EJP
European Journal of Pain

ORIGINAL ARTICLE

Role of the spinal TrkB-NMDA receptor link in the BDNF-induced long-lasting mechanical hyperalgesia in the rat: A behavioural study

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Funding sources
This work was supported by grant FB0807 from Centro para el Desarrollo de la Nanociencia y la Nanotecnología (CEDENNA), and Grant 021543CC from Dirección de Investigación Científica y Tecnológica (DICYT) of the University of Santiago of Chile. Proyectos Basal USA 1555 - Vridei 021743CC_PUBLIC Universidad de Santiago de Chile. We thank Ms. Cristina Arenas and Ms. Yaquelin González for technical support and animal care.

Conflicts of interest
None declared.

Accepted for publication
29 May 2017

doi:10.1002/ejp.1075

Abstract

Background: Intrathecal/intracisternal BDNF in rodents produces long-lasting hyperalgesia/allodynia, which implies BDNF plays a role in the establishment and maintenance of central sensitization. Both self-regeneration of endogenous BDNF and neuroplastic modifications of spinal NMDA receptors downstream TrkB signalling could be involved in such enduring hyperalgesia. We investigated to what extent BDNF by itself could participate in the generation and maintenance of mechanical hyperalgesia using pharmacological tools.

Methods: We studied sensitivity of mechanical hyperalgesia induced by a single intrathecal (i.t.) injection of BDNF (3 ng/10 μL i.t.) administered at time zero, for: (1) chronic NMDA receptor inhibition with subcutaneously implanted 7-day delivery osmotic pumps loaded with ketamine; (2) TrkB receptor inhibition with intraperitoneal (i.p.) cyclotraxine-B; and (3) chronic glial inhibition with repeated propentofylline i.t. injections. Nociceptive threshold to paw pressure, tested on days -3, 0, 3, 7, 10 and 14, was used as the index of central sensitization. Locomotor patterns and food and water consumption were assessed with LABORAS.

Results: Chronic ketamine prevented the mechanical hyperalgesia induced by BDNF, without affecting locomotion and food and water consumption. After pump depletion, a late hyperalgesic response to paw pressure stimulation emerged, which can be lastingly antagonized by cyclotraxine-B. Chronic propentofylline treatment irreversibly suppressed BDNF-induced hyperalgesia.

Conclusion: Activation of NMDA receptors downstream to TrkB signalling is essential for behavioural expression of the mechanical hyperalgesia induced by intrathecal BDNF. However, maintenance of the hyperalgesia depends mainly from self-regenerating glial BDNF rather than from a NMDA receptor-dependent form of neuroplasticity.

Significance: Intrathecal BDNF induces long-lasting central sensitization via a glial-likely BDNF self-regenerating mechanism, whose behavioural expression depends on downstream activation of NMDA receptors. This knowledge suggests that TrkB antagonists could represent an interesting lead for the development of novel therapeutic strategies for some chronic pain conditions.

1. Introduction

Brain-derived neurotrophic factor (BDNF) is crucially involved in the triggering of central sensitization. A single BDNF intrathecal injection can produce enduring mechanical hyperalgesia in rats and cold allodynia in mice lasting for more than 4 weeks post-injection, which can be both prevented and reverted by the TrkB antagonist cyclotraxin-B (Constandil et al., 2012; M'Dahoma et al., 2015). BDNF is released in spinal cord by C nociceptors in response to a peripheral inflammatory stimulus or to a nerve injury (Fukuoka et al., 2001; Malcangio and Lessmann, 2003), and only short bursts of high-frequency stimulation of the C-fibres bring BDNF-loaded vesicles to the plasma membrane in the synaptic space leading to their release (Lever et al., 2001). In neuropathic and inflammatory pain models, there are upregulated BDNF levels in sensory neurons and in the dorsal horn (Kerr et al., 1999; Fukuoka et al., 2001; Onda et al., 2003; Yajima et al., 2005). Enhanced concentration of BDNF in dorsal horn has also been reported in rats with thermal hyperalgesia (Miletic and Miletic, 2002). Additionally, BDNF is also produced by glial cells of the dorsal horn; e.g. ATP-activated microglia secrete BDNF, increasing allodynia to touch (Coull et al., 2005), and microglial BDNF contributes to spinal long-term potentiation and mechanical hypersensitivity in neuropathic pain (Zhou et al., 2011).

