

Fentanyl Enhancement of Carrageenan-induced Long-lasting Hyperalgesia in Rats

Prevention by the N-methyl-D-aspartate Receptor Antagonist Ketamine

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Background: Tissue damage may produce hyperalgesia, allodynia, and persistent pain. The authors recently reported that fentanyl elicits analgesia but also activates N-methyl-D-aspartate-dependent pain facilitatory processes opposing analgesia. In nonsuffering rats, this leads to a long-lasting enhancement in pain sensitivity. The current study assessed whether fentanyl could amplify carrageenan-induced hyperalgesia.

Methods: First, rats were injected once with carrageenan in a hind paw, with fentanyl (60 or 100 $\mu\text{g}/\text{kg}$ each given four times at 15-min intervals [4×60 or 4×100]) or saline. Second, rats were injected with carrageenan twice without fentanyl (7-day interval), with the second injection either in the previously injected paw or in the other paw. Third, rats were injected twice with carrageenan in the same hind paw: the first ketamine injection was given (10 mg/kg each given three times at 5-h intervals) with or without fentanyl ($4 \times 60 \mu\text{g}/\text{kg}$), and second injection was given without ketamine or fentanyl. The consequences of treatments on long-term hyperalgesia were examined by the paw-pressure vocalization test.

Results: The long-lasting hyperalgesia induced by the first carrageenan injection was dose-dependently enhanced in both duration and magnitude in 4×60 or $4 \times 100 \mu\text{g}/\text{kg}$ fentanyl-treated rats: 5 or 10 days, respectively, as compared with 2 days in saline-treated rats. Hyperalgesia observed in the hind paw contralateral to the first carrageenan injection was enhanced in fentanyl-treated rats. The second carrageenan injection, performed in any hind paw, induced an exaggerated hyperalgesia, especially in fentanyl-treated rats. Pretreatment with ketamine totally prevented the carrageenan- and fentanyl-induced enhancement of the long-lasting hyperalgesia.

Conclusion: Central sensitization in inflammatory pain states

is reinforced by an opiate treatment, which could be prevented by N-methyl-D-aspartate receptors blockade.

IT is well recognized that tissue damages often produce hyperalgesia (exaggerated nociceptive responses to noxious stimulation), allodynia (nociceptive responses to innocuous stimulation), and persistent spontaneous pain.¹⁻³ The establishment of these phenomena reflects a sensitization of the neuronal systems involved in the treatment of the nociceptive information. Pain sensitization can be produced at different levels: central or peripheral. Peripheral sensitization is elicited by increased sensitivity of the primary sensitive neurons innervating the injured tissue.^{2,4} Central sensitization is defined by increased spontaneous activity, reduced thresholds, or increased responsiveness to afferent inputs, prolonged after-discharges to repeated stimulation, and expansion of the peripheral receptive field of dorsal horn neurons.² Once pain sensitization is established, it does not require sustained inputs from the injured tissue, and it thus contributes by itself to the induction of pathologic pain states, *i.e.*, chronic pain. Many studies have shown a contribution of excitatory amino acids to injury-induced sensitization leading to hyperalgesia.^{2,3} Indeed, N-methyl-D-aspartate (NMDA) antagonists have been particularly effective in reducing persistent pain associated with central sensitization.³ For instance, the noncompetitive NMDA antagonist MK-801 reduces hyperalgesia developed in rats with a peripheral neuropathy^{5,6} or an adjuvant-induced inflammation.⁷ However, the cutaneous hyperalgesia observed in the rat incisional model is not sensitive to NMDA antagonists.^{8,9} On the other hand, many clinical studies have shown the effectiveness of the clinically available NMDA receptor antagonist, ketamine, for reducing postoperative pain.¹⁰

Although opiates are potent analgesics widely used in humans, clinical studies report that opiates may also elicit delayed hyperalgesia and allodynia.¹¹⁻¹⁶ Furthermore, experimental animal models demonstrate that opiates activate pain facilitatory systems, as revealed by long-lasting (days) hyperalgesia.^{1,17-24} Indeed, we recently¹⁹ showed that, in nonsuffering rats, administration of fentanyl, an opiate largely used in human surgery, induces long-lasting hyperalgesia subsequent to its analgesic effect. Interestingly, this phenomenon is totally prevented by pretreatment with the clinically available NMDA antagonist ketamine. This suggests that the devel-

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opment of long-lasting hyperalgesia induced by fentanyl is associated with the activation of the NMDA receptors.

Because both nociceptive inputs and opiates activate pain facilitatory systems *via* NMDA receptors, we hypothesized that opiates might enhance the hyperalgesia induced by tissue damages. To evaluate the effects of fentanyl on hyperalgesia induced by nociceptive inputs, we selected a model of carrageenan-elicited pain hyperalgesia.²⁵ Several behavioral and electrophysiologic studies have shown that carrageenan elicits an early edema followed by hyperalgesia lasting 1–4 days.^{25–27} Thus, the intraplantar injection of carrageenan is widely used for modeling localized inflammatory pain.²⁸

Therefore, we investigated in rats the effects of fentanyl on hyperalgesia induced by a single or two repeated carrageenan injections. As NMDA receptors are strongly involved in the development of both central pain sensitization and opiate-induced hyperalgesia, we also evaluated the effects of a ketamine pretreatment on the carrageenan-induced hyperalgesia in saline- and fentanyl-treated rats.

Materials and Methods

Animals

Experiments were performed on adult male Sprague-Dawley rats (IFFA-CREDO, L'Arbresle, France) weighing 300–400 g, housed in groups of four per cage with a 12-h light–12-h dark cycle (lights on at 7:00 AM) at a constant room temperature of $22 \pm 2^\circ\text{C}$. The animals had access to food and water *ad libitum*. Pharmacologic tests and care of the animals were conducted in accordance with the Animals Care and Use manual of the National Institutes of Health (1999). This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinki Declaration. Thus, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (J-P. L.).

