Research Paper

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Withdrawal from an opioid induces a transferable memory trace in the cerebrospinal fluid

Ruth Drdla-Schutting, Céline Heinl, Viktoria Hadschieff, Jürgen Sandkühler*

Abstract

Opioids are the most powerful analgesics available to date. However, they may also induce adverse effects including paradoxical opioid-induced hyperalgesia. A mechanism that might underlie opioid-induced hyperalgesia is the amplification of synaptic strength at spinal C-fibre synapses after withdrawal from systemic opioids such as remifentanil ("opioid-withdrawal long-term potentiation [LTP]"). Here, we show that both the induction as well as the maintenance of opioid-withdrawal LTP were abolished by pharmacological blockade of spinal glial cells. By contrast, the blockade of TLR4 had no effect on the induction of opioid-withdrawal LTP. D-serine, which may be released upon glial cell activation, was necessary for withdrawal LTP. D-serine is the dominant coagonist for neuronal NMDA receptors, which are required for the amplification of synaptic strength on remifentanil withdrawal. Unexpectedly, opioid-withdrawal LTP was transferable through the cerebrospinal fluid between animals. This suggests that glial-cell-derived mediators accumulate in the extracellular space and reach the cerebrospinal fluid at biologically active concentrations, thereby creating a soluble memory trace that is transferable to another animal ("transfer LTP"). When we enzymatically degraded D-serine in the superfusate, LTP could no longer be transferred. Transfer LTP was insensitive to pharmacological blockade of glial cells in the recipient animal, thus representing a rare form of glial cell-independent LTP in the spinal cord.

Keywords: Opioids, Withdrawal, Spinal cord, LTP, Glial cells

1. Introduction

Opioids provide analgesia by suppressing synaptic strength in excitatory nociceptive pathways. At spinal C-fibre synapses, they inhibit neurotransmitter release.[17] Paradoxically, opioids may also induce pain hypersensitivity in animals and humans, possibly by inducing long-term potentiation (LTP) at C-fibre synapses.[11]

Long-term potentiation at synapses between primary afferent C-fibres and neurons located in the superficial laminae of the spinal cord dorsal horn may underlie some forms of pain hypersensitivity.[49] There is growing appreciation that some forms of LTP involve complex communication and signalling between neurons and immunocompetent cells such as microglia and astrocytes.[5,6,16,34,61] Recently we showed that, contrary to widespread assumption, the activation of glial cells is sufficient for LTP induction ("gliogenic LTP").[31] Various diffusible glial mediators such as tumour necrosis factor (TNF), interleukin-1 (Il-1), or adenosine triphosphate (ATP) are sufficient to induce LTP at C-fibre synapses.[13,16,31] Glial cells are now considered active partners in nociceptive synaptic processing in general[14] and after cessation of long-term opioid treatment in particular.[2,3]

Some opioids, including remifentanil, may activate glial cells when applied for prolonged periods.[22,23,60] This may occur directly by acting on opioid receptors present on glial cells,[8,20,32] or by activation of the pattern recognition receptor toll-like receptor 4 (TLR4).[24,26,55] They could also activate glial cells indirectly as a downstream consequence of opioid effects on neurons.[15] Upon activation, glial cells release gliotransmitters, ie, soluble mediators including amino acids, cytokines, and chemokines, which are able to boost synaptic strength by acting on neuronal[12,37,38] or glial receptors.[16] Moreover, chronic opioid treatment may trigger neuronal plasticity, often involving the activation of glial cells.[9,46] Much less is known about the impact of short-term opioid treatment on glial cell function and synaptic plasticity. This lack of knowledge contrasts the frequent short-term use of opioids in the clinical context.

We have shown previously that withdrawal from a brief, systemic opioid application induces LTP at spinal C-fibre synapses ("opioid-withdrawal LTP").[11,18] This form of LTP occurs in the absence of any activity in primary afferents. It is presently unknown whether a brief opioid application is sufficient to activate glial cells to induce LTP. Here, we report that withdrawal from a brief opioid application induces LTP at C-fibre synapses through activation of spinal glial cells.

Unexpectedly, a brief opioid application created a memory trace in the cerebrospinal fluid that could be transferred from a donor animal to a recipient animal. It induced a form of LTP at C-fibre synapses ("transfer LTP"), which was insensitive to pharmacological blockade of glial cells in the recipient animal.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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2. Methods

2.1. Animals

All procedures were performed in accordance with European Communities Council directives (86/609/EEC) and were approved by the Austrian Federal Ministry of Science and Research (BMWF). Male Sprague-Dawley rats (Medical University of Vienna breeding facility; Himberg, Austria), weighing between 150 and 250 g, were used for all experiments. Animals were kept on a 12/12-hour light/dark cycle, housed 3 to 6 per cage and were provided food and water ad libitum.

2.2. Animal surgery in vivo

Isoflurane (4 vol%) in an N_2O/O_2 mixture (50:50) was administered through an induction chamber to induce anaesthesia. Animals were intubated using a 16 G cannula and mechanically ventilated at a rate of 75 strokes·min^{-1} using a tidal volume of 4 to 6 ml. Anaesthesia was maintained by 2 vol% isoflurane. Body core temperature was kept at 37.5°C with a feedback-controlled heating blanket. Deep surgical level of anaesthesia was verified by stable mean arterial blood pressure during noxious stimulation. Surgical procedures were performed as described in detail previously.[25] The spinal cord was exposed by laminectomy and continuously superfused with 5 mL artificial cerebrospinal fluid superfusate consisting of (in mM): 138 NaCl, 2 KCl, 1.8 $CaCl_2$, 10 HEPES, and 1 $MgCl_2$, pH 7.4 adjusted with KOH, measured osmolarity 290 mosmol·l^{-1} in a closed circuit system by means of a roller pump. Additional drugs could be dissolved in the superfusate as indicated. For transfer experiments, 5 mL of this superfusate were collected from a first animal, the *donor rat*, and transferred onto a second animal, the *recipient rat*, by replacing the superfusate circulating during baseline recordings.

At the end of each electrophysiological experiment, animals were decapitated under deep anaesthesia. The spinal cord was removed and shock-frozen for detection of a rhodamine B spot at the recording site under a fluorescence microscope. Only those experiments where the recording site was located in laminae I or II were analysed.

2.3. Drugs and drug administration

For in vivo recordings, pancuronium bromide (Pancuronium Inresa; Inresa, Freiburg, Germany) was administered as an intravenous (i.v.) infusion (2 μg·kg^{-1}·h^{-1}). Remifentanil (Ultiva; GlaxoSmithKline, Vienna, Austria) was dissolved in sterile 0.9% NaCl and applied as a 30 μg·kg^{-1} bolus injection followed by a 1-hour infusion at a rate of 450 μg·kg^{-1}·h^{-1}. Fentanyl dihydrogen citrate (Fentanyl-Janssen; Janssen-Cilag Pharma, Vienna, Austria) was applied as a 40 μg·kg^{-1} bolus injection, followed by a 1-hour infusion at a rate of 48 μg·kg^{-1}·h^{-1}. The microglia inhibitor minocycline dihydrochloride (60 mg·kg^{-1} dissolved in 0.9% NaCl; Sigma Aldrich, Steinheim, Germany) as well as the TLR4-specific inhibitor LPS-RS Ultrapure (100 μg·kg^{-1} dissolved in ddH_2O; Invitrogen, Carlsbad, CA) were applied as an i.v. bolus injection.