Interestingly, expression of the tropomyosin kinase B (TrkB) receptor for BDNF is also upregulated in the spinal dorsal horn of rats with inflammatory pain (Mannion et al., 1999) and in neuropathic mice (Narita et al., 2000). As has been pointed out, TrkB signalling is required for both the induction and maintenance of tissue and nerve injury-induced persistent pain (Wang et al., 2009), while signalling through the low-affinity receptor p75 mainly contributes to neuronal and glial cell damage, axonal degeneration and dysfunction during injury, and cellular stress (Ibáñez and Simi, 2012). Binding of BDNF to the TrkB receptor cytoplasmic region promotes the activation of the signalling pathways RAS-ERK, P13K-Akt and PLCγ1-PKC (Khan and Smith, 2015). In adult neurons, activation of these pathways regulates neural response and synaptic function in the dorsal horn output neurons through a variety of neuroplasticity mechanisms (Smith, 2014), including increased phosphorylation of NMDA receptor subunits NR1 (Slack et al., 2004; Liu et al., 2015) and NR2B (Ding et al., 2015), leading to the induction of late, protein-dependent long-term potentiation (LTP) in dorsal horn neurons (Zhou et al., 2008; Liu and Zhou, 2015). As a whole, these neuroplastic changes could mechanistically explain BDNF-mediated central sensitization and the related development of chronic pain. Nevertheless, because intrathecally administered BDNF has about a 1-h half-life in the rat (Soderquist et al., 2009), the fact that the TrkB receptor antagonist cyclotraxine-B readily reverses an already established process of hyperalgesia/allodynia induced by BDNF injected 1 week before (Constandil et al., 2012; M'Dahoma et al., 2015) suggests the interesting possibility that autogenerated endogenous BDNF could be involved in such a late effect of the TrkB antagonist.

Therefore, using the mechanical hyperalgesia caused by intrathecal administration of BDNF as a model of persistent pain we investigated, in combination with pharmacological tools, to what extent BDNF by itself participates in the maintenance of such enduring hyperalgesia, as well as the downstream involvement of NMDA receptors in the behavioural expression of the hyperalgesic response.

2. Methods

2.1 Animals

Adult male Sprague–Dawley rats (250–350 g), were kept under 12:12 h light:dark cycle, starting at 8:00 AM and food and water *ad libitum*. All experiments were performed according to the Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and under the Guide for Care and Use of Laboratory Animals (National Research Council, 2011). The experimental designs were approved by the Bioethics Committee of the University of Santiago of Chile, certificate number C-598. All the studies were performed with a double-blind design.

2.2 Experimental design and drug injection

Using algesimetric testing, we studied if the mechanical hyperalgesia induced by BDNF was influenced by NMDA receptor blockade with ketamine. NMDA receptors were antagonized with 10% (w/v) ketamine solution (Imalgene® 1000) administered with an Alzet® mini-osmotic pump model 1007D (DURECT Corporation, Cupertino, CA, USA). The osmotic pumps were loaded with 100 µL of ketamine or saline (NaCl 0.9%) and were aseptically implanted in the subcutaneous tissue of the dorsal region of the anaesthetized rat (isoflurane 3%) through a small

incision in the skin. This Alzet pump releases 0.5 ± 0.1 μL/h of saline or ketamine solution for 7 days (meaning 1.2 mg ketamine/day/rat), a daily ketamine amount that is not expected to induce psychomotor effects (Alvarez et al., 2003). BDNF (3 ng/10 μL) dissolved in artificial cerebrospinal fluid (ACSF: NaCl 120 mM, $MgCl_2$ 1 mM, NaH_2PO4 1.25 mM, KCl 3.1 mM, $CaCl_2$ 1 mM, Glucose 30 mM, $NaHCO_3$, 24 mM) or ACSF alone (10 μL) injection was administered intrathecally at time zero (3 days after inserting the ketamine pump) into the subarachnoid space between the L5 and L6 vertebrae (Mestre et al., 1994). The procedure was performed using a Hamilton syringe and a hypodermic needle of 26G × 1.2″. Entry into the subarachnoid space was evidenced by a slight movement of the rat tail, which is the result of a mechanical stimulation of cauda equina nerves by the needle passage. Labelling of groups formed is shown in the Results section (subheading 3.1), and in the legends of the figures. We also studied whether BDNF-induced hyperalgesia could be reverted with the TrkB antagonist cyclotraxine-B (a single i.p. dose of 40 mg/kg administered 4 days after the BDNF i.t. injection), as well as whether the glial inhibitor propentofylline microinjected daily for seven consecutive days (10 μg i.t. dissolved in ACSF), from day -3 to day 4, with respect to administration of BDNF at time zero) could influence BDNF-induced hyperalgesia.

