THE MEDULLARY DORSAL RETICULAR NUCLEUS AS A RELAY FOR DESCENDING PRONOCICEPTION INDUCED BY THE mGluR5 IN THE RAT INFRALIMBIC CORTEX

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Abstract—Metabotropic glutamate receptor 5 (mGluR5) activation in the infralimbic cortex (IL) induces pronociceptive behavior in healthy and monoarthritic rats. Here we studied whether the medullary dorsal reticular nucleus (DRt) and the spinal TRPV1 are mediating the IL/mGluR5-induced spinal pronociception and whether the facilitation of pain behavior is correlated with changes in spinal dorsal horn neuron activity. For drug administrations, all animals had a cannula in the IL as well as a cannula in the DRt or an intrathecal catheter. Heat-evoked paw withdrawal was used to assess pain behavior in awake animals. Spontaneous and heat-evoked discharge rates of single DRt neurons or spinal dorsal horn wide-dynamic range (WDR) and nociceptive-specific (NS) neurons were evaluated in lightly anesthetized animals. Activation of the IL/mGluR5 facilitated nociceptive behavior in both healthy and monoarthritic animals, and this effect was blocked by lidocaine or GABA receptor agonists in the DRt. IL/mGluR5 activation increased spontaneous and heat–evoked DRt discharge rates in healthy but not monoarthritic rats. In the spinal dorsal horn, IL/mGluR5 activation increased spontaneous activity of WDR neurons in healthy animals only, whereas heat-evoked responses of WDR and NS neurons were increased in both experimental groups. Intrathecally administered TRPV1 antagonist prevented the IL/mGluR5-induced pronociception in both healthy and monoarthritic rats. The results suggest that the DRt is involved in relaying the IL/mGluR5-induced spinal pronociception in healthy control but not monoarthritic animals. Spinally, the IL/mGluR5-induced behavioral heat hyperalgesia is mediated by TRPV1 and associated with facilitated heat-evoked responses of WDR and NS neurons. © 2017 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: infralimbic cortex, metabotropic glutamate receptor 5, experimental monoarthritis, pronociception, dorsal reticular nucleus, spinal TRPV1.

INTRODUCTION

Increased nociceptive sensitivity in chronic pain results from sensitization of peripheral and central pathways (Schaible et al., 2002). Central sensitization translates as hyperalgesia and allodynia resulting in hypersensitivity of nociceptive neurons to suprathreshold and previously subthreshold stimuli, respectively. Consequently, a pain facilitatory state arises due to changes in brain activity that can be detected through electrophysiological and imaging techniques (Apkarian, 2004; Metz et al., 2009; Woolf, 2011). These secondary neuroplastic changes, fundamental for the establishment and maintenance of chronic pain, occur throughout the pain matrix and range from frontal areas, such as the medial prefrontal cortex (mPFC), to caudal modulatory regions, such as the rostral ventromedial medulla (RVM) and the dorsal reticular nucleus (DRt) (Pertovaara et al., 1996; Ossipov et al., 2000; Lima and Almeida, 2002; Heinricher et al., 2009; Baron et al., 2013).

The DRt is a pain modulatory brain region better known for its facilitatory action (Almeida et al., 1996, 1999; Lima and Almeida, 2002; Martins et al., 2013). Electrolytic lesion or chemical block of the DRt increases tail-flick latency in healthy animals (Almeida et al., 1996) and decreases pain behavior in the formalin test (Almeida et al., 1999). DRt neurons receive afferent projections from spinal neurons (Almeida et al., 1993, 2000) activated by noxious stimulation (Almeida and Lima, 1997; Dugast et al., 2003). Its receptive fields

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Abbreviations: AMG, AMG 9810 (TRPV1 antagonist); ANOVA, analysis of variance; ARTH, kaolin/carrageenan induced monoarthritis; CHPG, (RS)-2-Chloro-5-hydroxyglycine (mGluR5 agonist); CNS, central nervous system; DRt, dorsal reticular nucleus; GABA, γ-aminobutyric acid; i.p., intraperitoneal; i.t., intrathecal; IL, infralimbic cortex; LIDO, lidocaine; mGluR5, metabotropic glutamate receptor 5; MPEP, 6-Methyl-2-(phenylethynyl)pyridine; mPFC, medial prefrontal cortex; MUSC, muscimol; NS, nociceptive-specific; ONDAN, ondansetron hydrochloride (5-HT3R antagonist); PAG, periaqueductal gray area; PFC, prefrontal cortex; PSTH, peristimulus time histogram; PWL, (heat-evoked hind) paw withdrawal latency; RVM, rostral ventromedial medulla; SAL, saline; SEM, standard error of the mean; TRPV1, transient receptor potential cation channel subfamily V member 1; WDR, wide-dynamic range.

encompass the entire body surface and are activated exclusively or preferentially by noxious stimuli (Villanueva et al., 1988, 1996). The DRt projects to several brain areas implicated in pain processing and modulation (Leite-Almeida et al., 2006) and also targets spinal dorsal horn neurons located in laminae I and IV-VI (Almeida et al., 1993, 2000; Tavares and Lima, 1994; Villanueva et al., 1995). This descending pathway seems to be directly involved in nociceptive facilitation in healthy (Almeida et al., 1999; Zhang et al., 2005; Amorim et al., 2015) and neuropathic animals (Sotgiu et al., 2008).

The DRt receives axonal projections from many brain regions implicated in pain processing and modulation (Almeida et al., 2002), including the infralimbic cortex (IL), a region shown to induce heat hyperalgesia in rodents after local activation of metabotropic glutamate receptor 5 (mGluR5) (David-Pereira et al., 2016). Conversely, mGluR5 block in the IL leads to heat analgesia, an effect observed only in rats with prolonged inflammatory pain, suggesting that experimental monoarthritis may lead to neuroplastic changes in the IL (David-Pereira et al., 2016). However, not much is known about the functional role of descending pathways between the IL and various spinally-projecting pain modulatory regions/relays, such as the DRt. In this work, electrophysiological and behavioral techniques were used to study whether the DRt is involved in relaying the descending pronociceptive effect induced by activation of the mGluR5 in IL in monoarthritic as well as healthy control animals.

We also attempted to assess which receptor mediates the IL/mGluR5-induced descending pronociceptive effect at the spinal cord level. In particular, we tested the potential involvement of the pronociceptive transient receptor potential cation channel subfamily V member 1 (TRPV1), best known for its important role in transduction of noxious signals in the peripheral terminals of primary afferent nociceptors (Caterina et al., 2000). In the spinal dorsal horn, TRPV1 is expressed on central terminals of nociceptive nerve fibers where it amplifies transmission on excitatory interneurons, and postsynaptically where it facilitates responses of presumed pain-relay neurons (Valtschanoff et al., 2001; Zhou et al., 2009). Earlier, it has been shown that pharmacological blocking of the spinal TRPV1 attenuates pain-related behavior in various arthritis models (Cui et al., 2006), but the role of spinal TRPV1 in descending facilitation of nociception is not yet known. Here we administered intrathecally a selective TRPV1 antagonist to assess whether spinal TRPV1 is involved in mediating the IL/mGluR5-induced descending pronociceptive effect in monoarthritic and/or healthy control animals.

EXPERIMENTAL PROCEDURES

Animals, anesthetics and ethical issues

The experiments were performed in adult Wistar Han male rats with 200–300 g (Envigo, Blackthorn, UK). The experimental protocol was approved by the Experimental Animal Ethics Committee of the Provincial Government of Southern Finland (Hämeenlinna, Finland; permission # ESAVI/7863/04.10.07/2013) and followed the European Community Council Directive 2010/63/EU concerning the use of animals for scientific purposes. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

For all surgical and electrophysiological procedures, anesthesia was induced through the intraperitoneal (i.p.) administration of sodium pentobarbitone (60 mg/kg; Mebunat, OrionPharma, Espoo, Finland). Anesthesia level was assessed by observation of pupil size, general muscle tone and by assessing withdrawal responses to noxious pinching; anesthesia was maintained by administering additional doses of sodium pentobarbitone (15–20 mg/kg) as required. During the electrophysiological experiments, anesthesia was kept at a level at which no spontaneous movement of extremities was observed.