Drugs

Fentanyl citrate, ketamine hydrochloride, and carrageenan λ (Sigma-Aldrich, Saint Quentin Fallavier, France) were dissolved in physiologic saline (0.9%). Fentanyl and ketamine were administered subcutaneously (1 ml/kg body weight, 60 or 100 $\mu\text{g}/\text{kg}$ and 10 mg/kg, respectively); control animals received an equal volume of saline injections. Carrageenan (0.2 ml of a 1% solution of carrageenan in saline) was prepared 24 h before each experiment. It was injected in one rat plantar hind paw subcutaneously, in nonanesthetized animals. Animals were placed in a plastic cage, and the injected paws were pulled through a hole at the base of the cage. Injections were performed with a 25-gauge needle.

Measurement of Nociceptive Threshold

Nociceptive thresholds in hand-held rats were determined by a modification of the Randall-Selitto method,²⁹ the paw-pressure vocalization test, in which a constantly increasing pressure is applied to the hind paw until the rat squeaks. The Basile analgesimeter (Apelex, Massy, France; stylus tip diameter, 1 mm) was used. A 600-g cutoff value was determined to prevent tissue damage.

General Procedure

After arrival in the laboratory, animals were left to become accustomed to the colony room for 4 days. To avoid stress resulting from the experimental conditions, which might affect measurement of the nociceptive threshold, the experiments were performed by the same experimenter in quiet conditions in a test room close to the colony room. For 2 weeks before the experiments, the animals were weighed daily, handled gently for 5 min, and placed in the test room for 2 h (from 11:00 AM to 1:00 PM), where they were left to become accustomed to the nociceptive apparatus. All experiments began at 11:00 AM and were performed on groups of eight animals during the light part of the cycle. To ensure nociceptive threshold stability, basal nociceptive threshold was measured on the 2 days preceding the planned experimental day (*i.e.*, on days -2 and -1). On the experimental day (day 0), basal nociceptive threshold was also determined once, before drug injections. Experiments were only initiated when no statistical change of the basal nociceptive threshold was observed for 3 successive days (days -2 , -1 , and 0; one-way analysis of variance [ANOVA], $P > 0.05$). The reference value of the basal nociceptive threshold for evaluating the pharmacologic effect of drugs was chosen as the measurement of the nociceptive threshold performed on day 0. The experimenter was unaware of the treatment used.

Measurement of the Nociceptive Threshold Changes Induced by Carrageenan Injections

On the experimental day, the basal nociceptive threshold was determined between 11:00 and 12:00 AM in all groups. In the 78 rats tested, mean baseline nociceptive thresholds \pm SD were 294 ± 50 and 296.9 ± 47 g for left and right hind paws, respectively. No statistical difference was observed between any groups (ANOVA, $P > 0.05$) or between the right and left paws (paired *t* test).

Two hours after this first measurement, the animals were administered subcutaneously a first (of four) saline injection (control group) or a first fentanyl injection (fentanyl group). Five minutes later, all animals received carrageenan administered intraplantarly in the left hind paw. The animals subsequently received the three last injections of saline (control group) or fentanyl (fentanyl group) separated by 15 min. Nociceptive thresholds of both ipsilateral and contralateral paws were measured 2 and 4 h after the carrageenan injection, and subse-

Table 1. Mean Diameters (in millimeters) of the Intraplantarily Carrageenan-injected Hind Paw in the Saline- or Fentanyl-treated Groups of Rats

	Basal	2 h	4 h	D ₁
Control	4.40 ± 0.20	10.86 ± 1.49*	11.09 ± 0.75*	8.41 ± 1.20*
Fentanyl 4 × 60 μg/kg	4.41 ± 0.21	8.06 ± 0.80*†	10.39 ± 0.77*	7.93 ± 0.63*
Fentanyl 4 × 100 μg/kg	4.36 ± 0.16	6.75 ± 0.86*†	9.35 ± 1.06*†	8.21 ± 0.63*

Results are expressed as mean ± SD (n = 8 in each group).

* $P < 0.05$ compared to the basal value of the considered group (one-way analysis of variance); † $P < 0.05$ compared to the corresponding value of the saline-treated rats (Newman-Keuls test).

quently daily. The hind-paw diameters were measured before each nociceptive threshold measurement to estimate the carrageenan-induced inflammation.

To study pain sensitization induced by nociceptive inputs, a second carrageenan injection was performed 7 days after the first one in saline-treated rats. In a first protocol, the second injection of carrageenan was performed in the same paw. In a second protocol, the second carrageenan injection was performed in the contralateral paw. The nociceptive thresholds of both ipsilateral and contralateral hind paws were measured 2 and 4 h after the carrageenan injection, and subsequently daily, until they returned to the basal value.

To study NMDA antagonist effect on inflammation- or opiate-induced pain sensitization, a first carrageenan injection was performed in rats treated with three ketamine injections (10 mg/kg each, administered subcutaneously) 30 min before and 4.5 and 9.5 h after the first saline or fentanyl (4 × 60 μg/kg) injection. Saline and fentanyl were administered as previously mentioned. The nociceptive threshold was measured 2, 4, 5.5, and 10.5 h after the carrageenan injection and subsequently daily. To study pain sensitization induced by nociceptive inputs, a second carrageenan injection was performed (without saline, fentanyl, or ketamine administration) 7 days after the first one. The nociceptive thresholds of each hind paw were measured 2 and 4 h after the carrageenan injection, and subsequently daily, until they returned to the basal value.

To avoid a putative enhancement of hyperalgesia caused by an excess of nociceptive inputs resulting from the evaluation of the nociceptive threshold after the first carrageenan injection, an additional experiment was conducted in fentanyl-treated rats (4 × 100 μg/kg) without performing a nociceptive threshold measurement after the first carrageenan injection (from day 0 to day +7). The nociceptive threshold was only measured after the second carrageenan injection on days +8–+27 in the contralateral paw (not previously injected).

Calculation and Statistical Analysis

Percentages of decreases of nociceptive thresholds were calculated as follows: $100 \times (\text{measured value} - \text{basal value})/\text{basal value}$. Algesic indexes, represented by the area above the curve, were calculated for each rat by

the trapezoidal method and expressed as a mean percentage (\pm SD) of the reference index (100%: algesic index associated with the first carrageenan injection of the saline-treated rats).