2.3 Mechanical nociception

The algesimetric evaluation was performed by measuring the paw pressure threshold using an analgesiometer (model 37215, Ugo Basile, Italy). Mechanical nociceptive testing consisted of applying an increasing pressure with a conical piece on the hind paw, up to the rat produced a limb withdrawal reflex, as described by Randall and Selitto (1957). This value, in grams, was recorded as a nociceptive threshold. A cut-off value of 600 g stimulation was used to avoid damage the animal's paw. Algesimetric scores were obtained before to implant the osmotic pump (day -3), just before to intrathecal BDNF/ACSF injection (day 0), and after 3, 7, 10 and 14 days of the BDNF/ACSF injection. The groups were comprised of six animals.

2.4 Locomotor patterns and activity, food and water consumption

The locomotor and motor activity patterns were evaluated using the Laboratory Animal Behavior Observation Registration and Analysis System (LABORAS, Metris, Netherlands) in the animals implanted with the 7-day delivery mini-osmotic pump loaded with ketamine or saline. Cumulated time spending locomotion (in min), maximum speed (in m/s), immobility time (in h) and number of rearings were evaluated continuously during a 15-h period (between 19:00 p.m. and 10:00 a.m., in a 12-h light/dark cycle with lights on at 08:00 a.m.) at days -3 and 0 (pre-pump scores), at day 4 and 7 (under-pump scores) and at days 10 and 14 (post-pump scores). Additionally, food and water consumption was measured in the same period on the same days. All measurements included six animals per group.

2.5 Data analysis

The results were expressed as means ± standard deviation (SD). Statistical analyses were made with the statistical software GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA), using the Kruskal–Wallis test for intragroup statistics followed by the *post-hoc* Dunn's multiple comparisons test, and using the Mann–Whitney test for intergroup statistics. In all comparisons, statistical significance was established at $p < 0.05$. Nonparametric statistics was chosen because animal groups were composed of six rats, a sample size that usually does not allow to demonstrate a Gaussian distribution of data group.

3. Results

3.1 NMDA receptor blockade depressed BDNF-induced hyperalgesia in the rat, but the hyperalgesia emerged after depletion of the ketamine pump

To study the involvement of NMDA receptors in the nociceptive response induced by TrkB activation, 7-day delivery ALZET pumps loaded with either a sub-analgesic dose of ketamine or saline were subcutaneously implanted in the rats. BDNF or ACSF was intrathecally administered 3 days after pump implantation, thus generating four groups of experimental animals: saline-ACSF, saline-BDNF, ketamine-ACSF, and ketamine-BDNF. Nociception was assessed in all groups by using paw pressure testing.

Control animals belonging to the saline-ACSF group did not show changes in the nociceptive threshold along time over the 14 days studied, evaluated by the paw pressure test ($p > 0.05$, Kruskal–Wallis test intragroup statistics). In contrast, animals

of the saline-BDNF group exhibited a significant reduction in the nociceptive threshold (e.g. mechanical hyperalgesia), from day 3 post-injection of BDNF until the day 14 ($p < 0.05$, Kruskal–Wallis test intragroup statistics; $*p < 0.05$, when comparing intragroup scores at days 3, 7, 10 and 14 against the pre-pump score at day -3 using *post-hoc* Dunn's multiple comparisons test; Fig. 1).

The ketamine-ACSF group did not show significant change in the nociceptive threshold along the study, as compared to the pre-ketamine score ($p > 0.05$, Kruskal–Wallis test intragroup statistics), indicating that the chronic ketamine treatment by its own did not modify the nociceptive threshold (Fig. 1). Nevertheless, the ketamine-BNDF group did not exhibit changes in the nociceptive threshold in the week following the administration of BDNF ($p > 0.05$, when comparing intragroup post-pump scores at days 3 and 7 against the under-pump T-3 score, Kruskal–Wallis intragroup statistics followed by *post-hoc* Dunn's multiple comparisons test), but a significant reduction in the nociceptive threshold was observed in this group on days 10 and 14 after BDNF injection, that is when ketamine was no longer available from the pump ($p < 0.05$, Kruskal–Wallis test intragroup statistics; $*p < 0.05$, when comparing intragroup post-pump scores at days 10 and 14 against the under-pump T-3 score using *post-hoc* Dunn's multiple comparisons test; Fig. 1). This latter experimental series shows that BDNF was not able to generate mechanical hyperalgesia when ketamine was available from the pump but, once the pump was empty, a hyperalgesic response to paw pressure stimulation emerged.