When performing the surgical procedures for insertion of chronic guide cannulas and/or an intrathecal catheter, anesthesia was induced and maintained as described above. After completion of the surgical procedure, animals were monitored until they were fully recovered. To prevent post-operative pain, animals were treated subcutaneously with 0.01 mg/kg of buprenorphine (Temgesic, Reckitt Benckiser, Berkshire, UK) twice a day for 3 days, and were allowed to recover for at least a week before the beginning of the behavioral experiments.

After the completion of the experiments, animals received a lethal dose of sodium pentobarbitone and the brains were removed for histological confirmation of cannula and/or electrode placement.

Induction of monoarthritis

Induction of monoarthritis (ARTH) was performed 28 days before the beginning of the experiments, as described in detail elsewhere (Pinto-Ribeiro et al., 2013). Briefly, 3% kaolin and 3% carrageenan (Sigma–Aldrich, St. Louis, MO, USA) were dissolved in distilled water and injected intrasynoviallly in the right knee joint at a volume of 0.1 mL. Mechanical hyperalgesia begins development a few hours after surgery and can be observed up to 8 weeks (Radhakrishnan et al., 2003; Amorim et al., 2014). ARTH development was verified 1–2 h prior to each behavioral/electrophysiological session in each animal. Only rats that audibly vocalized during each one of the five flexion–extension movements of the knee joint were considered to be monoarthritic and included in the ARTH group (Amorim et al., 2014). Saline solution (0.1 mL) was injected intrasynovially in the right knee joint of control animals (SHAM). SHAM animals did not vocalize during any of the five consecutive flexion–extension movements of the knee joint.

Procedures for intracerebral microinjections

For intracerebral drug administration, cannulas were implanted as described elsewhere (Pinto-Ribeiro et al., 2011). Rats were placed in a standard stereotaxic frame, the skull was exposed, one or two holes drilled and sterilized stainless-steel guide cannulas (26 gauge; Plastics

One, Roanoke, VA, USA) were implanted in the brain. The coordinates in this and other sections refer to the atlas of Paxinos and Watson (1986). The tip of the guide cannula was positioned 1 mm above the right IL [2.76 mm frontal to bregma; 0.6 mm lateral to midline; depth 4.2 mm (Fig. 1A, C–G)] and the right DRt [−14.04 mm frontal to bregma; 1.4 mm lateral to midline; depth 8.6 mm (Fig. 1B, H–L)], fixed to the skull with screws and dental acrylic cement, and the skin sutured around it. A dummy cannula (Plastics One) was inserted into the guide cannula to prevent contamination.

Test drugs were administered through a 33-gauge injection cannula (Plastics One) protruding 1 mm beyond the tip of the guide cannula and connected to a 5.0-μL Hamilton syringe by a polyethylene catheter (PE-10; Plastics One). The injection volume was 0.5 μL, with an expected drug injection spread within the brain of 1 mm in diameter (Myers, 1966). The efficacy of injection was monitored by noting the movement of a small air bubble through the tubing. The minimum duration for drug microinjection was of at least 20 s and the injection cannula was left in place for an additional 30 s to minimize drug solution return through the injection cannula.

Procedures for intrathecal injections

For spinal cord drug delivery at the lumbar level, intrathecal (i.t.) catheters (Intramedic PE-10, Becton Dickinson and Company, Sparks, MD, USA) were implanted as originally described by Størkson and colleagues (1996). The following day, the correct physiological placement of the catheter was confirmed by administering lidocaine (LIDO; 10 μL, 4%; OrionPharma, Espoo, Finland) with a 50-μL Hamilton syringe (Hamilton Company, Bonaduz, Switzerland). Only those rats that presented no motor impairment before LIDO injection but had bilateral paralysis of their hind limbs after i.t. administration of LIDO were used in further studies. After the test, animals where monitored until they regained motor control of their hind limbs. When administering the studied drugs i.t., the volume of drug injections was 10 μL.

Drugs

(RS)-2-chloro-5-hydroxyglycine (CHPG; mGluR5 agonist; Tocris, Bristol, UK), 6-Methyl-2-(phenylethynyl)pyridine (MPEP; mGluR5 antagonist; Tocris, Bristol, UK), 5-aminomethyl-3-hydroxyisoxazole (Muscimol – MUSC; GABA$_A$ receptor agonist; Tocris, Bristol, UK) and γ-aminobutyric acid (GABA, Tocris; Bristol, UK) solutions for intracerebral drug injection were prepared with sterilized saline solution 0.9% (B. Braun Oy, Espoo, Finland; pH 7.2). (2E)-N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-3-[4-(1,1-dimethylethyl)phenyl]-2-propenamide (AMG 9810; TRPV1 antagonist; Tocris, Bristol, UK) was dissolved in a solution of 5% ethanol + 5% Tween-80. LIDO (4%) was acquired as a solution (Orion). Previous studies showed that an intracerebral dose of 50 nmol of CHPG (Ansah et al., 2009; David-Pereira et al., 2016) and 50 nmol of MPEP (David-Pereira et al., 2016) are effective in activating/inhibiting mGluR5 in the rat and that intracerebral doses of 30 ng of MUSC and

50 nmol of GABA were effective in activating GABA receptors (Frye et al., 1983; Lacerda et al., 2003). An intrathecal dose of at least 15 μg of AMG 9810 (AMG) was shown to reverse mechanical and thermal hyperalgesia in a rat model of inflammatory pain (Yu et al., 2008). Alterations in nociceptive behavior were evaluated at fixed time points (Fig. 2) until the drug effect was no longer observed. The behavioral and electrophysiological results presented are for the peak effect of drugs, which was determined to be 30 min after drug injection. Control injections were performed with the respective vehicle (VEH) solutions.

BEHAVIORAL ASSESSMENT OF NOCICEPTION

Hargreaves model

Rats were habituated to the experimental conditions by performing daily handling sessions with the experimenter and by allowing them to spend 1 h daily in the testing room and apparatus during the week preceding any testing. Nociception in unanesthetized animals was determined by measuring hind paw withdrawal latency (PWL) following radiant heat stimulation (Hargreaves test; Plantar Test Device Model 37370, Ugo Basile, Varese, Italy). In each behavioral session, PWL was assessed before and at fixed intervals following intracerebral and/or i.t. drug administration (Fig. 2). At each time point, the measurements were repeated twice at an interval of 1 min. The mean of these values was used in further calculations. To avoid any damage to the skin, the cut-off time for radiant-heat exposure was set at 15 s.

Skin temperature

The temperature of the hind paws was measured before each PWL measurement by placing a contact thermode on the plantar skin of each hind paw (Physitemp, Model BAT-12, Physitemp Instruments Inc., Clifton, NJ, USA). This measurement was performed to exclude a drug-induced change in the skin temperature as a confounding factor when assessing radiant heat-induced response latencies (Luukko et al., 1994).

ELECTROPHYSIOLOGICAL RECORDINGS

DRt recordings

Single-unit recordings of DRt neurons were performed under sodium pentobarbitone anesthesia. Animals were breathing spontaneously and the body temperature was maintained within physiological range using a warming blanket.