To evaluate the time-course effects of treatments on the nociceptive threshold (basal reference value: pre-drugs value on day 0), one one-way ANOVA followed by *post hoc* analysis using the Newman-Keuls test was performed on day 0, and another was performed on the days after the treatments in each of the groups. We used the comparison of the algesic indexes to evaluate the amplitude of the effects in experiments using different pharmacologic treatments. The paired Student *t* test was used for comparing the algesic indexes of the first and second carrageenan injections in the same group. The Mann-Whitney test was used to compare the algesic index when the experiments involved two groups only. An ANOVA followed by *post hoc* analysis using the Newman-Keuls test was performed when the experiments involved more than two groups. The statistical significance criterion was $P < 0.05$.

Results

Fentanyl Enhancement of Carrageenan-induced Long-lasting Hyperalgesia

As expected, carrageenan injection induced edema in fentanyl- and non-fentanyl-treated rats (table 1). However, a reduced edema was observed in fentanyl-treated rats 2 and 4 h after carrageenan injection. No change was observed between saline- and fentanyl-treated rats on the days after the carrageenan injection (data not shown).

In the injected hind paw, carrageenan induced a nociceptive threshold decrease 2 and 4 h after administration (one-way ANOVA, $P < 0.05$; figs. 1A and B). A long-lasting hyperalgesia was induced, as revealed by a decrease of the nociceptive threshold for 2 days after the carrageenan injection (nadir, 19%; one-way ANOVA, $P < 0.05$; figs. 1A and B). As expected, fentanyl administration (four boluses of 60 μg/kg or 100 μg/kg) initially opposed the hyperalgesic effect of carrageenan (one-way ANOVA, $P < 0.05$; figs. 1A and B). This analgesic effect disappeared 4 h later. Subsequent daily measurements of the nociceptive threshold showed an amplification of the long-lasting hyperalgesia in fentanyl-treated

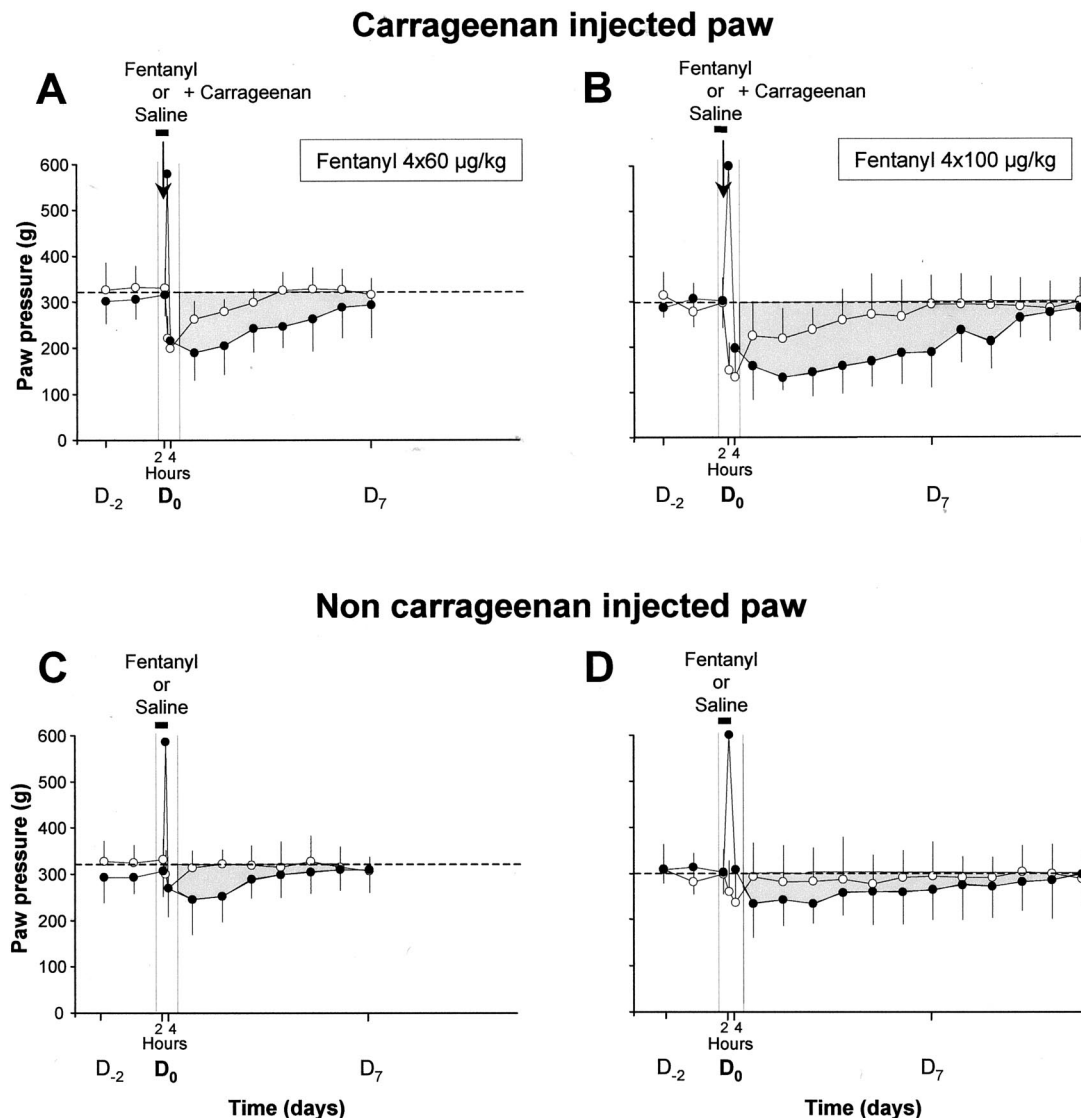


Fig. 1. Long-lasting effects of an intraplantar carrageenan injection on the nociceptive threshold. Modulation by fentanyl. Nociceptive thresholds of both hind paws (*A* and *B*: carrageenan injected paw; *C* and *D*: non-carrageenan-injected paw) were measured by the paw-pressure vocalization test and expressed as the mean \pm SD. The rats were injected in the left hind paw with carrageenan (0.2 ml, 1%) on day 0. Furthermore, four injections of fentanyl (4×60 [*A* and *C*] or 4×100 $\mu\text{g}/\text{kg}$ [*B* and *D*], administered subcutaneously; $n = 8$ each group) or saline ($n = 8$) were performed. The first of four fentanyl or saline injections was performed 5 min before the carrageenan injection. The following injections were administered every 15 min. The nociceptive thresholds of both hind paws were measured 2 and 4 h after the carrageenan injection on day 0, and subsequently once daily. Open circles = saline-treated group; filled circles = fentanyl-treated group; bold line indicates the time of saline or fentanyl administrations; thin arrow indicates the time and the paw injected by carrageenan.

rats as compared with the control carrageenan group (figs. 1*A* and *B*). This amplification depends on the dose of fentanyl: the decrease of the nociceptive threshold attained a maximal value of 41 and 57% and lasted 5 and 10 days for a fentanyl dose of 4×60 and 4×100 $\mu\text{g}/\text{kg}$, respectively (one-way ANOVA, $P < 0.05$).