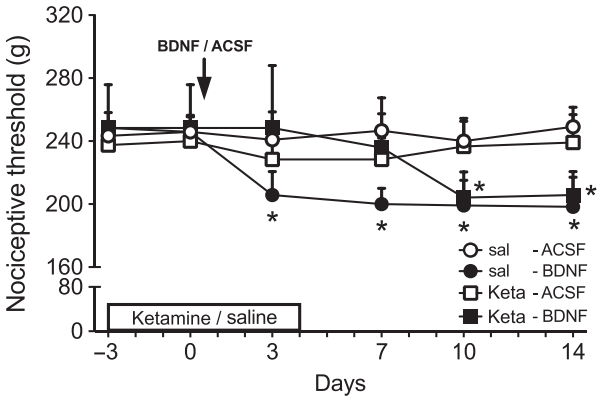

Figure 1 Effect of intrathecal BDNF in the rat nociceptive threshold to paw pressure, with and without ketamine blockade of NMDA receptors. Ketamine or saline was administered s.c. between days -3 and 4 (7-day duration mini-osmotic pump, as indicated by the horizontal bar), and BDNF or ACSF was injected i.t. at time zero (arrow). Each symbol represents the mean ± SD of nociceptive threshold scores (in g). $N = 6$ animals per group. $*p < 0.05$, as indicated by intragroup statistics comparing scores obtained at each observation day versus the corresponding pre-pump score at day-3 (Kruskal–Wallis test followed by the Dunn's multiple comparison test).

3.2 A single dose of the TrkB receptor antagonist cyclotraxine-B lastingly suppressed BDNF-induced hyperalgesia in rat

An additional ketamine-BNDF group was subjected to a single subcutaneous (s.c.) injection of the BDNF antagonist cyclotraxine-B on day 4 after BDNF i.t. (i.e. on day 7 after ketamine pump implantation, just when ketamine solution is running low), to study if the mechanical hyperalgesia emerging after ketamine pump depletion could be blocked by a single dose of the TrkB antagonist. Figure 2 shows that the late hyperalgesic response that appeared in the ketamine-BNDF group when the ketamine pump was depleted was completely prevented by cyclotraxin-B s.c. In fact, no statistical differences were found at any day when comparing the intragroup scores at days 3, 7, 10 and 14 against the pre-pump score at day-3 ($p > 0.05$, Kruskal–Wallis test intragroup statistics). This experiment indicates that a

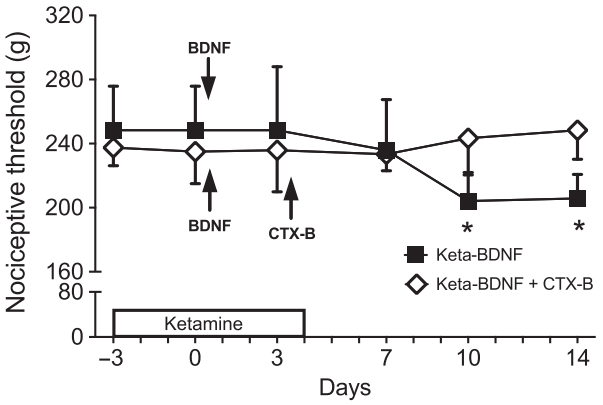

Figure 2 Effect of the TrkB receptor antagonist cyclotraxine-B on the late BDNF-induced hyperalgesic response emerging upon ketamine pump depletion. Ketamine was administered s.c. between days -3 and 4 (7-day duration mini-osmotic pump, as indicated by the horizontal bar), BDNF was injected i.t. at time zero (left arrows), and cyclotraxine-B was administered s.c. at day 4, when ketamine pump is running low (right arrow). Each symbol represents the mean ± SD of nociceptive threshold scores (in g). $N = 6$ animals per group. $*p < 0.05$, as indicated by intragroup statistics comparing scores obtained at each observation day versus the corresponding pre-pump score at day-3 (Kruskal–Wallis test followed by the Dunn's multiple comparison test). Note that the late hyperalgesic response that appeared when the ketamine pump was depleted was completely prevented by cyclotraxin-B.

TrkB ligand is involved in the late mechanical hyperalgesia, presumably spinal BDNF of glial origin.