All other drugs were dissolved in water and added directly to 5 mL of artificial cerebrospinal fluid superfusate to obtain the desired concentration as indicated: the μ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP, 10 μM), lipopolysaccharides from *Escherichia coli* (LPS O111:B4, 100 μg·ml^{-1}), the competitive N-methyl-D-aspartate receptor (NMDA receptor) antagonist D-2-amino-5-phosphonopentanoic acid (D-AP5, 100 μM), purchased from Ascent Scientific (Cambridge, United Kingdom); the glial cell inhibitor fluoroacetate (10 μM) and D-amino acid oxidase (DAAO, 1 U·ml^{-1}), purchased from Sigma Aldrich; and the Il-1 receptor antagonist Il-1Ra (80 ng·ml^{-1}) and the soluble TNF receptor (sTNFR, 1 μg·ml^{-1}), both purchased from R&D Systems (Minneapolis, MN).

2.4. Electrophysiological recordings

Electrophysiological recordings were performed as described in detail previously.[25] Briefly, C-fibre-evoked field potentials were recorded with glass electrodes from laminae I and II of the spinal cord dorsal horn in response to stimulation of sciatic nerve fibres (pulses of 0.5-ms duration at 25 V applied every 5 minutes using an electrical stimulator). The pipette solution consisted of (in mM): 138 NaCl, 2 KCl, 1.8 $CaCl_2$, 10 HEPES, 1 $MgCl_2$, and 0.2% rhodamine B. At the end of each electrophysiological experiment, the recording site was labelled by pressure application (300 mbar for 1 minute) of rhodamine B through the electrode.

2.5. Statistical analysis

Data were analysed using GraphPad Prism 6 (GraphPad Software, San Diego, CA) or SigmaStat 3.1 (Systat Software, Erkrath, Germany). For electrophysiological recordings, the area under the curve of C-fibre-evoked field potentials was determined offline using Clampfit 10 (Molecular Devices, San Jose, CA). The mean area under the curve of 5 consecutive field potentials before opioid application, or application of superfusate or drugs, respectively, served as a baseline control ("baseline recordings"). Responses were normalized to the baseline in every animal. Data were tested for normality using the Shapiro–Wilk test. A 2-sided paired *t* test was used to compare the effects of treatments at the time indicated in the text with baseline recordings.

In all cases, a *P*-value of <0.05 was considered as significant. Values are expressed as mean ± SEM. Sample size of each experimental group is given in the results section and can be found in the respective figures.

3. Results

3.1. Induction of opioid-withdrawal long-term potentiation requires activation of glial cells

Withdrawal from a brief, high-dose remifentanil infusion induced LTP lasting until the end of the recording period of 240 minutes in all animals tested (to 179 ± 14% of baseline recordings at 220-240 minutes, $P = 0.001$, paired *t* test; n = 11, **Fig. 1A**). The nonselective glial cell toxin fluoroacetate, which was applied directly onto the spinal cord dorsum 30 minutes before the intravenous remifentanil infusion, fully prevented opioid-withdrawal LTP (87 ± 10% of baseline recordings at 160-180 minutes, $P = 0.243$, paired *t* test; n = 11, **Fig. 1B**). Similarly, in the presence of the nonselective microglia inhibitor minocycline, C-fibre-evoked field potentials were not potentiated after opioid withdrawal (80 ± 12% of baseline recordings at 160-180 minutes, $P = 0.156$, paired *t* test; n = 13, **Fig. 1C**). Neither fluoroacetate nor minocycline alone had any effect on C-fibre-evoked field potentials when added to the superfusate (data not shown). These experiments suggest that functioning glial cells are required for the induction of opioid-withdrawal LTP.

3.2. Signalling pathways of opioid-withdrawal long-term potentiation

Previous studies suggested that opioids such as morphine might directly activate glial cells through binding to TLR4s.[24] Here,

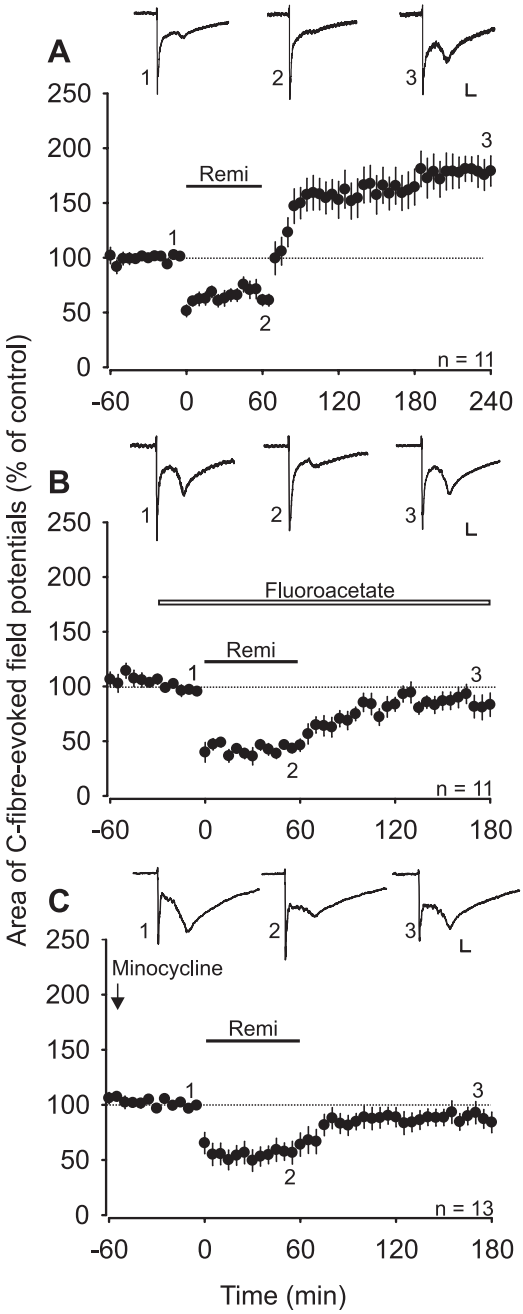

Figure 1. Spinal glial cells are necessary for the induction of opioid-withdrawal LTP. In all graphs, areas of C-fibre-evoked field potentials were normalized to baseline recordings (dotted line) and plotted against time (minutes). Insets show original traces recorded at indicated time points. Calibration bars indicate 0.2 mV and 10 ms. Black horizontal bars indicate periods of i.v. drug application; open horizontal bars indicate periods of spinal drug application. (A) Averaged time course of C-fibre-evoked field potentials recorded in 11 animals. Remifentanil was administered as a bolus (30 mg·kg^{-1}) followed by an infusion (450 mg·kg^{-1}·h^{-1} for 1 hour). (B) Spinal superfusion with fluoroacetate (10 μM) starting 30 minutes before the infusion of remifentanil and lasting throughout the recording period completely prevented withdrawal LTP. (C) Systemic injection of minocycline (60 mg·kg^{-1}, arrow) 60 minutes before remifentanil blocked withdrawal LTP in all animals tested. Data are expressed as mean ± SEM. Statistics: (A–C) paired *t* test; (A) $P = 0.001$; (B) $P = 0.243$; (C) $P = 0.156$. LTP, long-term potentiation.

inhibition of TLR4s using the specific inhibitor LPS-RS Ultrapure did not prevent the induction of opioid-withdrawal LTP (230 ± 30% of baseline recordings at 160-180 minutes, $P = 0.006$, paired *t* test; n = 9, **Fig. 2A**), demonstrating that opioid-withdrawal LTP is independent of TLR4 activation. We next tested potential glial-cell-derived signalling components that mediate opioid-withdrawal LTP. We applied specific blockers directly onto the spinal cord dorsum, which was continuously superfused with artificial cerebrospinal fluid. Withdrawal LTP was neither prevented by blockade of spinal interleukin-1 signaling with Il-1Ra (142 ± 12% of baseline recordings at 160-180 minutes, $P = 0.006$, paired *t* test; n = 10, **Fig. 2B**), nor by the blockade of spinal TNF-receptors with sTNFR (141 ± 13% of baseline recordings at 160-180 minutes, $P = 0.016$, paired *t* test; n = 9, **Fig. 2C**). However, when spinal D-serine was enzymatically degraded with DAAO, opioid-withdrawal LTP was fully prevented (114 ± 19% of baseline recordings at 160-180 minutes, $P = 0.431$, paired *t* test; n = 8, **Fig. 2D**). This shows that spinal D-serine, but not Il-1 or TNF, is required for the induction of LTP after withdrawal from systemic remifentanil application.