Animals were placed in a standard stereotaxic apparatus, the skull was exposed and holes were drilled to allow the placement of a guide cannula in the IL (2.76 mm frontal to bregma; 0.6 mm lateral to midline; depth 4.2 mm; Fig. 1A, C–G) and a recording electrode in the DRt (14.04 mm frontal to bregma; 1.4 mm lateral to midline; depth 8.6 mm; Fig. 1B, H–L). Single neuron activity was recorded with lacquer-coated tungsten electrodes (impedance 3–10 MΩ at 1KHz; FCH Inc., Bowdoin, ME, USA).
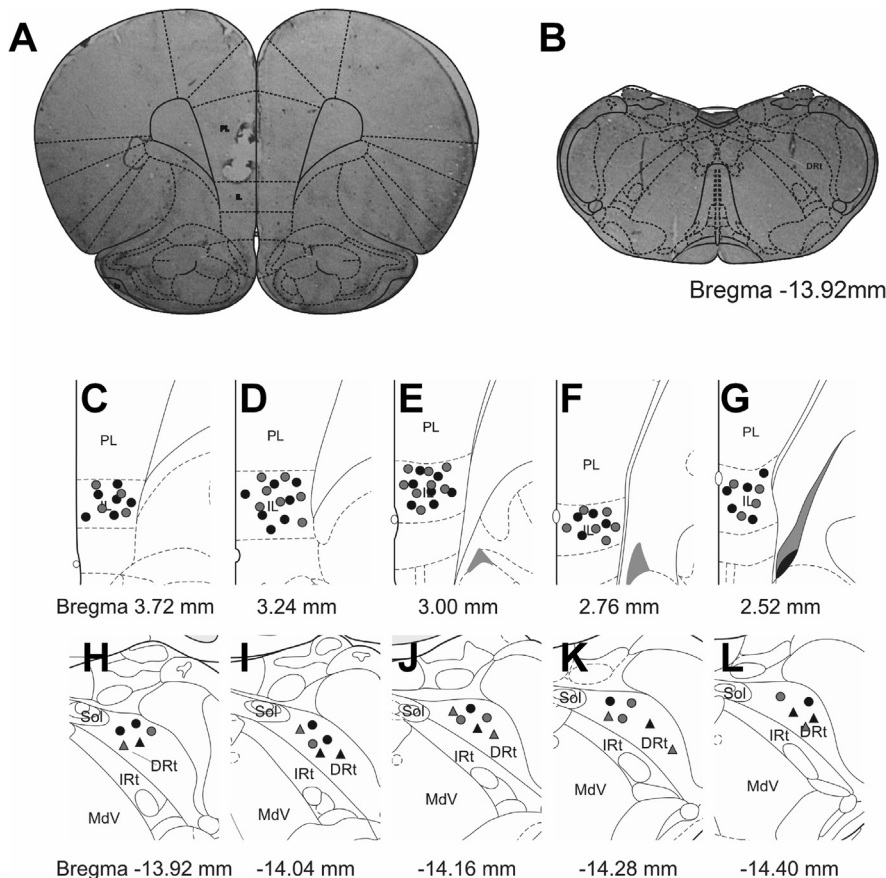
Fig. 1. Anatomical confirmation of drug injection and neuronal recordings. (A) Photomicrograph of an example of the cannula placement in the infralimbic cortex (IL) of the rat brain (AP: +3.72 mm from bregma) superimposed with the appropriate legend from Paxinos and Watson (2007) stereotaxic atlas. (B) Photomicrograph of an example of the recording site in the medullary dorsal reticular (DRt) of the rat brain (AP: −13.92 mm from bregma) superimposed with the appropriate legend from Paxinos and Watson (2007) stereotaxic atlas. (C-G) Schematic representation of other injection sites in the IL (C: +3.72 mm, D: +3.24 mm, E: +3.00 mm, F: +2.76 mm; G: +2.52 mm) in SHAM (black) and ARTH animals (gray). (H-L) Schematic representation of other injection (circles) or recording (triangles) sites in the DRt (H: −13.92 mm, I: −14.04 mm, J: −14.16 mm, K: −14.28 mm; L: −14.40 mm) in SHAM (black) and ARTH animals (gray). DRt – medullary dorsal reticular nucleus; IL – infralimbic cortex; IRt – intermediate reticular nucleus; MdV – medullary ventral reticular nucleus; PL – prelimbic cortex; Sol – nucleus of the solitary tract.

To search for DRt neurons, the response to a noxious heat stimulus applied to the plantar skin of the right hind paw was used (54 °C for 10 s; LTS-3 Stimulator, Thermal Devices Inc., Golden Valley, MN, USA). A piezoceramic movement detector (Siemens Elema Ab, Solna, Sweden) was taped to the skin of a flexor muscle in the hind limb to allow correlating the changes in the activity of DRt cells with withdrawal responses.

The evaluation of the response of DRt cells to peripheral noxious stimulation consisted of the following assessments: (i) spontaneous activity (first 20 s of recording without any stimulation); (ii) response to noxious heating of the hind paw (10 s); and (iii) latency of the heat-induced limb-reflex (time from the start of the heat stimulus to the first movement of the hind limb).

The signals from the recordings were amplified and filtered by using standard techniques. Data sampling and spike sorting were performed with a computer connected to a CED Micro 1401 interface and using Spike 2 software (Cambridge Electronic Design, Cambridge, UK). Multiple spikes were isolated based on spike shape parameters from the neuronal signals using the spike shape template functions in Spike2. To ensure the same neurons were evaluated before and after drug injection, the template generated in the first recording was used for spike sorting in all consecutive recordings.

Latency of DRt neuronal response to electric stimulation in the IL

To have an estimate of the time that it takes from activation of the IL to the activation of the DRt, the latency of DRt neuron response to electric stimulation of the IL was determined in two healthy control animals. For this purpose, recording and characterization of DRt neurons was performed as described in the above section. Additionally, a concentric bipolar stimulation electrode (SS80SNE-100, MicroProbes, Gaithersburg, MD, USA) was positioned in the right IL (2.76 mm frontal to bregma; 0.6 mm lateral to midline; depth 5.0 mm). After finding a neuron in the DRt that gave an excitatory response to noxious heat stimulation, single electrical stimuli (square pulses of 0.3-ms duration) were delivered in the IL using a constant-current stimulator (PSIU6 and Grass S88, Grass Instruments, Quincy, MA, USA). For assessment of latency and latency variation, a series of 10 stimuli at the intensity of 10.0 mA (real stimulus) or 0.0 mA (fake stimulus) were delivered consecutively at 2-s intervals. Peristimulus time histograms (PSTHs) were constructed from successive stimulation trials with 2-ms bins separately in the real and fake stimulus condition. PSTHs were normalized to give firing probability (spikes/bin). Based on earlier studies on corticofugal neurons, response of the DRt neurons was classified as a short latency response if the first impulse evoked by a real stimulus occurred in ≥20% of cases within 20 ms (Doig et al., 2014). In the present study, however, time window 0 ms – 9.5 ms could not be analyzed due to stimulus artifact produced by the currently used high stimulus intensity. Therefore, the response occurring between 9.5 ms and 20 ms was considered a short latency response in this study. Another criterion for possibly monosynaptic short latency response was that within 20 ms there was a histogram peak that had > 3 standard deviations higher firing probability in the real than the corresponding fake stimulus condition (Doig et al., 2014).
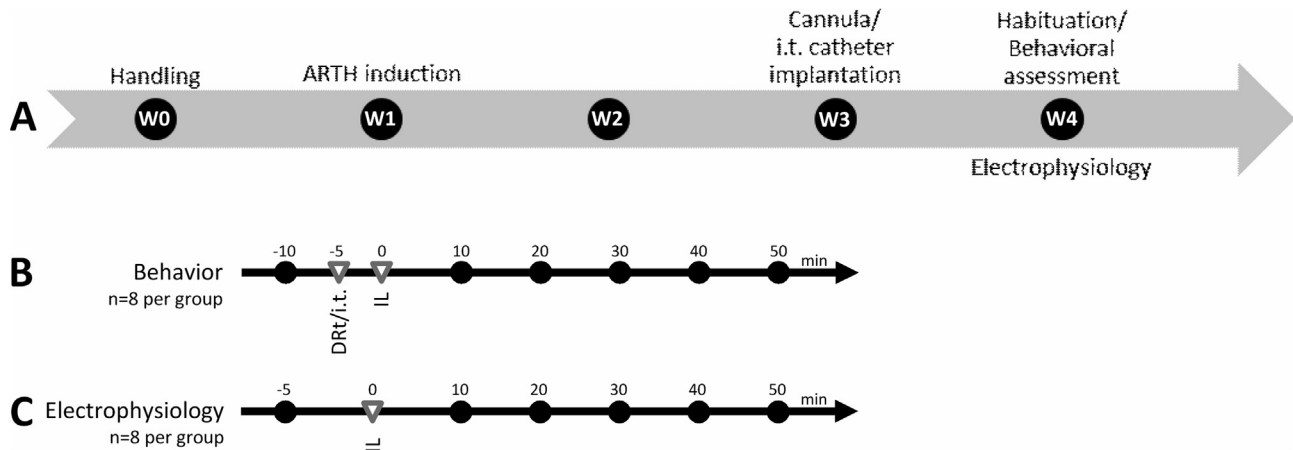
Fig. 2. Schematic representation of the experimental design timeline. (A) Rats were habituated to the laboratory and the experimenter for 5 days. After habituation, animals belonging to the monoarthritic (ARTH) group received an intra-synovial injection of 3% kaolin/carrageenan while control (SHAM) animals received an intra-synovial injection of saline solution. Two weeks after experimental monoarthritis induction, animals were implanted with a guide cannula in the infralimbic cortex (IL). After recovery (one week), rats were trained in the paw-withdrawal apparatus. (B) Behavioral testing was performed at the same time points for all the drug combinations. DRt/i.t. drug administration was performed 5 min before IL microinjection. (C) Electrophysiological recordings were performed at the same time points for all the drugs. DRt – dorsal reticular nucleus; IL – infralimbic cortex; i.t. – intrathecal; min – minutes; W0-4 – weeks 0 to 4.