3.3 Chronic intrathecal propentofylline treatment irreversibly depressed BDNF-induced hyperalgesia in rat

To study the involvement of a glial mediator in the nociceptive response induced by TrkB activation with BDNF, the glial inhibitor propentofylline was microinjected daily for seven consecutive days (10 μg propentofylline i.t. dissolved in ACSF, from day -3 to day 4), and BDNF was intrathecally injected at time zero.

Paw pressure testing showed that control animals (ACSF-ACSF group) did not show any change in nociceptive threshold along time over the 14 days studied ($p > 0.05$, Kruskal–Wallis test intragroup statistics), whereas the ACSF-BDNF group exhibited a significant hyperalgesia from day 3 post-BDNF injection until day 14 ($p < 0.05$, Kruskal–Wallis test intragroup statistics; $^*p < 0.05$, when comparing intragroup scores at days 3, 7, 10 and 14 against the pre-BDNF score at day -3 using *post-hoc* Dunn's multiple comparisons test; Fig. 3), thus confirming the ability of i.t. BDNF to induce mechanical hyperalgesia in the rat. Animals treated daily with i.t. propentofylline during 7 days (PPT-ACSF group) did not show significant change in the nociceptive threshold along the study ($p > 0.05$, Kruskal–Wallis test intragroup statistics), indicating that the chronic propentofylline treatment by its own did not modify the nociceptive threshold (Fig. 3). However, following intrathecal BDNF, no modification of paw pressure scores was observed in propentofylline treated animals (PPT-BDNF group) and no late hyperalgesic response emerged after finishing the propentofylline treatment ($p > 0.05$, Kruskal–Wallis test intragroup statistics). This experimental series indicates that treatment with i.t. propentofylline irreversibly inhibits i.t. BDNF-induced hyperalgesia in rats, thereby confirming the involvement of glial cells (i.e. a glial mediator) as a prerequisite for the expression of the pronociceptive effects of BDNF at the spinal cord level.

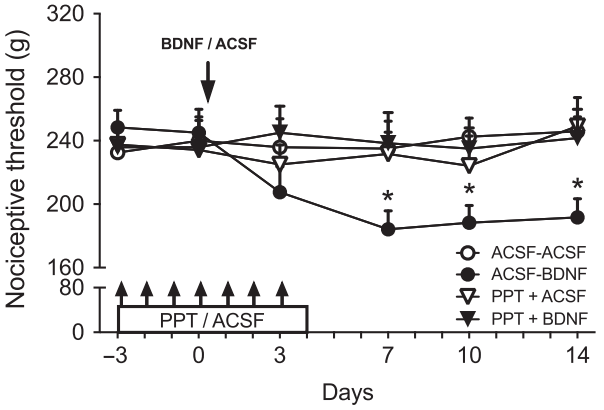

Figure 3 Effect of chronic intrathecal propentofylline treatment on the hyperalgesic response induced by intrathecal BDNF. Propentofylline or ACSF was administered s.c. between days -3 and 4 (daily microinjections for seven consecutive days, as indicated by the horizontal bar), and BDNF was injected i.t. at time zero (left arrows).Each symbol represents the mean ± SD of nociceptive threshold scores (in g). $N = 6$ animals per group. $^*p < 0.05$, as indicated by intragroup statistics comparing scores obtained at each observation day versus the corresponding pre-pump score at day-3 (Kruskal–Wallis test followed by the Dunn's multiple comparison test). Note that chronic propentofylline treatment irreversibly inhibits i.t. BDNF-induced hyperalgesia, while it had no effect on control ACSF rats.

3.4 NMDA receptors blockade neither change locomotor patterns and activity of rats, nor food consumption

Evaluation with LABORAS of rats under 7-day ketamine or saline treatment using Alzet mini-pumps (starting from day 0 until day 7), showed that there were neither significant intragroup differences ($p > 0.05$, Kruskal–Wallis test intragroup statistics) nor significant intergroup difference ($p > 0.05$, Mann–Whitney test intergroup statistics) in displacement during locomotion (Fig. 4A), the number of times engaged in locomotion (Fig. 4B), maximum speed (Fig. 4C), time of immobility (Fig. 4D) and number of rearings (Fig. 4E) at days -3 and 0 (pre-pump scores), at days 0 and 7 (under-pump scores) and at days 7 and 14 (post-pump scores). Food intake (Fig. 4F) increases steadily in both groups ($p < 0.05$, Kruskal–Wallis test intragroup statistics; $^*p < 0.05$, following *post-hoc* Dunn's multiple comparisons test), but no differences in this parameter were found between groups submitted to chronic ketamine or saline ($p > 0.05$, Mann–Whitney test intergroup statistics).