3.3. Maintenance of opioid-withdrawal long-term potentiation requires glial cells

To test whether the maintenance of opioid-withdrawal LTP requires active glial cells, we spinally applied fluoroacetate 1 hour after withdrawal from an intravenous remifentanil infusion. In the continuous presence of the glial cell toxin, the area of C-fibre-evoked field potentials returned to baseline within 2 hours (to 102 ± 16% of baseline recordings at 220-240 minutes, $P = 0.823$, paired *t* test; n = 9, **Fig. 3A**), suggesting that functional glial cells are required for LTP maintenance. Interestingly, spinal application of DAAO or the combined application of sTNFR and Il-1Ra had no effect on maintenance (156 ± 17% of baseline recordings at 240-260 minutes, $P = 0.006$, paired *t* test; n = 12, **Fig. 3B**; and 135 ± 13% of baseline recordings at 240-260 minutes, $P = 0.028$, paired *t* test; n = 10, **Fig. 3C**). Thus, although D-serine is required for LTP induction, it is not necessary for its maintenance. We then exchanged the superfusate circulating over the spinal cord dorsum at the recording site 1 hour after remifentanil withdrawal with fresh artificial cerebrospinal fluid. This significantly reduced but did not abolish opioid-withdrawal LTP (from 162 ± 11% at 100-120 minutes to 129 ± 9% at 220-240 minutes, $P = 0.031$, paired *t* test; n = 23, **Fig. 4A**). This suggests that the continuous release of mediators other than D-serine, Il-1, or TNF into the cerebrospinal fluid is required for full expression of opioid-withdrawal LTP.

3.4. Remifentanil-withdrawal creates a memory trace in the cerebrospinal fluid

We next tested whether the mediators that are released into the cerebrospinal fluid upon withdrawal from remifentanil are not only necessary for the full expression of withdrawal LTP, but are also sufficient to induce LTP. To this end, we collected the superfusates from donor animals 1 hour after withdrawal from remifentanil (**Fig. 4A**) and transferred them to the spinal cords of naive recipient animals. This induced an immediate onset facilitation of C-fibre-evoked field potentials ("transfer LTP") (to 176 ± 18% of baseline recordings at 160-180 minutes, $P = 0.015$, paired *t* test; n = 7, **Fig. 4B**). By contrast, the transfer of superfusate collected from untreated donor animals (n = 7, **Fig. 4C**) did not have any effects on synaptic strength in the recipient animals (105 ± 11% of baseline recordings at 160-180 minutes, $P = 0.692$, paired *t* test; n = 7, **Fig. 4D**). The superfusate in the donor animals was replaced by fresh artificial cerebrospinal fluid. This caused a significant

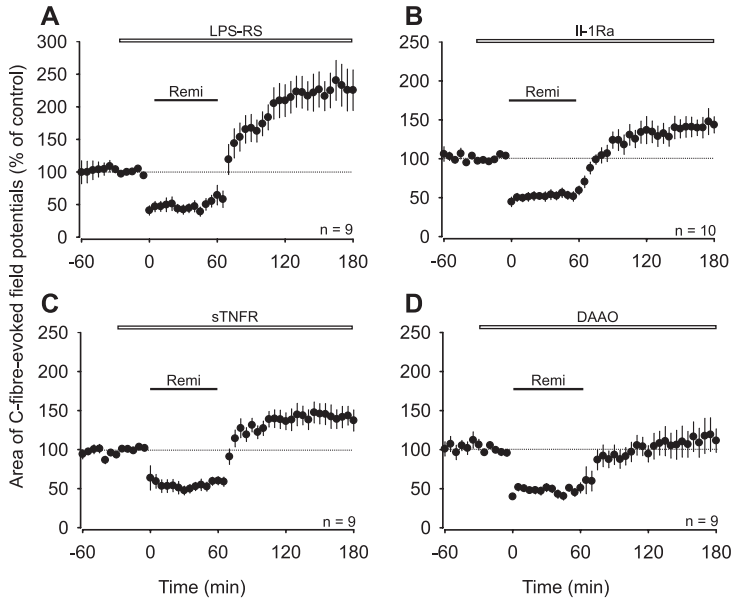

Figure 2. Signalling pathways involved in opioid-withdrawal LTP. In all graphs, areas of C-fibre-evoked field potentials were normalized to baseline recordings (dotted line) and plotted against time (minutes). Black horizontal bars indicate periods of i.v. drug application; open horizontal bars indicate periods of spinal drug application. (A) Systemic bolus injection of LPS-RS Ultrapure (100 μg·kg^{-1}) had no effect on the induction of opioid-withdrawal LTP. (B and C) Spinal superfusion with the Il-1 receptor antagonist Il-1Ra (80 ng·ml^{-1}) or topical application of the soluble TNF receptor (sTNFR; 1 μg·ml^{-1}) did not affect the induction of opioid-withdrawal LTP. (D) Spinal superfusion with DAAO (1 U·ml^{-1}) fully prevented the induction of LTP on opioid withdrawal. Data are expressed as mean ± SEM. Statistics: (A–D) paired *t* test; (A) $P = 0.006$; (B) $P = 0.006$; (C) $P = 0.016$; (D) $P = 0.431$. Il-1, interleukine-1; LTP, long-term potentiation; TNF, tumour necrosis factor.

reduction, but no reversal of remifentanil-withdrawal LTP (see above and **Fig. 4A**).

Together, these data suggest that withdrawal from remifentanil triggers the release of neuroactive substances from glial cells into the cerebrospinal fluid that are sufficient to amplify the strength at C-fibre synapses when transferred to the spinal cord dorsum of naive recipient animals.