At the contralateral hind-paw level, the carrageenan injection also induced a slight decrease of the nociceptive threshold 2 h (9%) and 4 h (18%) after its administration. No modification of the nociceptive threshold was observed on the following days (one-way ANOVA, $P > 0.05$; figs. 1*C* and *D*). As expected, fentanyl admin-

istration increased the nociceptive threshold 2 h after the carrageenan injection. However, a significant decrease of the nociceptive threshold was observed for 2 and 3 days for 4×60 and 4×100 $\mu\text{g}/\text{kg}$, respectively (one-way ANOVA, $P < 0.05$; figs. 1*C* and *D*).

Enhancement of Carrageenan-induced Long-lasting Hyperalgesia by a Second Carrageenan Injection in the Ipsilateral or Contralateral Paw

In a second series of experiments, we studied the effects of a second carrageenan injection when per-

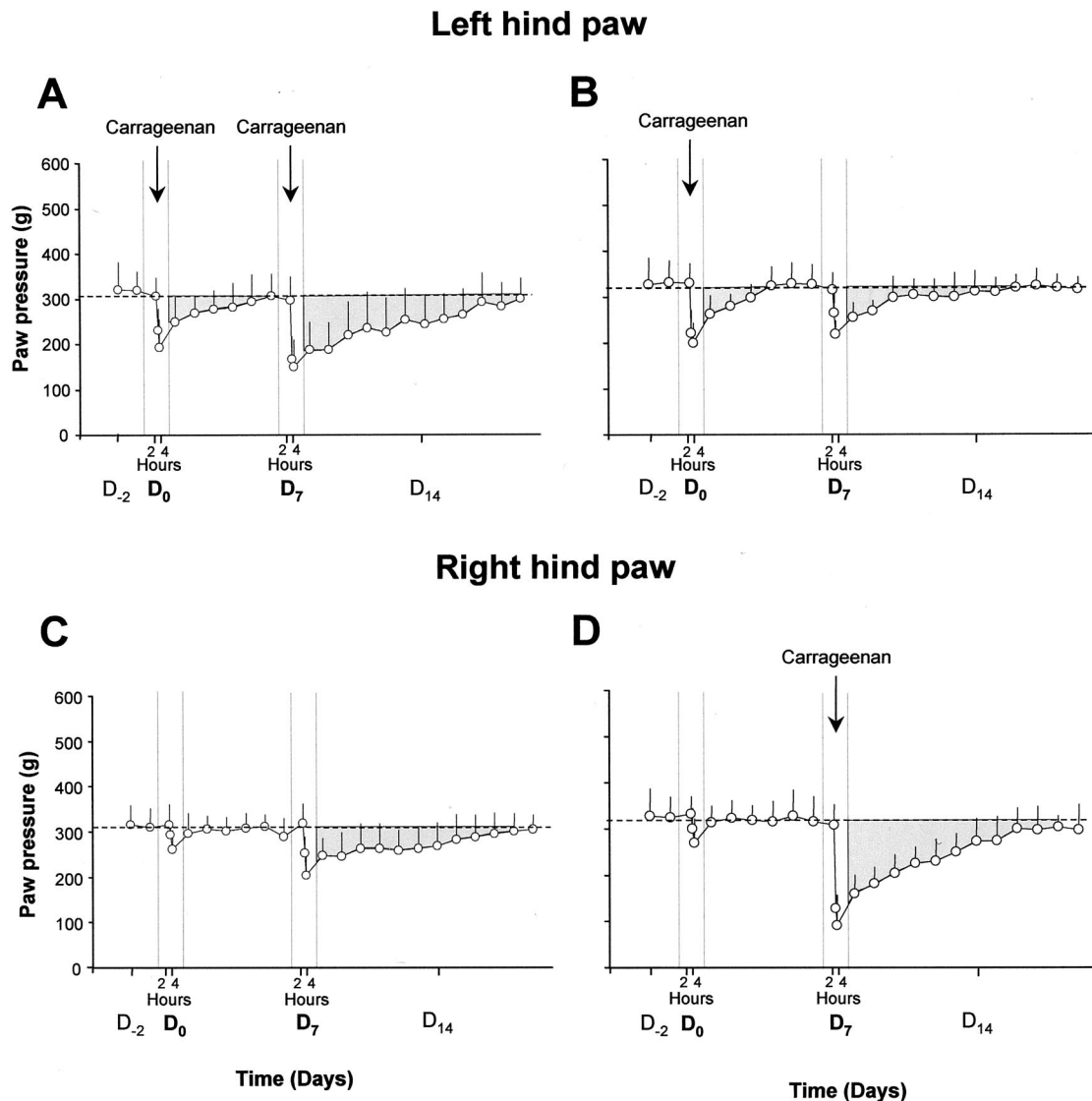


Fig. 2. Long-lasting effects of two intraplantar carrageenan injections on the nociceptive threshold. The nociceptive thresholds of both hind paws (A and B: left hind paw; C and D: right hind paw) were measured by the paw-pressure vocalization test and expressed as the mean \pm SD. On day 0, the rats' left hind paws were injected with carrageenan (0.2 ml, 1%). On day +7, a second carrageenan injection (0.2 ml of 1%) was performed either in the left (A and C) or right (B and D) hind paws. The nociceptive thresholds were measured 2 and 4 h after the carrageenan injection on days 0 and +7 and subsequently once daily. The thin arrow indicates the time and the paw injected by carrageenan.

formed in the same hind paw (left hind paw) or in the contralateral paw (right hind paw). Figures 2A and B show results obtained in the left hind paw, and figures 2C and D in right hind paw.