Spinal dorsal horn neuron recordings

Single-unit recordings of spinal dorsal horn neurons were performed under sodium pentobarbitone anesthesia. Animals were breathing spontaneously and the body temperature was maintained within physiological range using a warming blanket.

With the animal deeply anesthetized, a laminectomy was performed at the level of the T12–L2 vertebrae to expose the L4-L6 segments of the spinal cord. The dura was cut and a pool of skin formed and filled with warm mineral oil (Mineral Oil, Sigma–Aldrich Finland, Helsinki, Finland) to prevent dehydration. The animal was placed in a standard stereotaxic frame and two spinal clamps, one rostral and one distal to the laminectomy, were used to stabilize the preparation. Single neuron activity was recorded as previously described using lacquer-coated tungsten electrodes (Viisanen et al., 2012).

To search for spinal dorsal horn neurons, a mechanical innocuous stimulus was applied with a brush on the plantar skin of the ipsilateral hind paw (brushing), followed by noxious heat stimulation of the plantar skin of the right hind paw (54 °C for 10 s; LTS-3 Stimulator, Thermal Devices Inc., Golden Valley, MN, USA). If the neurons responded to both innocuous brush and noxious heat stimulation, the cell was classified as wide-dynamic range (WDR) neuron; if the neuron responded to the noxious thermal stimulation but failed to respond to the innocuous brushing, it was classified as a nociceptive-specific (NS) neuron (Willis and Coggeshall, 2004). Neurons that responded exclusively to innocuous stimuli were not further considered in this study. Only neurons that were considered to be in the spinal dorsal horn according to the recording depth from the cord surface (< 1000 μm) were further analyzed.

The evaluation of the response of spinal dorsal horn cells to peripheral noxious stimulation consisted of the following assessments: (i) spontaneous activity (first 20 s of recording without any stimulation) and (ii) response to noxious heating of the hind paw (10 s).

The signals from the recordings were amplified and processed as described in the previous section, using a computer connected to a CED Micro 1401 interface and using Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

Course of the behavioral study

Animals used in the behavioral studies were sub-divided in two experimental groups: (i) animals with intracerebral cannulas in the IL and in the DRt ($n_{SHAM} = 14$; $n_{ARTH} = 15$), and (ii) animals with an intracerebral cannula in the IL and an i.t. catheter ($n_{SHAM} = 8$; $n_{ARTH} = 7$). Three weeks after ARTH induction and at least one week after guide cannula/i.t. catheter implantation, animals were habituated to the Hargreaves test apparatus as described previously. Four weeks after ARTH induction, changes in nociceptive behavior of unanesthetized animals after drug administration in the IL and DRt/spinal cord (5 min between each administration) were determined (Fig. 2A) by assessing PWL before and 10, 20, 30, 40 and 50 min following the first drug administration. Additionally, at these time points the temperature of the hind paw plantar skin was also assessed (Fig. 2B). Drug testing was randomized among animals. The interval between behavioral assessments in each rat was of at least three days. All animals were treated with all drug combinations.

COURSE OF THE ELECTROPHYSIOLOGICAL STUDY

DRt neuron recordings

Electrophysiological recordings of DRt cells were performed 4 weeks after ARTH induction ($n_{SHAM} = 7$;

n_{ARTH} = 7). Pharmacological manipulations started after the spontaneous and noxious heat-evoked activity of responding cells had been recorded. In a single session, one to five neurons could be recorded simultaneously. The same cells were recorded throughout the whole session unless the neuron stopped responding for more than one hour, in which case another recording site was searched (Fig. 2C). In each session, a microinjection of SAL, CHPG or MPEP were administered at an interval of 1.5 h between injections. In general, one or two recording sessions were performed in each animal. At the end of the electrophysiological session, an electrolytic lesion was made in the last recording site to allow posterior confirmation of the recording site(s).

Latency of the DRt response to electric IL stimulation

The latency to response of DRt neurons to electric IL stimulation was measured in a separate experiment using two healthy control rats. Only one DRt neuron giving an excitatory response to noxious heat was tested in each animal. After completing the recording session, the animal was euthanized and the recording/ stimulation sites were determined as described above.

Spinal dorsal horn neuron recordings

Electrophysiological recordings of spinal dorsal horn neurons were performed four weeks after ARTH induction (n_{SHAM} = 7; n_{ARTH} = 8). Pharmacological manipulations started after the neuron was classified as a WDR or NS cell and its spontaneous and noxious heat-evoked activity had been recorded in a baseline condition (i.e., before drug injection; Fig. 2C). In a single session, one to three neurons could be recorded simultaneously. The same cells were recorded throughout the whole session unless the neuron stopped responding for more than one hour, in which case another recording site was searched. In each session, the interval between IL microinjections of SAL and CHPG was 1.5 h. In general, one or two recording sessions were performed in each animal. Recording sites within the spinal dorsal horn were estimated based on the depth from the cord surface.

Statistics

Statistical analyses were performed using a two-way analysis of variance (ANOVA) followed by t-test with a Bonferroni correction for multiple comparison, except for comparisons between two groups, which were made using Student t-test. Analyses were performed with GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA), and $p < 0.05$ was considered to represent a significant difference. Data are presented as mean ± standard error of the mean (SEM). Both behavioral and electrophysiological data are presented as the difference (Δ) between values measured 30 min after drug administration and values measured before injection.

$$\Delta = 30 \text{ min} - (\text{baseline})$$

RESULTS

Blocking the DRt prevents heat hyperalgesia after IL/ mGluR5 activation in both SHAM and ARTH animals

The effect of CHPG, an mGluR5 agonist, in the IL, was assessed in both SHAM and ARTH animals, while the DRt and the descending pathway relaying through it were blocked through the local microinjection of LIDO, MUSC or GABA. Baseline PWLs before drug injections were not significantly different between ARTH and SHAM groups (t_{173} = 1.111, p = 0.268; Fig. 3D). Overall, LIDO microinjection altered PWL (effect of drug microinjection: $F_{3,89}$ = 42.34, $p < 0.0001$), independent of the experimental group (interaction of drug administration vs experimental group: $F_{3,89}$ = 0.171, p = 0.915). Post hoc tests indicate that administration of CHPG alone in the IL decreased PWL, LIDO alone in the DRt increased PWL, whereas the combination of CHPG in the IL and LIDO in the DRt did not alter PWL of SHAM or ARTH animals (Fig. 3A). Selectively blocking DRt neuronal activity with MUSC and GABA administration held results similar to the general inhibition with LIDO: drug microinjection altered PWL (effect of MUSC microinjection: $F_{3,104}$ = 39.89, $p < 0.0001$; effect of GABA microinjection: $F_{3,101}$ = 40.09, $p < 0.0001$), independent of the experimental group tested (effect of MUSC microinjection: $F_{3,104}$ = 0.311, p = 0.817; effect of GABA microinjection: $F_{3,101}$ = 0.165, p = 0.920). Post hoc tests indicate that the combination of CHPG in the IL and MUSC (Fig. 3B) or GABA (Fig. 3C) in the DRt did not alter the PWL of SHAM or ARTH animals.

