamine-deficient diet opposes hyperalgesia, tolerance and the increased anxiety-like behavior associated with heroin withdrawal in rats. Pharm Biochem Behav. 2013;103:510–19.
71. Cipolla B, Guilli F, Moulinoux JP. Polyamine-reduced diet in metastatic hormone refractory prostate cancer (HRPC) patients. Biochem Soc Trans. 2003;31:384–87.
72. Cipolla BG, Havouis R, Moulinoux JP. Polyamine contents in current foods: basis for polyamine reduced diet and a study of its long-term observance and tolerance in prostate carcinoma patients. Amino Acids. 2007;33:203–12.
73. Neuman MD, Baterman BT, Wunsch HP. Inappropriate opioid prescription after surgery. Lancet. 2019;393:1547–57.
74. Strang J, Volkow ND, Degenhardt L, Hickman M, Johnson K, Koob GF, et al. Opioid use disorder/. Nat Rev Dis Prim. 2020;6:3.

ACKNOWLEDGEMENTS

We thank Jacques-Philippe Moulinoux, Ph.D., Professor (Faculté de Médecine and CHU de Rennes, Université de Rennes 1, 35043 Rennes, France) for gifting the Polyamine deficient rodent nutrient used in some experiments of this study. We are also grateful to Dr Romuald Nargeot for his invaluable assistance with our statistical analyses.

AUTHOR CONTRIBUTIONS

GS and EL designed experiments. EL conducted behavioral studies with the help of MBB. Tissue sample preparation, mRNA level determinations and data analyses were performed by AM and MP. GS, EL and JS interpreted the data and wrote the paper. All authors discussed the results and commented on the paper.

FUNDING

This work was supported by institutional and departmental resources from the Université de Bordeaux and Université de René Descartes Paris 5. We also received support from the 'Conseil Régional d'Aquitaine Bordeaux', the 'Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation', and the 'Centre Nationale de la Recherche Scientifique'. The Universities of Bordeaux and Rennes 1 have a patent (co-inventors J.P. Moulinoux and G. Simonnet) for a human form of polyamine deficient diet adapted from standard rodent nutrient ingredients.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41386-021-01200-5.

Correspondence and requests for materials should be addressed to Guy Simonnet.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.