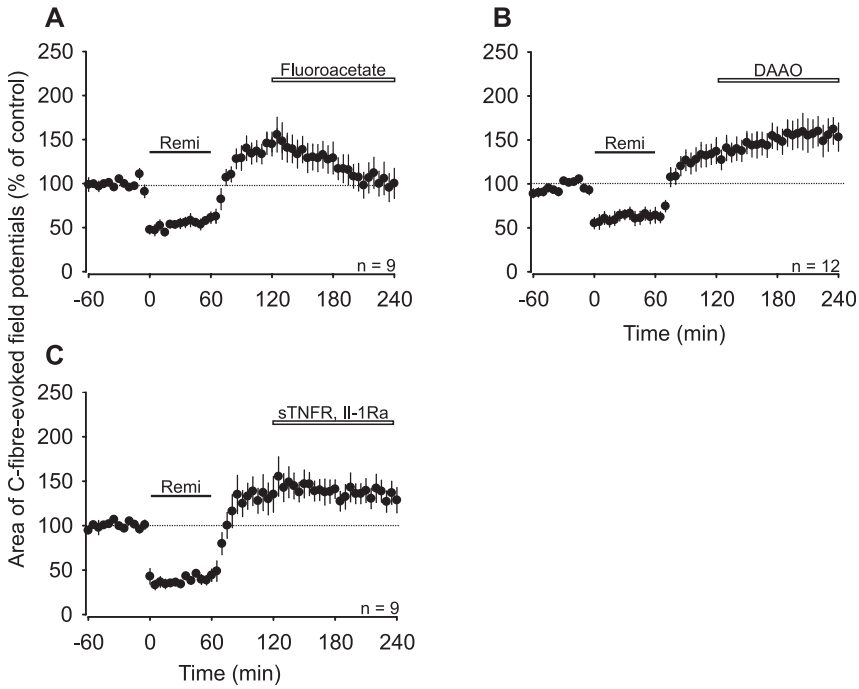

Figure 3. Maintenance of opioid-withdrawal LTP depends on spinal glial cells but not on D-serine. In all graphs, areas of C-fibre-evoked field potentials were normalized to baseline recordings (dotted line) and plotted against time (minutes). Black horizontal bars indicate periods of i.v. drug application; open horizontal bars indicate periods of spinal drug application. (A) Spinal application of the glial toxin fluoroacetate (10 μM) 1 hour after withdrawal from high-dose remifentanil completely reversed opioid-withdrawal LTP. (B) Degradation of D-serine by DAAO (1 U·ml^{-1}) added to the superfusate did not affect maintenance of withdrawal LTP. (C) Blockade of TNF or Il-1 signalling had no effect on maintenance of withdrawal LTP. Data are expressed as mean ± SEM. Statistics: (A–C): paired *t* test; (A) $P = 0.823$; (B) $P = 0.006$; (C) $P = 0.028$. Il-1, interleukine-1; LTP, long-term potentiation; TNF, tumour necrosis factor.

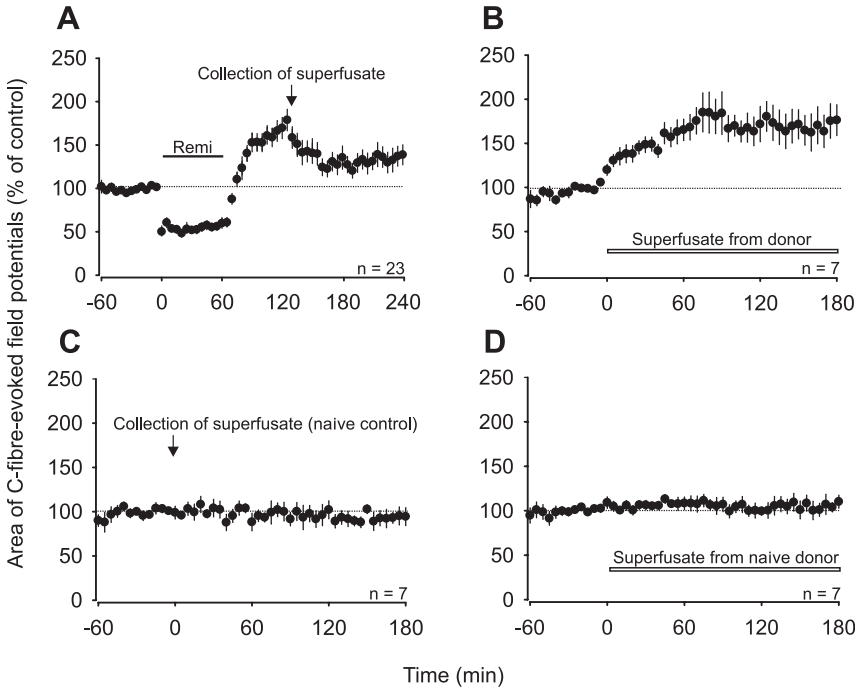

Figure 4. Superfusate collected from donor animals after induction of opioid-withdrawal LTP induces transfer LTP at spinal C-fibre synapses of untreated recipient animals. In all graphs, areas of C-fibre-evoked field potentials were normalized to baseline recordings (dotted line) and plotted against time (minutes). The black horizontal bar indicates periods of i.v. drug application; open horizontal bars indicate periods of spinal drug application. (A) The collection of superfusate (arrow) from donor animals 1 hour after opioid withdrawal significantly attenuated the magnitude of withdrawal LTP. (B) Superfusate (collected from donor animals in A) induced transfer LTP when applied to the spinal cord dorsum of untreated recipients (at time point zero). (C and D) Transfer of superfusate collected from untreated animals (in C, collected at time point zero, arrow) to the spinal cord of naive recipient animals (in D, applied at time point zero) did not have any effect on C-fibre-evoked field potentials. Data are expressed as mean ± SEM. Statistics: (A–D) paired *t* test; (A) $P = 0.031$; (B) $P = 0.015$; (C) $P = 0.253$; (D) $P = 0.692$. LTP, long-term potentiation.

3.5. Transfer long-term potentiation is insensitive to pharmacological blockade of glial cells, and requires D-serine and NMDA receptors in the recipient animals

When the superfusate was collected 1 hour after remifentanil withdrawal from donor animals in which spinal glial cells were blocked by fluoroacetate (data not shown) and transferred to recipients, no change in C-fibre-evoked field potentials was observed in the recipient animals (96 ± 13% of baseline recordings at 160-180 minutes, $P = 0.644$, paired *t* test; n = 6, **Fig. 5A**). By contrast, blockade of glial cells in the spinal cord of recipient animals using fluoroacetate did not prevent the facilitation of synaptic strength at spinal C-fibre synapses induced by the application of superfusate collected from donor animals 1 hour after remifentanil-withdrawal (to 155 ± 11% of baseline recordings at 160-180 minutes, $P = 0.019$, paired *t* test; n = 6, **Fig. 5B**). This suggests that glial cell activation in the donor, but not in the recipient animals, is required for the induction of transfer LTP.

The activation of the NMDA receptor plays an essential role in the induction of LTP throughout the central nervous system. To further elucidate the mechanisms of transfer LTP, the recipient animals were pretreated with the NMDA receptor antagonist D-AP5, which was added to the superfusate. This fully prevented the induction of transfer LTP on application of superfusate collected from donor animals 1 hour after remifentanil withdrawal in all recipient animals tested (109 ± 15% of baseline recordings at 160-180 minutes, $P = 0.592$, paired *t* test; n = 6, **Fig. 5C**). The binding of a coagonist such as the amino-acid transmitter D-serine to its dedicated receptor-binding site is pivotal for NMDA-receptor activation. Here, transfer LTP was completely prevented when DAAO was added to the superfusate before its application onto the spinal cord dorsum of the recipient animal (102 ± 7% of baseline recordings at 160-180 minutes, $P = 0.896$, paired *t* test; n = 6, **Fig. 5D**). This demonstrates that similar to the induction of withdrawal LTP, transfer LTP requires D-serine.

3.6. Is transfer long-term potentiation a specific feature of withdrawal from remifentanil?

We next tested whether transfer LTP could also be observed when the donor animals were treated with an opioid other than remifentanil. We collected the superfusate from donor animals 1 hour after CTOP-precipitated withdrawal from systemic fentanyl. This treatment induced LTP at C-fibre synapses (to 202 ± 33% of baseline recordings at 100-120 minutes, $P = 0.016$, paired *t* test; n = 7, **Fig. 6A**). We then transferred the superfusate to recipient animals. This induced transfer LTP in all the animals tested (166 ± 15% of baseline recordings at 160-180 minutes, $P = 0.014$, paired *t* test; n = 5, **Fig. 6B**). In these experiments, CTOP might have been present in the superfusate of the donor animals. To exclude that the transfer of the superfusate including CTOP enhanced synaptic strength in C-fibres of the recipient animal eg, by disinhibition, we added CTOP to the superfusate of the recipient animals 30 minutes before the transfer. CTOP itself had no effect on the recipient animal.