The temperature of the plantar skin was not affected by drug administrations (effect of drug microinjection: $F_{7,99}$ = 1.213, p = 0.303), independent of the experimental group (interaction of drug administration vs experimental group: $F_{7,99}$ = 0.334, p = 0.937; Fig. 3E).

mGluR5 activation in the IL increases spontaneous and evoked activity of DRt neurons in SHAM, but not in ARTH animals

In general, DRt neurons were spontaneously active and their receptive fields covered large areas of the body including the right sided hind limb and the tail. Receptive field stimulation with a noxious thermal stimulus but not innocuous brushing of the skin produced an excitatory response in DRt neurons (Fig. 4A).

The activity of nociceptive DRt cells was recorded before and after the administration of CHPG in the IL. In ARTH animals, the baseline spontaneous activity of DRt neurons before drug administrations was significantly higher than in SHAM animals (t_{77} = 2.156, p = 0.034; Fig. 4B), whereas there were no differences in the heat-evoked baseline responses between SHAM and ARTH animals (t_{89} = 0.1085, p = 0.914; Fig. 4C). Administration of CHPG in the IL significantly changed the spontaneous activity of DRt neurons (effect of drug administration: $F_{1,69}$ = 11.08, p = 0.001); this effect varied with the experimental group (interaction between
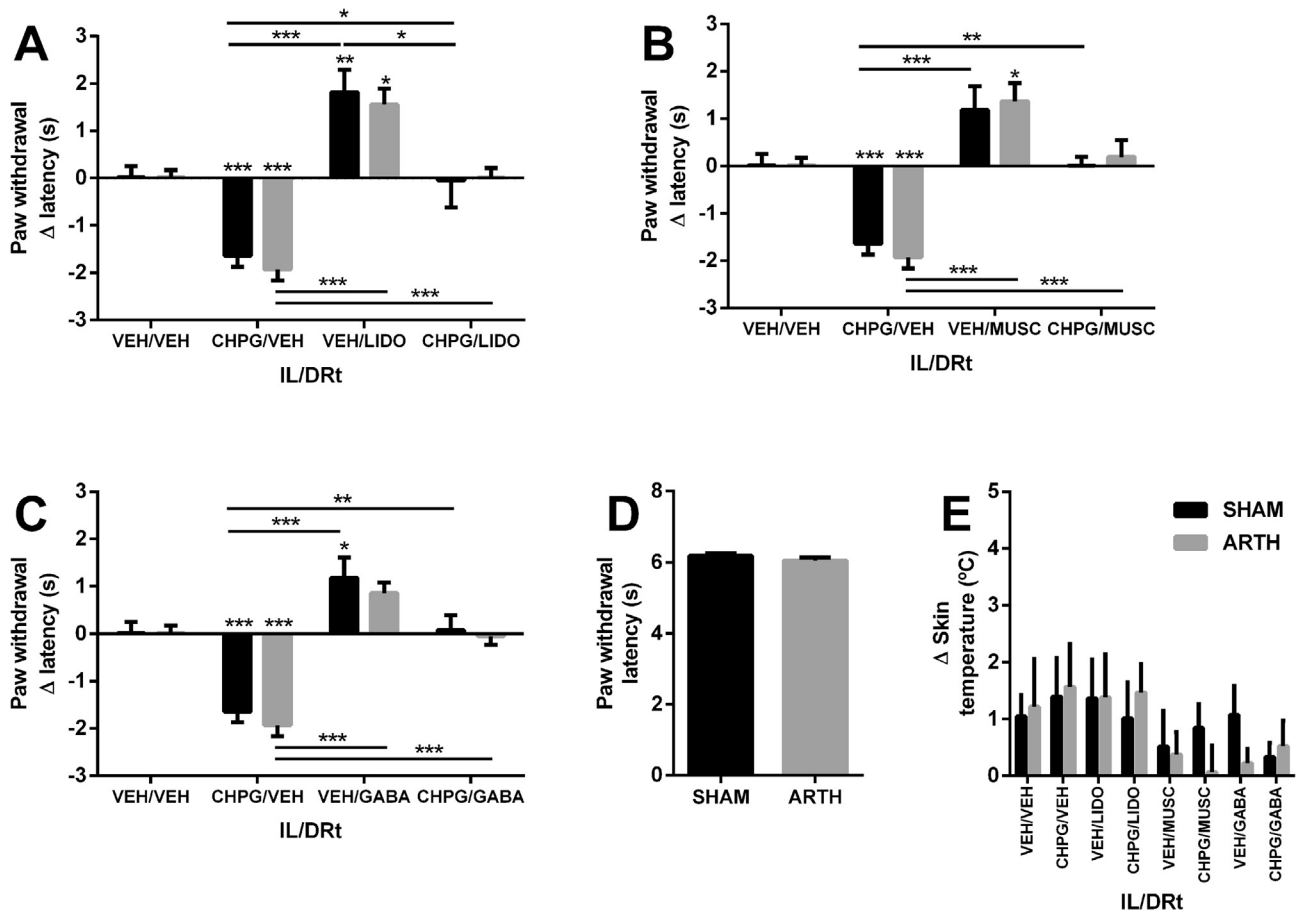
Fig. 3. (A–C) Paw withdrawal latency (PWL) variation 30 min after drug microinjection into the infralimbic cortex (IL) and dorsal reticular nucleus (DRt) of SHAM and ARTH animals. Effects of vehicle (VEH) in the IL and in the DRt; CHPG (mGluR5 agonist) in the IL and VEH in the DRt; VEH in the IL and lidocaine (LIDO)/muscimol (MUSC)/GABA in the DRt; and CHPG in the IL and LIDO/MUSC/GABA in the DRt. (D) PWL before drug microinjection in the IL and DRt in control (SHAM) and arthritic (ARTH) groups. (E) Skin temperature (°C) variation 30 min after drug injection into the IL and DRt of SHAM and ARTH animals. Δ – (PWL30 min)-(PWL-5 min). Graphs A, B, C and E show Δ mean + SEM; graph D shows mean + SEM. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. (VEH/VEH: $n_{SHAM} = 20$; $n_{ARTH} = 19$; CHPG/VEH: $n_{SHAM} = 18$; $n_{ARTH} = 15$; VEH/LIDO: $n_{SHAM} = 6$; $n_{ARTH} = 6$; CHPG/LIDO: $n_{SHAM} = 6$; $n_{ARTH} = 7$; VEH/MUSC: $n_{SHAM} = 10$; $n_{ARTH} = 10$; CHPG/MUSC: $n_{SHAM} = 10$; $n_{ARTH} = 10$; VEH/GABA: $n_{SHAM} = 9$; $n_{ARTH} = 9$; CHPG/GABA: $n_{SHAM} = 7$; $n_{ARTH} = 12$).

drug administration and experimental group: $F_{1,69} = 4.258$, $p = 0.043$). *Post hoc* tests show CHPG increased the spontaneous activity of DRt neurons in SHAM but not ARTH animals, while VEH administration did not alter spontaneous activity of DRt neurons in any of the experimental groups (Fig. 4D).

CHPG administration significantly altered heat-evoked DRt responses (effect of drug administration: $F_{1,71} = 10.48$, $p = 0.002$) and this effect varied with the experimental group (interaction between drug administration and experimental group: $F_{1,71} = 6.293$, $p = 0.014$). Similarly to what was observed for the spontaneous activity, *post hoc* tests showed that the heat-evoked DRt neuronal discharge increased in SHAM but not ARTH animals after IL administration of CHPG (Fig. 4E).

Administration of MPEP in the IL had no effect upon the spontaneous and evoked activity of DRt neurons (effect of drug administration upon spontaneous activity: $F_{1,49} = 1.541$, $p = 0.22$; effect of drug administration upon evoked activity: $F_{1,47} = 0.352$; $p = 0.56$), independent of the experimental group (interaction

between drug administration and experimental group for spontaneous activity: $F_{1,49} = 0.992$, $p = 0.32$; interaction between drug administration and experimental group for evoked activity: $F_{1,47} = 0.599$, $p = 0.44$; Fig. 4F, G).