When performed in the same hind paw (left hind paw), a second carrageenan injection (day +7) induced an enhanced long-lasting hyperalgesia. Indeed, a peak decrease (39%) of the nociceptive threshold was observed the day after this second carrageenan injection. The nociceptive threshold was significantly lower than its basal value for 8 days as compared with 1 day after the first carrageenan injection (one-way ANOVA, $P < 0.05$; fig. 2A). The second carrageenan injection also exaggerated the nociceptive threshold decrease of the nonin-

jected hind paw on the experimental day and elicited a longer-lasting (6 days) hyperalgesia (nadir, 22%; one-way ANOVA, $P < 0.05$; fig. 2C).

When the second carrageenan injection was performed in the contralateral hind paw (day +7; right hind paw previously noninjected), it decreased its nociceptive threshold by 61 and 72% for 2 and 4 h, respectively, after administration of the carrageenan. Subsequent daily measurements of the nociceptive threshold also showed an enhancement of long-lasting hyperalgesia. The decrease of the nociceptive threshold lasted 8 days (one-way ANOVA, $P < 0.05$; fig. 2D) as compared with 2 days after the first carrageenan injection, and reached 51%.

When injected in the non-previously carrageenan-in-

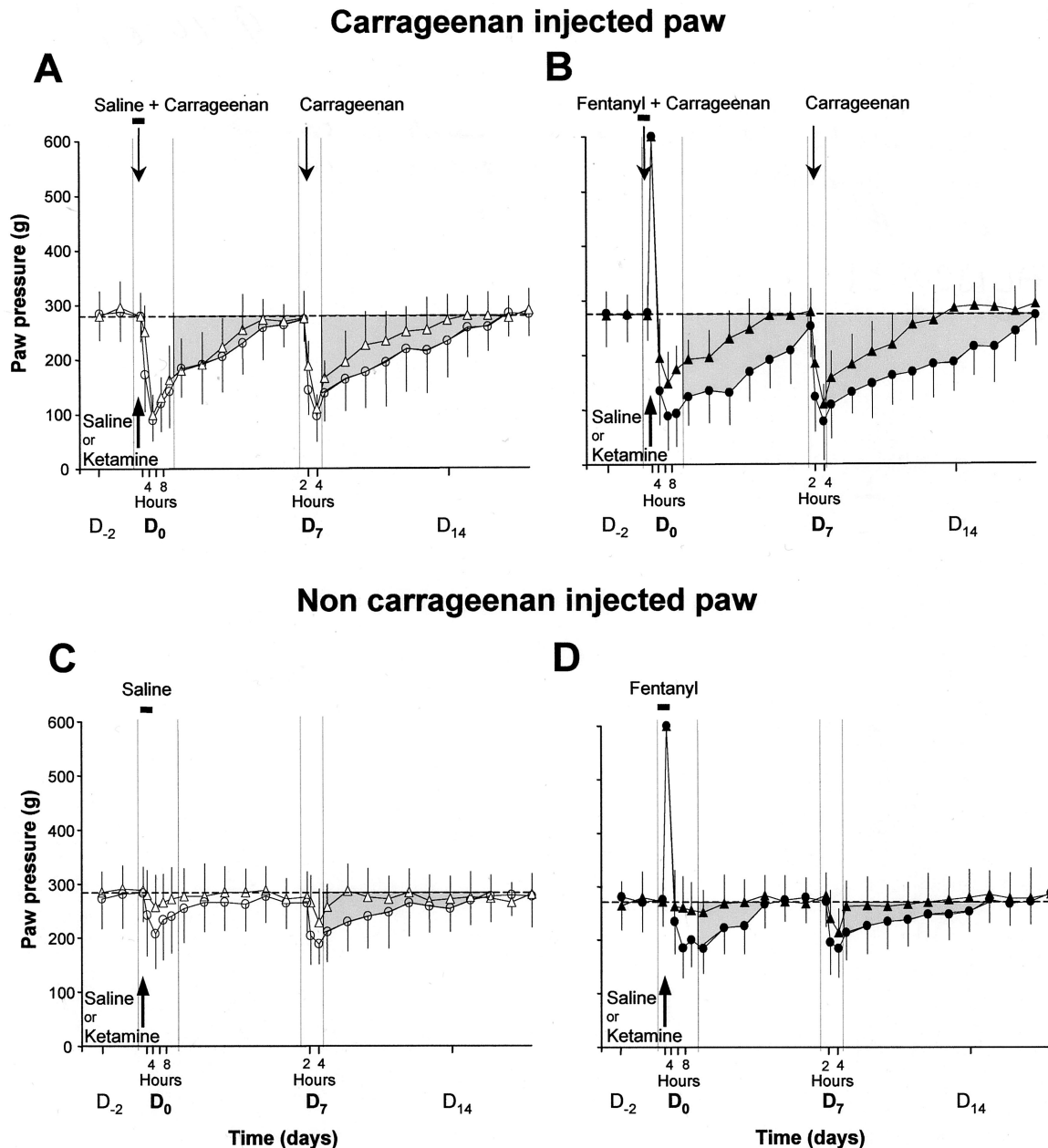
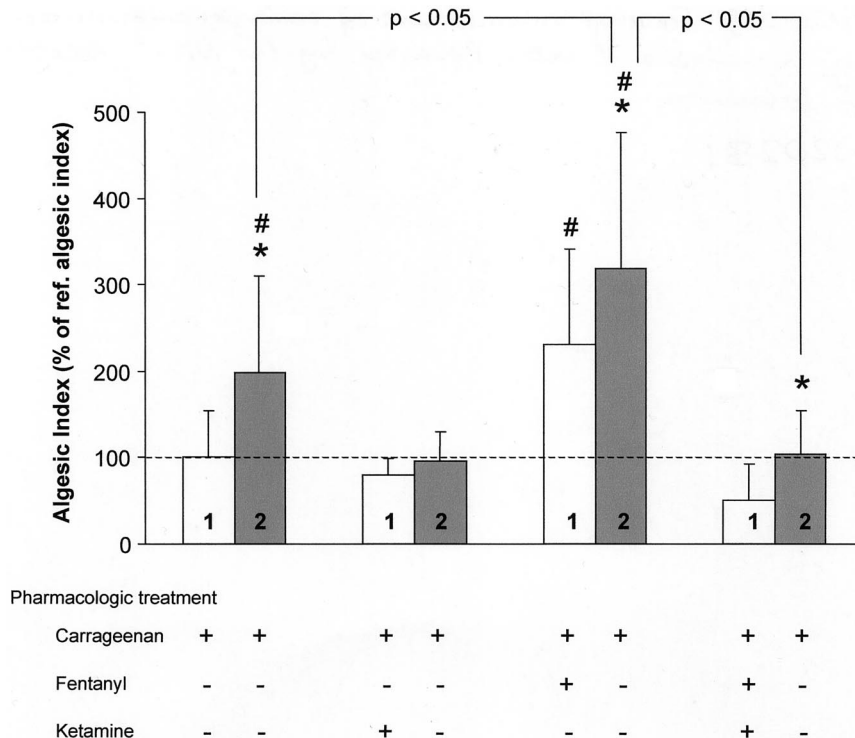