3.7. Lipopolysaccharide treatment induces long-term potentiation, but no "transfer long-term potentiation"

Lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria, is an agonist at TLR4s, which are expressed on glial cells.[55] When we applied LPS directly onto the spinal cord dorsum, synaptic strength in donor animals was potentiated (to 168 ± 21% of baseline

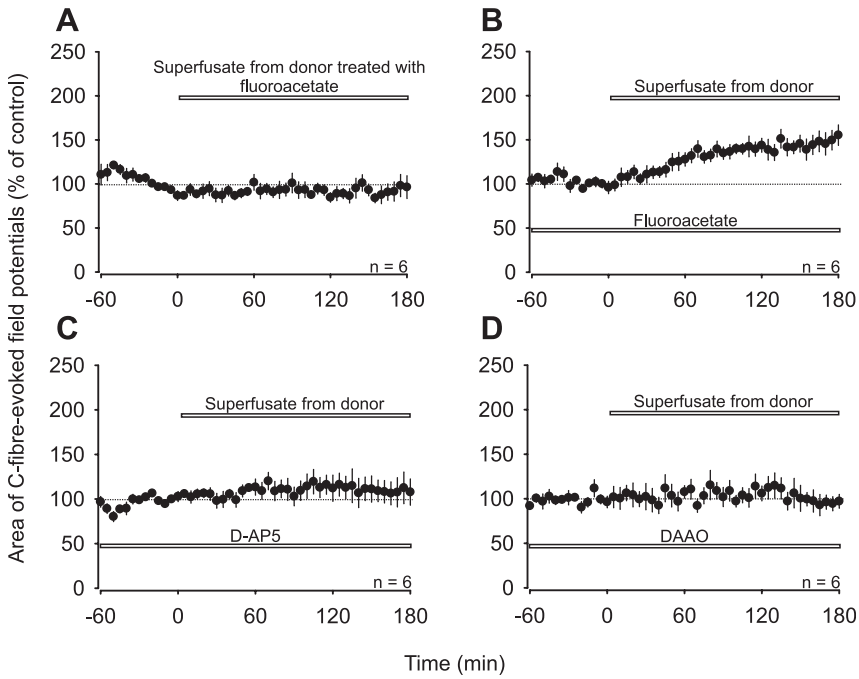

Figure 5. Transfer LTP requires glial cells in donor but not in recipient animals and involves NMDA receptors and D-serine signalling. In all graphs, areas of C-fibre-evoked field potentials were normalized to baseline recordings (dotted line) and plotted against time (minutes). Open horizontal bars indicate periods of spinal drug application. (A) Superfusate collected from donors 1 hour after withdrawal from high-dose remifentanil in the presence of fluoroacetate (10 μM, donors not shown) has no effect on synaptic strength when transferred to recipients (at time point zero, open bar). Recipient animals were pretreated with fluoroacetate to exclude any effects of the glial toxin itself. (B) Superfusate collected from donor animals (shown in Fig. 4A) applied at time point zero induced transfer LTP in recipient animals (time point zero) that were pretreated with spinal fluoroacetate. (C and D) No change in synaptic strength was observed in recipient animals when the superfusate (from donor animals shown in Fig. 4A) was applied in the continuous presence of D-AP5 (50 μM, C) or DAAO (1 U·ml^{-1}, *D*) at time point zero. Data are expressed as mean ± SEM. Statistics: (A–D) paired *t* test; (A) $P = 0.664$; (B) $P = 0.019$; (C) $P = 0.592$; (D) $P = 0.896$. LTP, long-term potentiation.

recordings at 220-240 minutes, $P = 0.01$, paired *t* test; n = 11, **Fig. 6C**). Interestingly, when we collected the superfusate from donor animals which developed LTP due to LPS which circulated over the spinal cord dorsum for 4 hours and transferred the superfusate to the spinal cord of recipient animals, synaptic strength remained unaltered in recipient animals (92 ± 7% of baseline recordings at 160-180 minutes, $P = 0.41$, paired *t* test; n = 7; **Fig. 6D**). These experiments were performed in the presence of fluoroacetate to prevent glial cell activation by LPS in the recipient animals. To test whether D-serine is involved in LPS-induced potentiation of synaptic strength, we added DAAO to the superfusate 30 minutes before the LPS treatment. This did not affect the induction of LTP by LPS (148 ± 8% at 220-240 minutes, $P = 0.004$, paired *t* test; n = 5; **Fig. 6E**), suggesting that D-serine is not involved in this form of plasticity.

Thus, opioids such as remifentanil and fentanyl, but not LPS, trigger the release of active ingredients such as D-serine into the cerebrospinal fluid that are sufficient for the induction of transfer LTP in a naive recipient animal.

4. Discussion

In this study, we demonstrate that withdrawal from a brief, systemic application of remifentanil activated glial cells at the spinal cord level. This created a transferable, pronociceptive memory trace in the cerebrospinal fluid.

4.1. Opioid-withdrawal long-term potentiation is gliogenic

Research over the past years has shed light on the mechanisms underlying opioid-induced plasticity at different levels of the nociceptive system, which may ultimately result in opioid-induced hyperalgesia (OIH). The type of opioid used, the dose and route of administration, as well as the duration of treatment may all affect the properties of OIH.[1,46] Short-term opioid treatments are often used in the clinical setting. Surprisingly little is known about the effects of a brief opioid administration and the withdrawal thereof on the properties and functions of glial cells or synapses.

We have shown recently that withdrawal from a brief systemic administration of an opioid induces LTP at spinal C-fibre synapses.[11,18] This opioid-withdrawal LTP represents a cellular mechanism leading to the amplification of nociception and potentially also to OIH. Thus even a brief systemic opioid treatment generates a pronociceptive memory trace at the level of the spinal cord dorsal horn. Our study demonstrates that glial cells are key to this form of opioid-induced synaptic plasticity.

It is now well established that glial cells are necessary for some forms of synaptic plasticity. Recently, we discovered that the activation of spinal cord dorsal horn glial cells can also be sufficient to induce plasticity at spinal C-fibre synapses ("gliogenic LTP").[31] Opioids too can activate glial cells, and glial cell activation is necessary for the development of OIH.[15]

Opioids might activate glial cells either directly or indirectly in different ways. Opioids may bind directly to glial TLR4 receptors[24,26,55] but see Ref. 36, or to glial μ-opioid receptors[8,20,32] but see Refs. 7, 29, 50. Opioids may also activate glial cells indirectly through signalling cascades downstream of neuronal μ-opioid receptor activation.[28,41,56] A study by Hutchinson et al.[22] reported that remifentanil activation of TLR4 contributes to drug reinforcement. Our present findings demonstrate that induction of remifentanil-withdrawal LTP requires activation of glial cells but not TLR4. Opioid-withdrawal LTP rather depends on the