The time spent between the beginning of the noxious heat stimulus and observing a change in neuronal activity was not significantly altered by CHPG administration (effect of drug administration: $F_{2,36} = 2.604$, $p = 0.088$), and was independent of the experimental group (interaction between drug administration and experimental group: $F_{2,36} = 0.011$, $p = 0.989$). However, DRt neurons in ARTH animals had a significantly shorter latency to the onset of the heat-evoked response than DRt neurons in SHAM animals (effect of experimental group: $F_{1,36} = 4.966$, $p = 0.032$; Fig. 4H).

Response latency of DRt neurons to electrical activation of the IL

Recordings of two nociceptive DRt neurons in two healthy control animals indicated that the median latencies of the
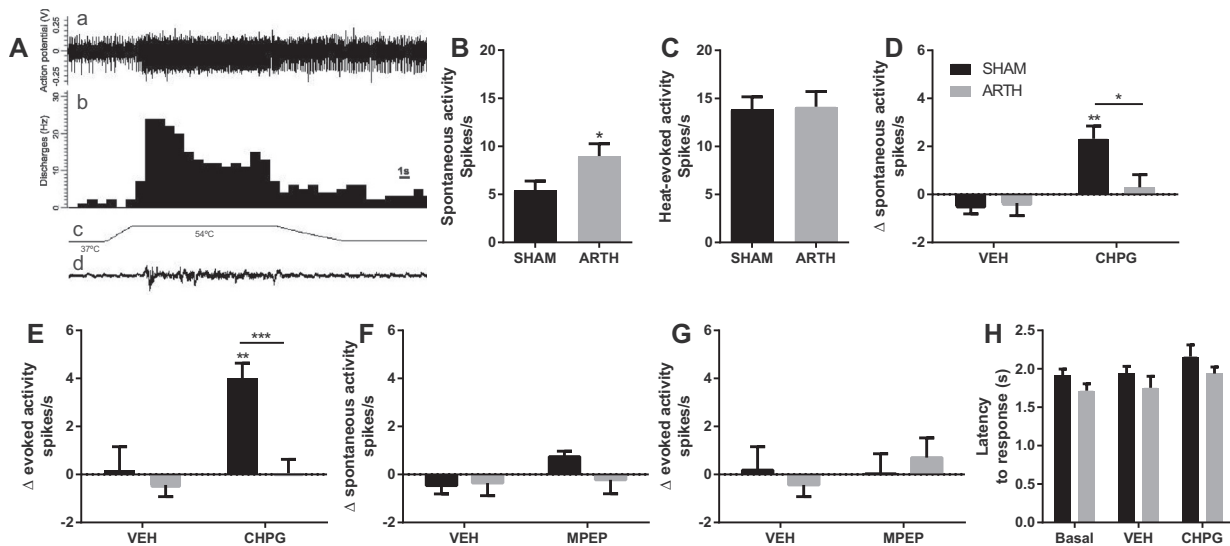
Fig. 4. Effect of mGluR5 activation/inactivation in the infralimbic cortex (IL) upon the spontaneous and heat-evoked activity of dorsal reticular nucleus (DRt) cells. (A) Example of an original recording of a DRt neuron in response to noxious heating of the right hind paw. a – raw data of neuronal responses; b – peristimulus time histogram showing the discharge of a DRt neuron; c – heat stimulus that starts from the baseline temperature of 37 °C and peaks at 54 °C; d – movement detection of a flexor muscle in the hind limb during hind paw heat stimulation. (B) Spontaneous activity of DRt neurons in healthy (SHAM) and arthritic (ARTH) animals. (C) Evoked activity of DRt neurons in healthy and arthritic animals. (D) Effect of IL CHPG administration upon the spontaneous activity of DRt neurons in SHAM and ARTH animals. (E) Effect of IL CHPG administration upon the noxious heat-evoked activity of DRt neurons in SHAM and ARTH animals. (F) Effect of IL MPEP administration upon the spontaneous activity of DRt neurons in SHAM and ARTH animals. (G) Effect of IL MPEP administration upon the noxious heat-evoked activity of DRt neurons in SHAM and ARTH animals. (H) Latency from the beginning of stimulation to cell/muscle response to stimulation. VEH – vehicle; CHPG – mGluR5 agonist; MPEP – mGuR5 antagonist; Δ – (activity 30 min) – (activity-5 min). Graphs B, C and F show mean + SEM; graphs D-G show Δ mean + SEM. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. (VEH: n_{SHAM} = 15, n_{ARTH} = 20; CHPG: n_{SHAM} = 19, n_{ARTH} = 22; MPEP: n_{SHAM} = 8, n_{ARTH} = 10).

first impulse followed by electric stimulation of IL were 14 ms (interquartile range: 12–65 ms) and 15 ms (14–34 ms), whereas the corresponding median latencies followed by fake stimulation of IL were 663 ms (160–1387 ms) and 650 ms (165–864 ms). Within 20 ms from the real IL stimulus, the firing probability was >3 SDs higher than within the same time window after the fake IL stimulus (Fig. 5).

MGLUR5 ACTIVATION IN THE IL INCREASES DISCHARGE RATES OF SPINAL DORSAL HORN NEURONS IN BOTH SHAM AND ARTH ANIMALS

Wide-dynamic range (WDR) neurons

Recordings of the studied WDR neurons were performed in the deep spinal dorsal horn as indicated by the recording depth that varied from 500 to 1000 μm from the cord surface. The receptive fields of the studied neurons covered the plantar skin of the hind paw and some surrounding areas (e.g. partial/complete toes or heel; Fig. 6A).

The baseline spontaneous activity of spinal WDR neurons before drug administration was higher in the ARTH than SHAM group (t_{39} = 2.505, p = 0.017; Fig. 6B). Drug administration in the IL had no significant overall effect on the spontaneous activity of WDR neurons (effect of drug administration: $F_{1,47}$ = 1.280, p = 0.264). However, the drug effect varied with the experimental group (interaction between drug administration and experimental group: $F_{1,47}$ = 4.271,

p = 0.044). *Post hoc* tests showed the spontaneous activity of WDR neurons in SHAM animals was increased after CHPG administration, while in ARTH animals no drug-induced changes of spontaneous activity were observed (Fig. 6C).

Drug administration in the IL altered the heat-evoked response of spinal WDR neurons (effect of drug administration: $F_{1,43}$ = 16.61, p = 0.0002), independent of the experimental group (interaction between drug administration and experimental group: $F_{1,43}$ = 0.1525, p = 0.698). *Post hoc* tests show that the heat-evoked responses of spinal WDR neurons both in SHAM and ARTH groups were significantly increased after IL administration of CHPG and that in the SHAM group the increase of the heat-evoked response was significantly higher than in the ARTH group (Fig. 6D).

Nociceptive specific (NS) neurons

Recordings of the studied NS neurons were performed in the superficial spinal dorsal horn as indicated by the recording depth that varied from 50 to 250 μm from the cord surface. The receptive fields of the studied neurons covered the plantar skin of the hind paw and some surrounding areas (e.g. partial/complete toes or heel; Fig. 6E).