Fig. 3. Long-lasting effects of the first and second carrageenan injections on the nociceptive threshold. Modulation by ketamine or fentanyl. The nociceptive thresholds of both hind paws (**A** and **B**: carrageenan-injected paw; **C** and **D**: non-carrageenan-injected paw) were measured by the paw-pressure vocalization test and expressed as the mean \pm SD. On day 0, the rats were injected in the left hind paw with carrageenan (0.2 ml, 1%). Furthermore, four injections of fentanyl (4×60 [**B** and **D**] administered subcutaneously; $n = 8$ each group) or saline (**A** and **C**; $n = 8$) were performed. The first of four fentanyl or saline injections was performed 5 min before the carrageenan injection. The following injections were administered every 15 min. Ketamine administration was performed as a series of injections: 30 min before and 4.5 and 9.5 h after the first fentanyl injection. The nociceptive thresholds of both hind paws (**A** and **B**: left paw; **C** and **D**: right paw) were measured 2, 4, 5.5, and 10.5 h after the carrageenan injection on day 0 and subsequently once daily. On day +7, a second carrageenan injection (without ketamine, fentanyl, or saline) was injected in the same hind paw. The nociceptive thresholds of both hind paws were measured 2 and 4 h after the carrageenan injection on day +7 and subsequently once daily. Open circles = saline-saline-treated; open triangles = ketamine-saline-treated; filled circles = saline-fentanyl-treated; filled triangle = ketamine-fentanyl-treated groups; the bold arrow indicates the time of ketamine or saline treatments; bold line indicates time of saline or fentanyl administrations; thin arrow indicates the time and the paw injected by carrageenan.

jected hind paw on day +7, carrageenan also evoked a large decrease of the nociceptive threshold at the other hind-paw level previously injected on day 0. Indeed, we observed a decrease of the nociceptive threshold in the

contralateral paw: 18 and 33% for 2 and 4 h, respectively, after administering carrageenan. Long-lasting hyperalgesia was also observed during 2 days (nadir, 21%; one-way ANOVA, $P < 0.05$; fig. 2B).

Fig. 4. Algesic indexes of the long-lasting effects of the first and second carrageenan injections on the nociceptive threshold. Modulation by ketamine or fentanyl. The algesic indexes were calculated (see Material and Methods) using the variations of the nociceptive threshold of the carrageenan-injected paw measured after the first (open square) or the second (filled square) carrageenan injections as shown in figure 3. The plus symbol corresponds to pharmacologic treatments (carrageenan, fentanyl, ketamine) and the minus symbol to saline injections. Newman-Keuls test, $P < 0.05$: first versus second long-lasting hyperalgesia induced by carrageenan injections. *Paired Student t test was used for comparing the algesic indexes of the first and second carrageenan injections in the same group. #Newman-Keuls test, $P < 0.05$ compared with the reference algesic index: long-lasting hyperalgesia induced by the first carrageenan injection in saline-saline-treated rats.



Preventive Effect of Ketamine on the Enhancement of Carrageenan-induced Long-lasting Hyperalgesia Elicited by a Second Carrageenan Injection or by Fentanyl

Experimental results and comparative algesic indexes are shown in figures 3 and 4, respectively. At the dose used in this study, ketamine only induced slight side effects, evidenced by circling behavior and head weaving, which are limited to the first 10 min after each ketamine injection. In saline-treated rats, ketamine had no preventive effect on long-lasting hyperalgesia induced by a first carrageenan injection. However, ketamine totally prevented the enhancement of long-lasting hyperalgesia resulting from a second carrageenan injection. Indeed, long-lasting hyperalgesia associated with the second carrageenan injection lasted only 4 days (nadir, 40%) in ketamine-treated rats versus 7 days (nadir, 50%) in non-ketamine-treated rats (one-way ANOVA, $P < 0.05$; fig. 3A).

In fentanyl-treated rats (injections of $4 \times 60 \mu\text{g/kg}$ fentanyl were only associated with the first carrageenan injection), the second carrageenan injection induced an exaggerated hyperalgesia that lasted 9 days (nadir, 61%) as compared with the one observed with the first carrageenan injection (fig. 3B; one-way ANOVA, $P < 0.05$). The algesic index also exceeded the one observed with the second carrageenan injection in saline-treated rats (Newman-Keuls, $P < 0.05$; fig. 4).

In fentanyl-treated rats, similar enhancement of hyperalgesia induced by the second carrageenan injection was observed whether or not the nociceptive thresholds were measured after the first carrageenan injection from

day 0 to day +7 (algesic index, $910 \pm 183\%$ vs. $751.7 \pm 334\%$, measured vs. not measured rats; Mann-Whitney test, $P > 0.05$).

In fentanyl-treated rats, ketamine totally prevented the enhancement of hyperalgesia observed after the first carrageenan injection in non-ketamine-treated rats. Indeed, long-lasting hyperalgesia associated with the first carrageenan injection lasted only 2 days (nadir, 29%) in ketamine-treated rats versus 6 days (nadir, 55%) in non-ketamine-treated rats (one-way ANOVA, $P < 0.05$; fig. 3B). In these ketamine-fentanyl-treated rats (ketamine and fentanyl injections were only associated with the first carrageenan injection), the second carrageenan injection did not induce an enhancement of the long-lasting hyperalgesia because it lasted only 4 days (nadir, 41%) as compared with the one observed in rats that did not receive fentanyl or ketamine with the first carrageenan injection (one-way ANOVA, $P > 0.05$; fig. 3B).