4. Discussion

The present study showed that, in rats: (1) blockade of NMDA receptors by sub-analgesic chronic ketamine treatment can prevent the mechanical hyperalgesia induced by intrathecal BDNF administration but, once ketamine is no longer available because of exhausting of the ketamine pump, a late mechanical hyperalgesia emerged; (2) emergence of this late hyperalgesic response after depletion of the ketamine

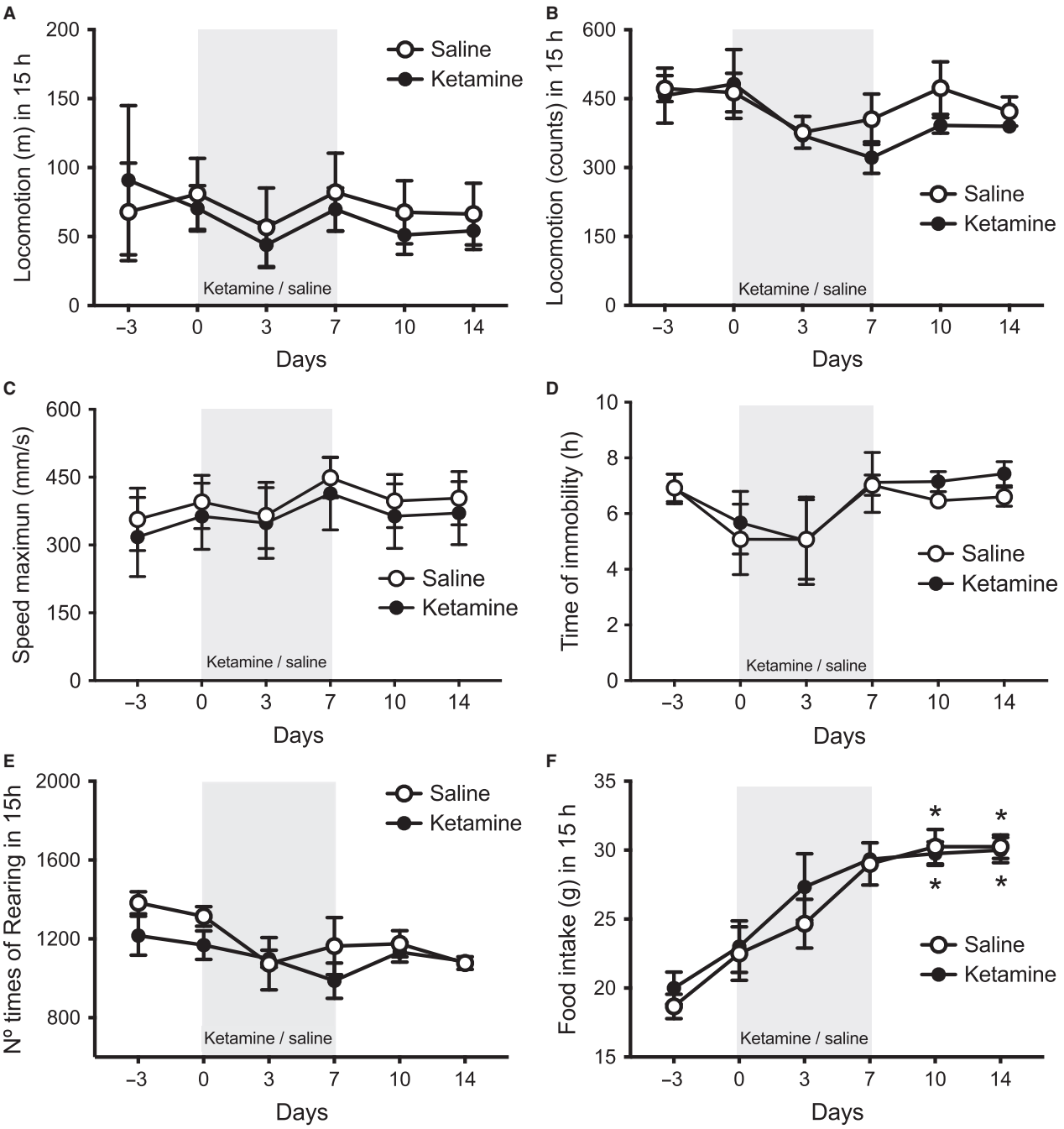

Figure 4 Effect of ketamine blockade of spinal NMDA receptors on locomotor and motor activity patterns, food and water consumption of rats. Ketamine or saline was administered s.c. for 7 days with mini-osmotic pumps, as indicated by the grey zone). (A) displacement during locomotion, (B) number of times engaged in locomotion, (C) maximum speed, (D) time of immobility, (E) number of rearings, (F) food intake. Intragroup statistics (Kruskal–Wallis test) showed that there were no changes in the parameters measured at any time in ketamine-treated and control rats (A–E), except for a steady increase in food intake (F) in both ketamine-treated and control rats (*$p < 0.05$, Kruskal–Wallis test followed by Dunn's multiple comparisons test). However, no intergroup differences were found in food intake when compared ketamine-treated and control rats each other ($p > 0.05$, Mann–Whitney test intergroup statistics) in similar time points (F).

pump can be lastingly suppressed by blockade of TrkB receptors with cyclotraxine-B; and (3) glial inhibition with chronic intrathecal propentofylline treatment irreversibly prevented the mechanical hyperalgesia induced by intrathecal BDNF administration. In addition, it was shown that chronic

ketamine neither modified locomotor patterns and motor activity of rats as measured in LABORAS software, nor food consumption of the animals.

The fact that chronic ketamine treatment prevented the mechanical hyperalgesia induced by intrathecal BDNF is consistent with previous observations indicating that spinal NMDA receptors, downstream to TrkB signalling, are importantly involved in the behavioural expression of BDNF-induced central sensitization underlying some chronic pain conditions. In this regard, it has been reported that, in the spinal cord, BDNF triggers phosphorylation of NMDA receptor subunits NR1 (Slack et al., 2004; Liu et al., 2015) and NR2B (Ding et al., 2015) leading to the induction of late, protein-dependent long-term potentiation (LTP) in dorsal horn neurons (Zhou et al., 2008; Liu and Zhou, 2015), and that these events are mechanistically associated with behavioural pain responsiveness in neuropathic experimental models of chronic pain (Geng et al., 2010; Ding et al., 2015). Nevertheless, the anti-hyperalgesic effect of ketamine on the paw pressure threshold of BDNF-injected rats was transient and reversible, as indicated by the appearance of mechanical hyperalgesia once the ketamine pump was depleted. This means that the long-lasting hyperalgesic state triggered by intrathecal BDNF, at least during the first 2 weeks, was not maintained by a NMDA-dependent central sensitization process, but by a BDNF-TrkB-dependent spinal mechanism sited upstream to NMDA receptors.