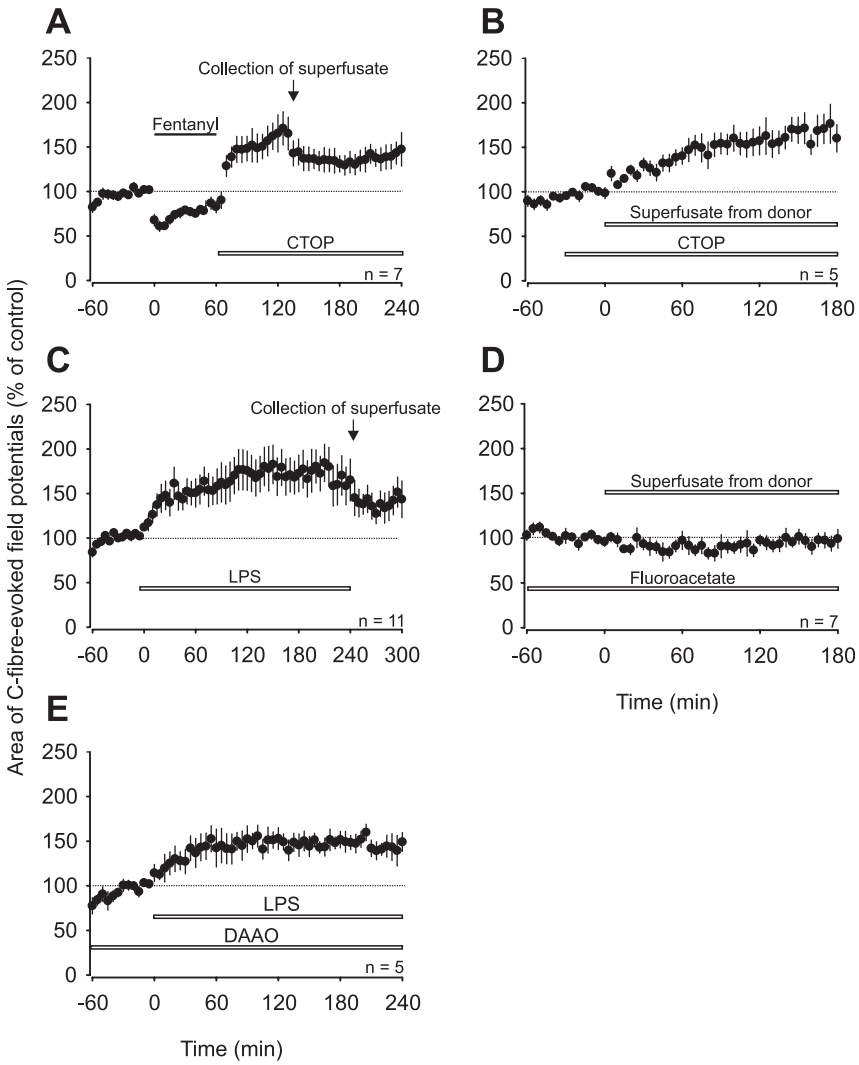

Figure 6. Application of superfusate collected after precipitated withdrawal from fentanyl but not after spinal LPS treatment induces transfer LTP at C-fibre synapses of recipient animals. In all graphs, areas of C-fibre-evoked field potentials were normalized to baseline recordings (dotted line) and plotted against time (minutes). Black horizontal bars indicate periods of i.v. drug application; open horizontal bars indicate periods of spinal drug application. (A) Spinal application of the μ-opioid receptor antagonist CTOP to the spinal cord dorsum after termination of an intravenous infusion of fentanyl (40 $\mu g \cdot kg^{-1}$ bolus injection, followed by 1 hour infusion at a rate of 48 $\mu g \cdot kg^{-1} \cdot h^{-1}$) induced withdrawal LTP. One hour after withdrawal, the superfusate was collected (arrow). (B) Superfusate from donor animals in (A) induced transfer LTP in recipient animals that where pretreated with CTOP (10 μM). (C) Spinal treatment with LPS (100 $\mu g \cdot ml^{-1}$) induced a long-lasting potentiation of synaptic strength. After 4 hours, the superfusate was collected (arrow). (D) Superfusate from donor animals in (C) had no effect on synaptic strength in recipient animals that were pretreated with fluoroacetate. (E) The continuous presence of DAAO (1 $U \cdot ml^{-1}$) in the superfusate had no effect on the induction of LTP after spinal LPS treatment. Data are expressed as mean ± SEM. Statistics: (A–E) paired *t* test; (A) $P = 0.016$; (B) $P = 0.014$; (C) $P = 0.016$; (D) $P = 0.014$; (E) $P = 0.004$. LPS, lipopolysaccharide; LTP, long-term potentiation.

activation of spinal opioid receptors.[11,18] Thus, our present and previous findings suggest that remifentanil activates glial cells through spinal opioid receptors and thereby triggered the release of pronociceptive substances into the cerebrospinal fluid.

Our present data suggest that opioid-withdrawal LTP constitutes another subtype of gliogenic LTP in male rats. It is currently unknown whether opioid-withdrawal LTP also exists in females.

4.2. Signalling mechanisms of gliogenic opioid-withdrawal long-term potentiation

Previous studies suggest that chronic but not acute morphine treatment leads to an increase in the expression of proinflammatory cytokines in the spinal cord and brain.[10,27,28,43,45] In particular, the proinflammatory glial-cell-derived cytokines TNF and Il-1 have been identified as key players in the pain enhancement after withdrawal from long-lasting opioid treatment.[57] All previously identified forms of gliogenic LTP likewise required Il-1 and TNF signalling.[16,31] Interestingly, in this study, the pharmacological blockade of Il-1 receptors or inhibition of TNF had no effect on the LTP induction after withdrawal from a brief remifentanil application. This indicates that distinct mechanisms and signalling pathways are activated upon withdrawal after long-term vs short-term opioid application. Gliogenic LTP is not a uniform phenomenon mechanistically.

We showed previously that opioid-withdrawal LTP is NMDA-receptor-dependent.[11,18] An important coagonist at NMDA receptors is D-serine. It is necessary, but not sufficient for activity-dependent forms of LTP at spinal C-fibre synapses.[31] Here, opioid-withdrawal LTP was abolished when D-serine was degraded by DAAO, suggesting that this amino acid is necessary for the induction of NMDA-receptor-dependent withdrawal LTP.

The cellular source of D-serine is currently under debate. Although its origin was initially attributed to astrocytes,[35,42,51] recent studies suggest an expression of the D-serine synthesizing enzyme serine racemase in neurons.[30,47,59] In any case, astrocytes are unequivocally involved, as D-serine production depends on L-serine, which is of astrocytic origin.[58]

In this study, minocycline prevented the induction of withdrawal LTP. Minocycline is considered to impair functions of microglia, which in turn are required for the activation of astrocytes under pathological conditions.[33,39] In addition, minocycline can also impair astrocytic functions directly.[40] Thus, minocycline could either indirectly or directly prevent the release of D-serine from astrocytes after withdrawal from remifentanil.

4.3. Maintenance of opioid-withdrawal long-term potentiation deepens on glial cell activity, but not on D-serine signalling

Here, we show that glial cell activity is necessary for the maintenance of opioid-withdrawal LTP. The glial cell toxin fluoroacetate fully impeded the maintenance of opioid-withdrawal LTP. This is in striking contrast to activity-dependent forms of LTP, where the spinal blockade of glial cells by fluoroacetate does not affect its maintenance.[31] When we collected the superfusate from donor animals 1 hour after the withdrawal from remifentanil, withdrawal LTP was reduced, but not abolished. One could thus speculate that ongoing production of glial cell-dependent D-serine is required for LTP maintenance. However, the degradation of D-serine by DAAO, which blocked LTP induction, had no effect on its maintenance, demonstrating that induction and maintenance of opioid-withdrawal LTP at spinal C-fibre synapses involve distinct mechanisms.

Spinal glial cells are indispensable for both opioid-withdrawal LTP (this study) and for OIH after opioid withdrawal.[4,21,44] This is in line with the hypothesis that gliogenic LTP can cause OIH.

4.4. A transferable memory trace is generated on withdrawal from systemic opioids

Upon activation, gliotransmitters act locally but can also spread through the parenchyma to reach remote synapses.[19,52] Our data suggest that after opioid withdrawal, glial cell-derived modulators such as D-serine can reach the cerebrospinal fluid, accumulate in the superfusate at biologically active concentrations, and induce synaptic plasticity when transferred between animals.

The spinal cord superfusate taken from animals that were treated with lipopolysaccharide, a TLR4 agonist, failed to induce transfer LTP in recipient animals. This indicates that activation of glial cells by TLR4 differs substantially from glial cell activation through other mechanisms, eg, through opioid receptors, although both forms of activation lead to gliogenic LTP.