Baseline spontaneous activity of spinal NS neurons was higher in the ARTH than the SHAM group (t_{31} = 3.881, p = 0.0005; Fig. 6F). Drug administration in the IL had no significant effect on the spontaneous activity of spinal NS neurons (effect of drug administration: $F_{1,20}$ = 0.602, p = 0.467), independent
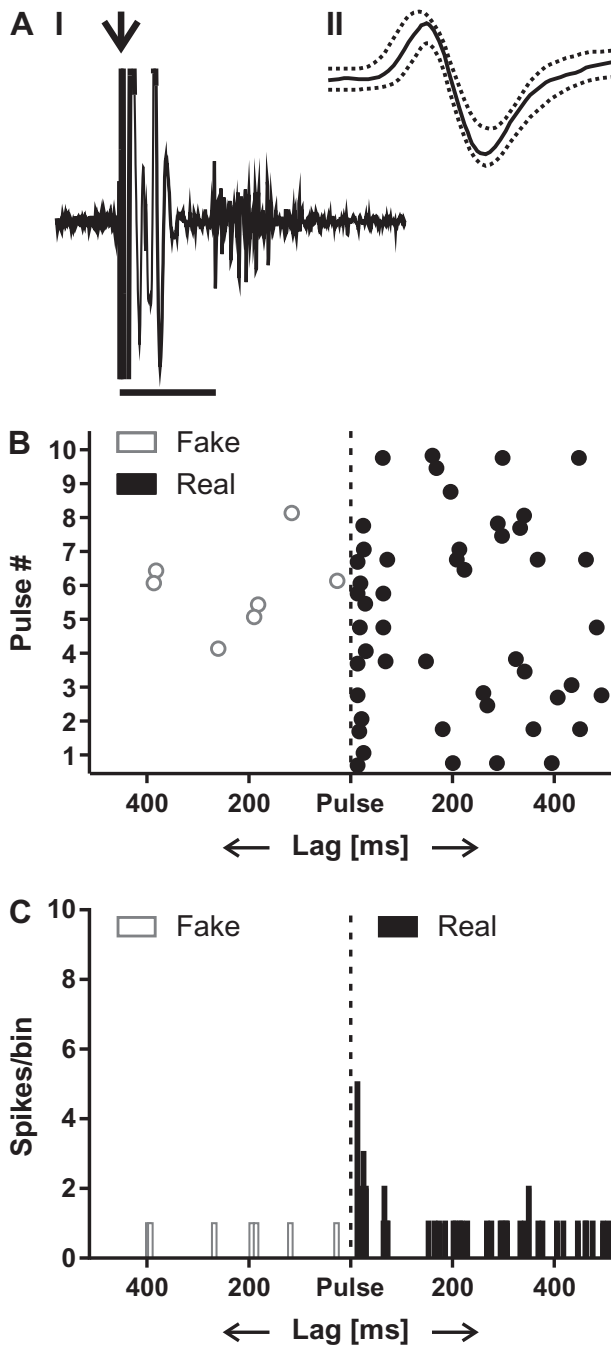
Fig. 5. Recording responses of a nociceptive dorsal reticular nucleus (DRt) neuron to electric stimulation of the infralimbic cortex (IL). (A, I) Ten consecutive superimposed responses to single pulse IL stimuli (0.3 ms, 10 mA, 0.5 Hz). The arrow indicates the electric stimulus that is followed by a stimulus-evoked noise signal lasting 9.5 ms. The horizontal calibration bars represents 17 ms. (A, II) The shape of the single action potential recorded in the DRt, and its template (dotted lines). (B) Raster plot of DRt neuron spiking following 10 consecutive real (10 mA) or fake (0 mA) IL stimulations. Responses following real IL stimulations are shown to the right from the midline, and responses to the fake IL stimulations are shown to the left from the midline. (C) Poststimulus time histograms of 10 repeated real (to the right from the midline) vs fake (to the left from the midline) IL stimulations. Bin width: 2 ms.

of the experimental group (interaction between drug administration and experimental group: $F_{1,20} = 0.009$, $p = 0.928$; Fig. 6G).

In contrast to spontaneous activity, heat-evoked responses of spinal NS neurons were significantly altered after CHPG microinjection in the IL (effect of drug administration: $F_{1,23} = 9.496$, $p = 0.005$). This effect did not vary with the experimental group (interaction between drug administration and experimental group: $F_{1,23} = 0.531$, $p = 0.474$; Fig. 6H).

Behavioral hyperalgesia after IL/mGluR5 activation is mediated by spinal TRPV1

Baseline PWLs were not significantly different between SHAM and ARTH animals before i.t. administration of AMG, a TRPV1 antagonist ($t_{52} = 1.320$, $p = 0.193$; Fig. 7A).

When studying the role of spinal TRPV1 in IL-mediated pronociception, there was an overall change in PWL after drug administration (effect of drug microinjection: $F_{3,46} = 16.33$, $p < 0.0001$), independent of the experimental group (interaction between drug administration and experimental group: $F_{3,46} = 0.039$, $p = 0.990$). *Post hoc* analysis showed CHPG in the IL significantly decreased PWL both in SHAM and ARTH animals. I.t. AMG alone had no significant influence on PWL in either the SHAM or ARTH group, while AMG blocked pronociception induced by IL administration of CHPG (Fig. 7B). Paw skin temperature was not altered by drug administrations (effect of drug microinjection: $F_{3,36} = 0.154$, $p = 0.926$) in any of the experimental groups (interaction between drug administration and experimental group: $F_{3,36} = 0.425$, $p = 0.737$; Fig. 7C).

DISCUSSION

In this work, we confirmed that mGluR5 activation in the IL by intracortical microinjection of CHPG enhances behavioral hyperalgesia and spinal neuronal activity in healthy (SHAM) and monoarthritic (ARTH) rats. Moreover, we showed for the first time that the medullary DRt is a relay nucleus for the IL/mGluR5-induced descending pronociceptive effect in healthy animals, but not in monoarthritic rats. The IL/mGluR5-induced behavioral hyperalgesia to heat was accompanied by facilitation of heat-evoked responses of spinal dorsal horn WDR and NS neurons in both experimental groups. Interestingly, pharmacological inhibition of spinal TRPV1 prevented the IL/mGluR5-induced hyperalgesia in both SHAM and ARTH groups suggesting that spinal TRPV1 is mediating the descending pronociceptive effect.