Because intrathecally administered BDNF has only about a 1-h half-life in the rat (Soderquist et al., 2009), BDNF-induced hyperalgesia is likely maintained by a yet unspecified pre-NMDA mechanisms, involving some endogenous molecule with the ability of both: (1) to bind TrkB receptors, because of the sensitivity of such enduring hyperalgesia to cyclotraxine-B; and (2) to self-regenerate, because of the long-lasting effect generated (more than a week). Although neurotrophin-4 could also bind to TrkB receptors, neurotrophin-4 appears to have no effect on activity-dependent synaptic plasticity or neuropathic pain (Heppenstall and Lewin, 2001; Yajima et al., 2002). Instead, the neurotrophin BDNF probably holds a key position as the putative unknown molecule: (1) First, BDNF can promote its own synthesis in neurons, since it has been shown that stimulation of TrkB receptors can autonomously induce delayed BDNF-mRNA expression in an activity-dependent manner in rat cortical neurons (Yasuda et al., 2007) and, in addition, BDNF binding to TrkB has proven to serve as a self-amplifying autocrine factor during axon development (Cheng et al., 2011); (2) Secondly, endogenous BDNF can also be autogenerated in microglia, because microglia can be activated by BDNF (Zhou et al., 2011) and, in turn, activated microglia can release BDNF (Coull et al., 2005). Specifically, BDNF induces sustained elevation of intracellular Ca^{2+} in rodent microglia, and this calcium rise in turn induces the phosphorylation and activation of p38 MAPK, particularly the isoform expressed by microglia (Zhou et al., 2011), and MAPK phosphorylation results in microglial cell BDNF synthesis and release (Trang et al., 2009; Yang et al., 2012). Such a positive feedback loop of autocrine BDNF from microglia causes prolonged microglia activation (Zhang et al., 2014). An alternative (but not exclusive) ATP-dependent, BDNF self-regenerating positive feedback loop could also be envisioned, because i.t. BDNF can depolarize neurons (Blum et al., 2002) leading to the release of the microglial activator ATP in the spinal cord, which in turn could activate spinal microglia (Coull et al., 2005) via P2X4 receptors to produce more BDNF (Trang et al., 2009; Beggs et al., 2012; Jin et al., 2014). However, the neural–glial interactions initiated by exogenous BDNF in the spinal cord are complex and difficult to dissect because both dorsal horn neurons (Masuda et al., 2016) and microglia (Imura et al., 2013) express the VNUT transporter and release activity-dependent ATP, together with expressing almost all of P2X and P2Y purinergic receptors; (3) Thirdly, the fact that chronic intrathecal propentofylline treatment, which is known to produce long-lasting inhibition of glial cells, irreversibly depressed the pre-NMDA mechanism mediating the BDNF-induced hyperalgesic response strongly suggests that the unknown spinal mediator of hyperalgesia was, indeed, BDNF of glial origin. Although inhibition of spinal microglia with intrathecal propentofylline is an unspecific pharmacological manipulation able to depress the production of a variety of pronociceptive microglial mediators (e.g. cytokines) in addition to BDNF, the fact that both procedures – the propentofylline treatment and the cyclotraxine-B injection – are sufficient to block independently the mechanical hyperalgesia induced by intrathecal BDNF points to the involvement of glial BDNF as the relevant glial mediator underlying the hyperalgesic response. This notion is further supported by a recent study reporting long-lasting spinal BDNF-mRNA overexpression (more than 10 days) in the lumbar enlargement of rats receiving a single i.t. injection of BDNF, together with a clear-cut increase in the density of the