4.5. Transfer long-term potentiation is a neuronal mechanism

Blockage of spinal NMDA receptors by D-AP5 fully blocked the induction of transfer LTP because it did block other known forms of LTP at spinal C-fibre synapses.[48] Functional NMDA receptors may be expressed both on neurons and on glial cells.[54] The spinal application of the glial cell toxin fluoroacetate in the recipient animal did not have any effect on transfer LTP, suggesting that in the recipient animals, NMDA receptors on neurons but not glial cells are involved.

Thus, although glial cells are necessary to create the soluble memory trace in the donor, neuronal mechanisms are sufficient for the induction of transfer LTP in the recipient animals. This is a unique feature of transfer LTP because all other tested forms of LTP at spinal C-fibre synapses depend on glial cell activation.[31]

5. Conclusions

Glial cells play a pivotal role in many different forms of pain, as well as in adverse events of chronic opioid application such as tolerance, hyperalgesia, and withdrawal. Here, we have shown that a brief opioid treatment for just 1 hour is sufficient to activate spinal cord glial cells in male rats. Once activated, glial cells release highly active substances that can modulate other glial cells or neurons, possibly by activating a positive feedback loop that further boosts synaptic strength at spinal C-fibres. D-serine was necessary for the induction but not for the maintenance of opioid-withdrawal LTP. When released, it reaches the cerebrospinal fluid in a concentration that is sufficiently high to enable a NMDA-receptor-dependent facilitation of synaptic strength when transferred to the spinal cord of naive animals. The spread of gliotransmitters such as D-serine through the cerebrospinal fluid might provide an explanation for the clinical observation that OIH may be widespread.[53] Additionally, gliotransmitters could spread further into other areas of the central nervous system and thereby contribute to other supraspinally mediated adverse effects of opioids.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

[1] Angst MS, Clark JD. Opioid-induced hyperalgesia: a qualitative systematic review. Anesthesiology 2006;104:570–87.
[2] Burke NN, Fan CY, Trang T. Microglia in health and pain: impact of noxious early life events. Exp Physiol 2016;101:1003–21.
[3] Burma NE, Bonin RP, Leduc-Pessah H, Baimel C, Cairncross ZF, Mousseau M, Shankara JV, Stemkowski PL, Baimoukhametova D, Bains JS, Antle MC, Zamponi GW, Cahill CM, Borgland SL, De KY, Trang T. Blocking microglial pannexin-1 channels alleviates morphine withdrawal in rodents. Nat Med 2017;23:355–60.
[4] Burma NE, Leduc-Pessah H, Trang T. Genetic deletion of microglial Panx1 attenuates morphine withdrawal, but not analgesic tolerance or hyperalgesia in mice. Channels (Austin) 2017;11:487–94.
[5] Chu YX, Zhang Y, Zhang YQ, Zhao ZQ. Involvement of microglial P2X7 receptors and downstream signaling pathways in long-term potentiation of spinal nociceptive responses. Brain Behav Immun 2010;24:1176–89.
[6] Chu YX, Zhang YQ, Zhao ZQ. Involvement of microglia and interleukin-18 in the induction of long-term potentiation of spinal nociceptive responses induced by tetanic sciatic stimulation. Neurosci Bull 2012;28:49–60.