At the contralateral hind-paw level, ketamine, when administered at the time of the first carrageenan injection, prevented hyperalgesia induced by the first or second carrageenan injections in saline- or fentanyl-treated rats. A reduced hyperalgesia was only observed 4 h after the second carrageenan injection in both saline- and fentanyl-treated rats (one-way ANOVA, $P < 0.05$; figs. 3C and D).

Discussion

The main finding of this study is that fentanyl strongly enhanced carrageenan-induced long-lasting hyperalgesia

and reinforced pain sensitization as demonstrated by an exaggerated response to a second carrageenan injection. Interestingly, ketamine, an NMDA antagonist drug that had no analgesic effect *per se* at the tested dose, prevented both the sensitization of inflammation-induced hyperalgesia and its enhancement by fentanyl.

Opiates provide an unsurpassed utility for the relief of severe pain. However, we recently reported¹⁹ that fentanyl, an opiate widely used for human surgery, also induced delayed hyperalgesia for days thereafter: the higher the fentanyl dose, the greater this hyperalgesic effect. However, these results were observed in nonsuffering rats. A widely used and well-established rat model for inflammatory pain is the carrageenan model,²⁸ which shows hyperalgesia peaking after 1–4 h (acute pain) and lasting 24–96 h (long-lasting hyperalgesia).^{25–27} It has been suggested that this evolution may be compared with the time course of postoperative pain.³⁰ The current study shows, for the first time, that fentanyl not only had an analgesic effect but also strongly enhanced long-lasting inflammation-induced hyperalgesia. In agreement with our previous study on nonsuffering rats,¹⁹ higher fentanyl doses resulted in larger enhancement of hyperalgesia. Although the experimental carrageenan model may be different from inflammatory responses involved in developing and maintaining postoperative pain, our results are in agreement with clinical data showing that patients receiving a large dose of intraoperative fentanyl or remifentanyl have greater postoperative pain scores and morphine consumption than those receiving smaller doses of these opiates.^{15,16}

Our observation that the non-carrageenan-injected posterior paw (contralateral paw) also showed decrease of the nociceptive threshold for several days after the first carrageenan injection in fentanyl-treated rats suggests that the enhancement of pain sensitivity resulted from a central sensitization process. It is well recognized that one of the main characteristics of postinjury nociceptive sensitization is that it leads to long-term changes^{2,31,32} not only at the site of injury (primary hyperalgesia), but also in the surrounding noninjured tissue (secondary hyperalgesia).^{2,33} Primary hyperalgesia is caused by peripheral neuronal mechanisms believed to result from sensitization of nociceptors.⁴ Secondary hyperalgesia is the consequence of functional changes in the central nervous system leading to reduction of the nociceptive threshold.^{34,35} In recent studies using carrageenan, it has been demonstrated that a first inflammation can enhance the pain related to a second inflammation (7 days later) in the ipsilateral or contralateral paw.^{25,30,36} In our study, daily measurements of the nociceptive threshold provided accurate determination and comparison of the time course of delayed hyperalgesia evoked by the first and second carrageenan injections. We observed that delayed hyperalgesia associated with the second carrageenan injection in the same paw

largely exceeded both the magnitude and duration of the hyperalgesia associated with the first carrageenan injection. Interestingly, we also showed that enhancement of long-lasting hyperalgesia associated with the second carrageenan injection was observed when carrageenan was injected in the contralateral (not previously injected) hind paw. Moreover, whatever the second injection site (ipsilateral or contralateral to the first injection), the second carrageenan injection simultaneously induced sustained hyperalgesia in both injected and noninjected paws. Although these results do not preclude that an increase in the peripheral nociceptors' excitability may contribute to inflammation-induced pain sensitization,³ they do suggest that pain sensitization was mainly from a central origin. Taken as a whole, these data indicate that the repetition of two carrageenan injections is a very attractive model for evaluating both the characteristics of a pain sensitization process induced by nociceptive inputs associated with inflammation and the effectiveness of a pharmacologic treatment to prevent such a process.

An interesting observation is that fentanyl-treated rats at the time of a first carrageenan injection showed an exaggerated nociceptive response to a second carrageenan injection performed alone 7 days later when nociceptive thresholds (response to a brief nociceptive stimulus) were recovered. Such an enhancement cannot be explained by an excess of nociceptive inputs or by an avoidance response resulting from a conditioned processing associated with repetitive nociceptive stimuli, because it was also observed in fentanyl-treated rats unexposed to repeated nociceptive stimuli from day 0 to day +7 after the first carrageenan injection. This indicates that fentanyl-treated rats were not returned to their initial pain sensitivity state, but are in a new biologic condition associated with central hypersensitivity, *i.e.*, pain sensitization for new inflammatory nociceptive inputs. Our study suggests that opiates had reinforced a nociceptive memory,³⁷ which may facilitate the establishment of chronic pain.

Development of optimal pain treatments requires that animal models be studied, providing both a relevant assessment of long-lasting hyperalgesia characteristics and an evaluation of pharmacologic treatments that might prevent pain sensitization. If a great number of investigators have hitherto studied the capability of various drugs for preventing carrageenan-induced pain at zenith hyperalgesia (3–4 h),^{38–40} the treatment of long-lasting hyperalgesia related to pain sensitization is poorly documented. Although opiates are very potent analgesics to relieve acute pain associated with tissue injury, our study indicates that they are not good candidates for alleviating long-lasting changes in the pain integrative process³⁸ as they enhance carrageenan-induced hyperalgesia and facilitate the establishment of pain sensitization. There is a large body of evidence that NMDA