microglial activation marker phospho-p38 within the dorsal horn, provided the rats were previously subjected to sustained unilateral mechanical, non-nociceptive stimulation of the hindlimb (M'Dahoma et al., 2015). In this study, it was also reported that no modification in NR2B transcription was found on day 10 following i.t. BDNF (M'Dahoma et al., 2015). This observation, together with the fact that in the present study the mechanical hyperalgesia returned after depletion of the ketamine pump, do not support the idea of a NMDA-dependent neuroplastic mechanism to explain the maintenance of hyperalgesia induced by intrathecal administration of BDNF, at least for the first 2 weeks following the BDNF i.t., but instead reinforces the possibility that the hyperalgesic response to BDNF i.t. is later maintained by a glial-like BDNF self-regenerating mechanism, upstream of the NMDA receptors.

Importance of the spinal BDNF–TrkB interaction in the triggering and maintenance of central sensitization in chronic pain animal models is receiving increased attention for the past few years. For example, intrathecal injection of both the unspecific tyrosine kinase inhibitor K252a and the BDNF scavenger TrkB-Fc prevented the injury-associated painful response in the sciatic nerve ligation neuropathic rat model (Miletic and Miletic, 2008). Similarly, intrathecal TrkB-Fc significantly attenuated the development of mechanical allodynia in neuropathic mice (Renn et al., 2011) and intrathecal administration of K252a significantly alleviated mechanical hypersensitivity in paclitaxel-treated neuropathic rats (Nakahashi et al., 2014). Finally, cyclotraxine-B partially reversed mechanical hyperalgesia in sciatic nerve ligation neuropathic rats (M'Dahoma et al., 2015). These data altogether with the present results, support the idea that the BDNF neurotrophin plays a cardinal role in physiopathological mechanisms underlying neuropathic pain, and suggest that targeting BDNF–TrkB interactions with TrkB antagonists could represent an interesting lead for the development of novel therapeutic strategies for some chronic pain conditions.

In conclusion, the present results show that activation of NMDA receptors downstream to TrkB signalling is essential for behavioural expression of the mechanical hyperalgesia induced by intrathecal BDNF, but maintenance of the hyperalgesia depends mainly on a self-regenerating mechanism of glial BDNF rather than from a NMDA receptor-dependent form of neuroplasticity. This suggests that TrkB antagonists could represent an interesting lead for the development of novel therapeutic strategies for some chronic pain conditions.

Author contributions

J.L.M. and L.C. designed research; J.L.M., D.G. and T.P. performed research; J.L.M., D.G. and L.V. contributed to acquisition of data; J.L.M., A.H., T.P., L.V. and L.C. analysed data; J.L.M., A.H. and L.C. wrote the paper.

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