[7] Corder G, Tawfik VL, Wang D, Sypek EI, Low SA, Dickinson JR, Sotoudeh C, Clark JD, Barres BA, Bohlen CJ, Scherrer G. Loss of μ opioid receptor signaling in nociceptors, but not microglia, abrogates morphine tolerance without disrupting analgesia. Nat Med 2017;23:164–73.
[8] Cui Y, Chen Y, Zhi JL, Guo RX, Feng JQ, Chen PX. Activation of p38 mitogen-activated protein kinase in spinal microglia mediates morphine antinociceptive tolerance. Brain Res 2006;1069:235–43.
[9] Dacher M, Nugent FS. Opiates and plasticity. Neuropharmacology 2011; 61:1086–96.
[10] Doyle HH, Eidson LN, Sinkiewicz DM, Murphy AZ. Sex differences in microglia activity within the periaqueductal gray of the rat: a potential mechanism driving the dimorphic effects of morphine. J Neurosci 2017; 37:3202–14.
[11] Drdla R, Gassner M, Gingl E, Sandkühler J. Induction of synaptic long-term potentiation after opioid withdrawal. Science 2009;325:207–10.
[12] Gao YJ, Ji RR. Targeting astrocyte signaling for chronic pain. Neurotherapeutics 2010;7:482–93.
[13] Gong QJ, Li YY, Xin WJ, Zang Y, Ren WJ, Wei XH, Li YY, Zhang T, Liu XG. ATP induces long-term potentiation of C-fiber-evoked field potentials in spinal dorsal horn: the roles of $P2X_4$ receptors and p38 MAPK in microglia. Glia 2009;57:583–91.
[14] Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol 2014;14:217–31.
[15] Grace PM, Maier SF, Watkins LR. Opioid-induced central immune signaling: implications for opioid analgesia. Headache 2015;55: 475–89.
[16] Gruber-Schoffnegger D, Drdla-Schutting R, Honigsperger C, Wunderbaldinger G, Gassner M, Sandkühler J. Induction of thermal hyperalgesia and synaptic long-term potentiation in the spinal cord lamina I by TNF-α and IL-1β is mediated by glial cells. J Neurosci 2013;33: 6540–51.
[17] Heinke B, Gingl E, Sandkühler J. Multiple targets of μ-opioid receptor mediated presynaptic inhibition at primary afferent Aδ- and C-fibers. J Neurosci 2011;31:1313–22.
[18] Heinl C, Drdla-Schutting R, Xanthos DN, Sandkühler J. Distinct mechanisms underlying pronociceptive effects of opioids. J Neurosci 2011;31:16748–56.
[19] Hirase H, Iwai Y, Takata N, Shinohara Y, Mishima T. Volume transmission signalling via astrocytes. Philos Trans R Soc Lond B Biol Sci 2014;369: 20130604.
[20] Horvath RJ, Romero-Sandoval EA, De Leo JA. Inhibition of microglial $P2X_4$ receptors attenuates morphine tolerance, Iba1, GFAP and μ opioid receptor protein expression while enhancing perivascular microglial ED2. PAIN 2010;150:401–13.
[21] Hutchinson MR, Lewis SS, Coats BD, Skyba DA, Crysdale NY, Berkelhammer DL, Brzeski A, Northcutt A, Vietz CM, Judd CM, Maier SF, Watkins LR, Johnson KW. Reduction of opioid withdrawal and potentiation of acute opioid analgesia by systemic AV411 (ibudilast). Brain Behav Immun 2009;23:240–50.
[22] Hutchinson MR, Northcutt AL, Hiranita T, Wang X, Lewis SS, Thomas J, van SK, Kopajtic TA, Loram LC, Sfregola C, Galer E, Miles NE, Bland ST, Amat J, Rozeske RR, Maslanik T, Chapman TR, Strand KA, Fleshner M, Bachtell RK, Somogyi AA, Yin H, Katz JL, Rice KC, Maier SF, Watkins LR. Opioid activation of toll-like receptor 4 contributes to drug reinforcement. J Neurosci 2012;32:11187–200.
[23] Hutchinson MR, Shavit Y, Grace PM, Rice KC, Maier SF, Watkins LR. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. Pharmacol Rev 2011;63:772–810.
[24] Hutchinson MR, Zhang Y, Shridhar M, Evans JH, Buchanan MM, Zhao TX, Slivka PF, Coats BD, Rezvani N, Wieseler J, Hughes TS, Landgraf KE, Chan S, Fong S, Phipps S, Falke JJ, Leinwand LA, Maier SF, Yin H, Rice KC, Watkins LR. Evidence that opioids may have toll-like receptor 4 and MD-2 effects. Brain Behav Immun 2010;24:83–95.
[25] Ikeda H, Stark J, Fischer H, Wagner M, Drdla R, Jäger T, Sandkühler J. Synaptic amplifier of inflammatory pain in the spinal dorsal horn. Science 2006;312:1659–62.
[26] Jacobsen JH, Watkins LR, Hutchinson MR. Discovery of a novel site of opioid action at the innate immune pattern-recognition receptor TLR4 and its role in addiction. Int Rev Neurobiol 2014;118:129–63.
[27] Jin H, Li YH, Xu JS, Guo GQ, Chen DL, Bo Y. Lipoxin A_4 analog attenuates morphine antinociceptive tolerance, withdrawal-induced hyperalgesia, and glial reaction and cytokine expression in the spinal cord of rat. Neuroscience 2012;208:1–10.
[28] Johnston IN, Milligan ED, Wieseler-Frank J, Frank MG, Zapata V, Campisi J, Langer S, Martin D, Green P, Fleshner M, Leinwand L, Maier SF, Watkins LR. A role for proinflammatory cytokines and fractalkine in analgesia, tolerance, and subsequent pain facilitation induced by chronic intrathecal morphine. J Neurosci 2004;24:7353–65.
[29] Kao SC, Zhao X, Lee CY, Atianjoh FE, Gauda EB, Yaster M, Tao YX. Absence of μ opioid receptor mRNA expression in astrocytes and microglia of rat spinal cord. Neuroreport 2012;23:378–84.
[30] Kartvelishvily E, Shleper M, Balan L, Dumin E, Wolosker H. Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. J Biol Chem 2006;281:14151–62.
[31] Kronschläger M, Drdla-Schutting R, Gassner M, Honsek S, Teuchmann HL, Sandkühler J. Gliogenic LTP spreads widely in nociceptive pathways. Science 2016;354:1144–8.
[32] Leduc-Pessah H, Weilinger NL, Fan CY, Burma NE, Thompson RJ, Trang T. Site-specific regulation of P2X7 receptor function in microglia gates morphine analgesic tolerance. J Neurosci 2017;37:10154–72.
[33] Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Munch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 2017;541:481–7.
[34] Ma JY, Zhao ZQ. The involvement of glia in long-term plasticity in the spinal dorsal horn of the rat. Neuroreport 2002;13:1781–4.
[35] Martineau M, Baux G, Mothet JP. D-serine signalling in the brain: friend and foe. Trends Neurosci 2006;29:481–91.
[36] Mattioli TA, Leduc-Pessah H, Skelhorne-Gross G, Nicol CJ, Milne B, Trang T, Cahill CM. Toll-like receptor 4 mutant and null mice retain morphine-induced tolerance, hyperalgesia, and physical dependence. PLoS One 2014;9:e97361.
[37] McMahon SB, Malcangio M. Current challenges in glia-pain biology. Neuron 2009;64:46–54.
[38] Milligan ED, Watkins LR. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci 2009;10:23–36.
[39] Miyoshi K, Obata K, Kondo T, Okamura H, Noguchi K. Interleukin-18-mediated microglia/astrocyte interaction in the spinal cord enhances neuropathic pain processing after nerve injury. J Neurosci 2008;28: 12775–87.
[40] Möller T, Bard F, Bhattacharya A, Biber K, Campbell B, Dale E, Eder C, Gan L, Garden GA, Hughes ZA, Pearse DD, Staal RG, Sayed FA, Wes PD, Boddeke HW. Critical data-based re-evaluation of minocycline as a putative specific microglia inhibitor. Glia 2016;64:1788–94.
[41] Narita M, Suzuki M, Narita M, Yajima Y, Suzuki R, Shioda S, Suzuki T. Neuronal protein kinase Cγ-dependent proliferation and hypertrophy of spinal cord astrocytes following repeated *in vivo* administration of morphine. Eur J Neurosci 2004;19:479–84.
[42] Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, Oliet SH. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell 2006;125:775–84.
[43] Raghavendra V, Rutkowski MD, DeLeo JA. The role of spinal neuroimmune activation in morphine tolerance/hyperalgesia in neuropathic and sham-operated rats. J Neurosci 2002;22:9980–9.
[44] Raghavendra V, Tanga FY, DeLeo JA. Attenuation of morphine tolerance, withdrawal-induced hyperalgesia, and associated spinal inflammatory immune responses by propentofylline in rats. Neuropsychopharmacology 2004;29:327–34.
[45] Raghavendra V, Tanga FY, DeLeo JA. Complete Freunds adjuvant-induced peripheral inflammation evokes glial activation and proinflammatory cytokine expression in the CNS. Eur J Neurosci 2004;20:467–73.
[46] Roeckel LA, Le Coz GM, Gavériaux-Ruff C, Simonin F. Opioid-induced hyperalgesia: cellular and molecular mechanisms. Neuroscience 2016; 338:160–82.
[47] Rosenberg D, Kartvelishvily E, Shleper M, Klinker CM, Bowser MT, Wolosker H. Neuronal release of D-serine: a physiological pathway controlling extracellular D-serine concentration. FASEB J 2010;24: 2951–61.
[48] Ruscheweyh R, Wilder-Smith O, Drdla R, Liu XG, Sandkühler J. Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. Mol Pain 2011;7:20.
[49] Sandkühler J. Models and mechanisms of hyperalgesia and allodynia. Physiol Rev 2009;89:707–58.
[50] Sanna MD, Ghelardini C, Galeotti N. Activation of JNK pathway in spinal astrocytes contributes to acute ultra-low dose morphine thermal hyperalgesia. PAIN 2015;156:1265–75.
[51] Schell MJ, Molliver ME, Snyder SH. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc Natl Acad Sci U S A 1995;92:3948–52.
[52] Syková E, Chvatal A. Glial cells and volume transmission in the CNS. Neurochem Int 2000;36:397–409.

[53] Tompkins DA, Campbell CM. Opioid-induced hyperalgesia: clinically relevant or extraneous research phenomenon? Curr Pain Headache Rep 2011;15:129–36.
[54] Verkhratsky A, Kirchhoff F. NMDA receptors in glia. Neuroscientist 2007; 13:28–37.
[55] Wang X, Loram LC, Ramos K, de Jesus AJ, Thomas J, Cheng K, Reddy A, Somogyi AA, Hutchinson MR, Watkins LR, Yin H. Morphine activates neuroinflammation in a manner parallel to endotoxin. Proc Natl Acad Sci U S A 2012;109:6325–30.
[56] Wang Z, Ma W, Chabot JG, Quirion R. Cell-type specific activation of p38 and ERK mediates calcitonin gene-related peptide involvement in tolerance to morphine-induced analgesia. FASEB J 2009;28: 2576–86.
[57] Watkins LR, Hutchinson MR, Johnston IN, Maier SF. Glia: novel counter-regulators of opioid analgesia. Trends Neurosci 2005;28:661–9.
[58] Wolosker H. Serine racemase and the serine shuttle between neurons and astrocytes. Biochim Biophys Acta 2011;1814:1558–66.
[59] Wolosker H, Balu DT, Coyle JT. The rise and fall of the d-Serine-Mediated gliotransmission hypothesis. Trends Neurosci 2016;39:712–21.
[60] Ye L, Xiao L, Yang SY, Duan JJ, Chen Y, Cui Y, Chen Y. Cathepsin S in the spinal microglia contributes to remifentanil-induced hyperalgesia in rats. Neuroscience 2017;344:265–75.
[61] Zhong Y, Zhou LJ, Ren WJ, Xin WJ, Li YY, Zhang T, Liu XG. The direction of synaptic plasticity mediated by C-fibers in spinal dorsal horn is decided by Src-family kinases in microglia: the role of tumor necrosis factor-α. Brain Behav Immun 2010;24:874–80.