receptors are involved in the development and maintenance of central sensitization.^{1-3,41} Moreover, it has been reported that NMDA receptor antagonists also prevent opiate-induced hyperalgesia.^{18,20,23,42} This suggests that a “balanced analgesia” with ketamine could be a fruitful strategy to prevent pain sensitization induced by both nociceptive inputs and opiates. At the dose used in this study, a single series of ketamine injection associated with the first carrageenan injection had neither an analgesic nor a preventive effect on primary hyperalgesia induced by the first carrageenan injection (ipsilateral paw). These results are in agreement with previous data showing that NMDA receptor antagonists do not prevent the development of enhanced responses to mechanical stimuli after a single plantar incision in non-opiate-treated rats.^{8,9} Nevertheless, our study shows that ketamine prevented the secondary hyperalgesia observed in the noninjected paw (contralateral paw) and was also effective in preventing hyperalgesia enhancement induced by a second carrageenan injection or by fentanyl. This is in agreement with a clinical study showing that ketamine has no effect on primary hyperalgesia but reduces for several days the area of mechanical hyperalgesia surrounding the surgical incision in humans.⁴³ This ketamine preventive effect confirms that NMDA receptors are actually recruited by inflammation^{7,39,44} and mainly involved in the initiation of central sensitization rather than in primary hyperalgesia. This means that analgesic potency is not an essential criteria for preventing the establishment of pain sensitization because some pharmacologic agents, such as NMDA receptor antagonists, which are devoid of any effect on acute “physiologic” pain, may be effective in changing the course of central sensitization and thereby influence “pathologic” pain such as the long-lasting hyperalgesia observed in this study.⁴⁵

Although it is clear that hyperalgesia involves prolonged alteration of spinal nociceptive processing,^{46,47} it has been reported that descending facilitatory influences from the rostro ventromedial medulla significantly contribute to secondary, but not primary hyperalgesia.⁴⁸ Intra-rostro ventromedial medulla injection of NMDA receptor antagonist was found to selectively attenuate secondary hyperalgesia after intraarticular carrageenan injection.⁴⁸ It was recently reported that tonic descending facilitation from the rostro ventromedial medulla-mediated opiate-induced hyperalgesia in rats implanted subcutaneously with pellets or osmotic minipumps delivering morphine.⁴⁹ Taken as a whole, this suggests that rostro ventromedial medulla descending facilitatory influences may play a critical role in fentanyl-induced pain sensitization observed in this study in suffering rats, and that NMDA antagonists may partly act at a supraspinal level for preventing this phenomenon. It is commonly recognized that μ -opioid receptor stimulation triggers indirectly the activation of NMDA receptors by reducing

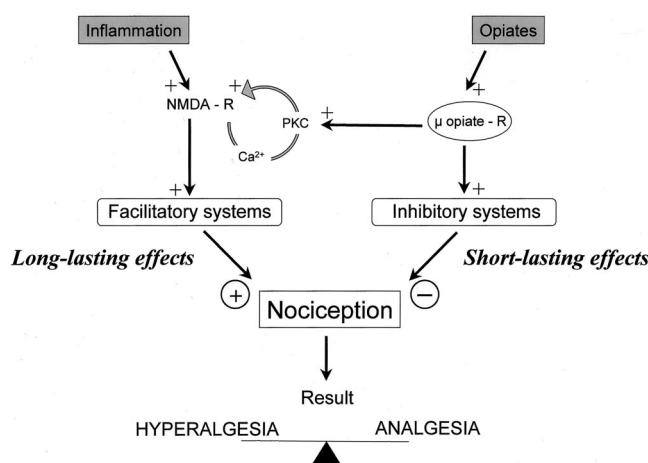


Fig. 5. Schematic view of the proposed opposing effects of opiates on nociception. Inflammatory processes induce pain sensitization (hyperalgesia) by activation of an NMDA-dependent sensitization loop.^{1,2} Opiates would activate not only pain inhibitory systems (eliciting analgesia) but also pain facilitatory systems (eliciting hyperalgesia).^{18-21,24} The latter would also act through the glutamatergic NMDA receptors.^{1,18-20} It was suggested that the interaction between μ -opioid and NMDA receptors results from increased protein kinase C γ activity.^{50,51} This hypothetical diagram would explain fentanyl enhancement of pain sensitization induced by carrageenan. NMDA-R = N-methyl-D-aspartate receptor; μ opiate-R = μ opiate receptor; PKC γ = protein kinase C γ .

Mg^{2+} via intracellular protein kinase C γ activation (fig. 5).^{1,50,51} Moreover, it has been reported that mice lacking protein kinase C γ display normal responses in the first phase of the inflammatory model as a formalin test, but almost completely fail to develop a prolonged phase of pain behavior.⁵² This and the current study may support the view that protein kinase C-mediated phosphorylation of NMDA receptors plays a key role in pain sensitization induced by inflammation and opiates. Studies at a cellular level are in progress in our laboratory to evaluate this hypothesis.

The results of this study suggest that the mechanisms eliciting central sensitization by opiates share some common pathways with those underlying central sensitization elicited by nociceptive inputs, especially those associated with pain inflammation. In clinical studies, it is very difficult to differentiate pain components associated with nociceptive inputs induced by tissue damages from those resulting from a putative enhancement by opiate administration. This might explain why opiate-induced pain sensitization is poorly documented. Because different mechanisms may account for hyperalgesia after surgery, it would be fruitful to evaluate consequences of opiate-induced pain sensitization on the rat incisional pain model,⁵³ a very interesting model of postoperative pain. Although some studies reported advantageous effects of preoperative opiate administration, especially in surgery using low doses of intraoperative opiates or various anesthetics,^{2,38,45} our results may also explain why there is little evidence for preemptive analgesia,

especially with opiates in human surgery. Results from this study confirm that, as suggested,^{19,54} opiate administration before surgery may contribute to "preemptive hyperalgesia, not analgesia."⁵⁴ This does not mean that we must abandon the use of large doses of opiates, especially before and during surgery. From a clinical point of view, our results suggest that NMDA receptor antagonist therapy may be a beneficial therapy for preventing or reducing the development of pain sensitization, especially when opiates are used. Despite the apparent logic appeal of using analgesics for preemptive analgesia, the current study provides better understanding as to why a great number of studies report preemptive effects of ketamine by using low doses that have no analgesic effect *per se*.¹⁰ Because ketamine can produce unwanted hemodynamic and psychological effects,¹⁰ pharmacologic studies are in progress to develop new NMDA receptor antagonists with fewer side effects, allowing for better systematic use in humans.

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