As previously reported (David-Pereira et al., 2016), CHPG injection in the IL facilitated nociceptive spinally-organized behavior in both SHAM and ARTH animals. Interestingly, a recent study showed the opposite effect when an mGluR5-positive allosteric modulator was administered in the IL of rats with K/C induced-monoarthritis, restoring mechanical hind limb withdrawal threshold of monoarthritic rats to pre-arthritis values (Kiritoshi et al., 2016). The disparity between the two
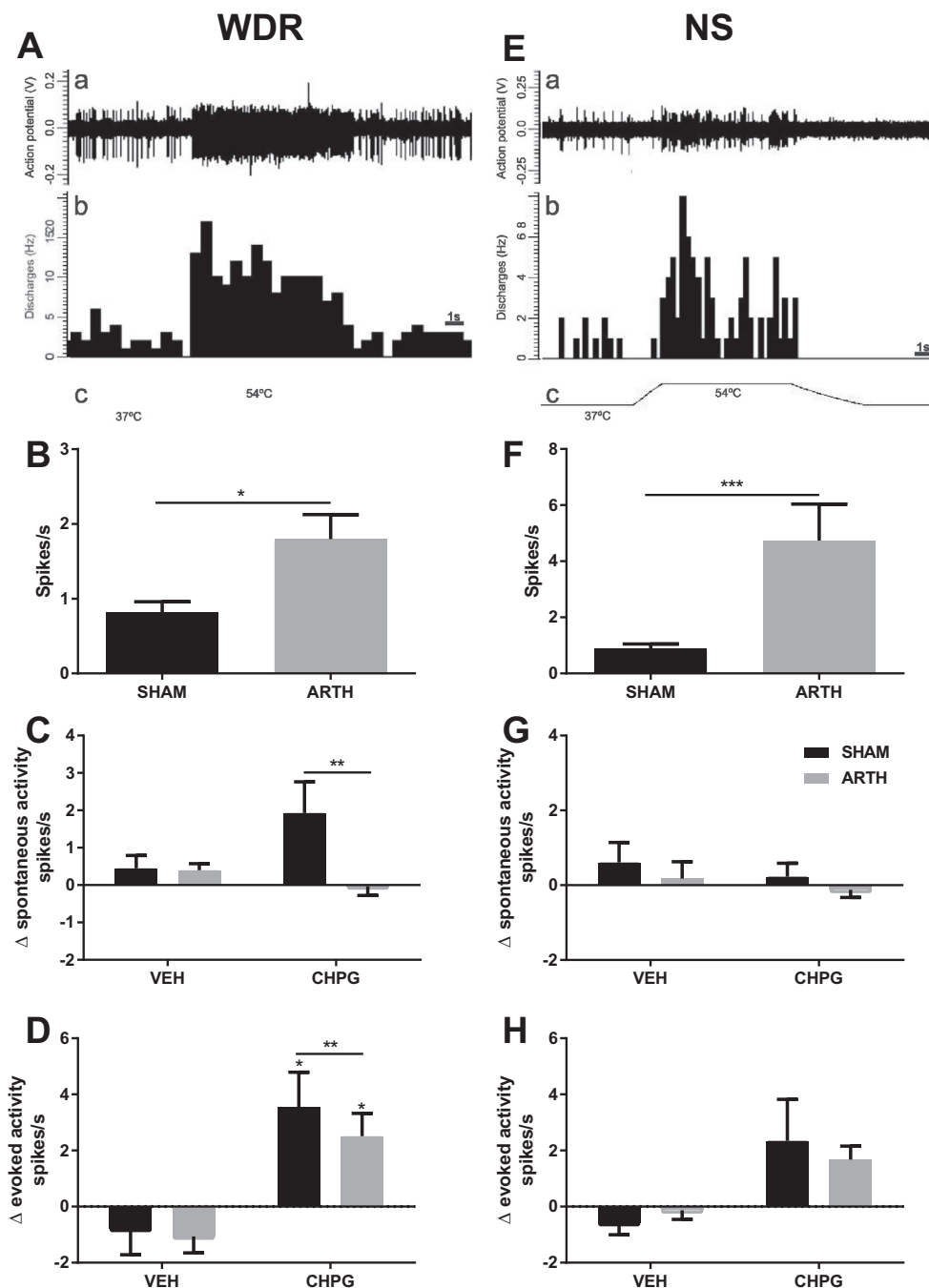
Fig. 6. Effect of mGluR5 activation in the infralimbic cortex (IL) upon the spontaneous and heat-evoked activity of L4-L6 spinal dorsal horn nociceptive neurons. (A) Example of an original recording of a wide-dynamic range (WDR) neuron in response to noxious heating of the right hind paw. a – raw data of neuronal responses; b – peristimulus time histogram showing the discharge of a spinal dorsal horn neuron; c – heat stimulus that starts from the baseline temperature of 37 °C and peaks at 54 °C. (B) Spontaneous activity of WDR neurons in control (SHAM) and arthritic (ARTH) animals. (C) Effect of IL drug administration upon the spontaneous activity of spinal WDR neurons in SHAM and ARTH animals. (D) Effect of IL drug administration upon the noxious heat-evoked activity of spinal WDR neurons in SHAM and ARTH animals. (E) Example of an original recording of a nociceptive specific (NS) neuron in response to noxious heating of the right hind paw. a – raw data of neuronal responses; b – peristimulus time histogram showing the discharge of a spinal dorsal horn neuron; c – heat stimulus that starts from the baseline temperature of 37 °C and peaks at 54 °C. (F) Spontaneous activity of NS neurons in SHAM and ARTH animals. (G) Effect of IL drug administration upon the spontaneous activity of spinal NS neurons in SHAM and ARTH animals. (H) Effect of IL drug administration upon the noxious heat-evoked activity of spinal NS neurons in SHAM and ARTH animals. VEH – vehicle; CHPG – mGluR5 agonist; Δ – (activity 30 min)-(activity-5 min). Graphs B and F show mean + SEM; graphs C, D, G and H show Δ mean + SEM. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. (WDR: VEH: $n_{SHAM} = 8$, $n_{ARTH} = 12$; CHPG: $n_{SHAM} = 12$, $n_{ARTH} = 21$. NS: VEH: $n_{SHAM} = 6$, $n_{ARTH} = 8$; CHPG: $n_{SHAM} = 6$, $n_{ARTH} = 9$).
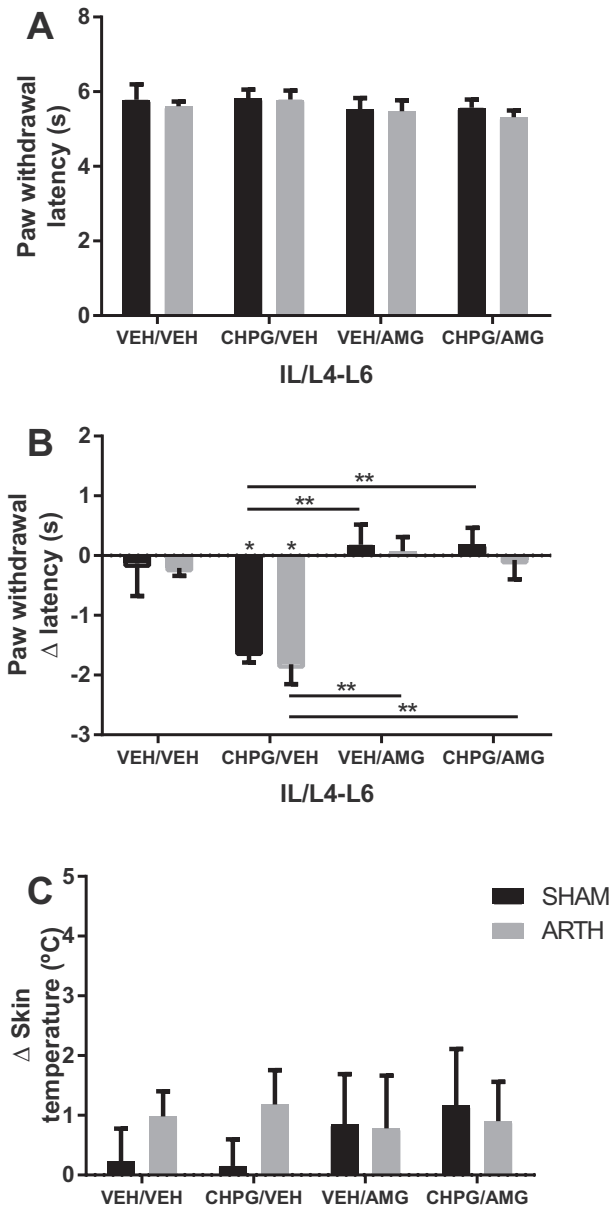
Fig. 7. (A) Paw withdrawal latency (PWL) before drug microinjection into the infralimbic cortex (IL) and the spinal cord (L4-L6) of control (SHAM) and arthritic (ARTH) animals. (B) PWL variation 30 min after drug microinjection in the IL and L4-L6 in SHAM and ARTH animals. Effects of vehicle (VEH) in the IL and in the L4-L6; CHPG (mGluR5 agonist) in the IL and SAL in the L4-L6; SAL in the IL and AMG-9810 (AMG; TRPV1 antagonist) in the L4-L6; and CHPG in the IL and AMG in the L4-L6. (C) Skin temperature (°C) variation 30 min after drug injection into the IL and L4-L6 of SHAM and ARTH animals. Δ − (PWL30 min)-(PWL-5 min). Graph A shows mean + SEM; graphs B and C show Δ mean + SEM; $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. (VEH/VEH: $n_{SHAM} = 5$, $n_{ARTH} = 7$; CHPG/VEH: $n_{SHAM} = 7$, $n_{ARTH} = 6$; VEH/AMG: $n_{SHAM} = 8$, $n_{ARTH} = 7$; CHPG/AMG: $n_{SHAM} = 7$, $n_{ARTH} = 7$).

studies could result from the site, type or, particularly, time of stimulation; Kiritoshi and colleagues tested IL mGluR5 function on nociception by stimulating the knee joint of rats with mechanical pressure – primary hypersensitivity – 6 h after K/C injection. Contrastingly, we stimulated the distal hind paw with noxious heat 4 weeks after ARTH induction. Although at earlier time points the

K/C model of monoarthritis is associated with both primary and secondary hyperalgesia (Urban et al. 1999), at later stages monoarthritic rats only exhibit primary mechanical hyperalgesia (Sluka and Westlund, 1993; Ren and Dubner, 1999; Amorim et al., 2014). Interestingly, these alterations in behavior are mirrored by changes of neurotransmitter levels in the spinal cord. For example, glutamate expression correlates with secondary hyperalgesia, and substance P and calcitonin gene-related peptide expressions correlate with primary hyperalgesia, reflecting a transition from acute to chronic inflammatory pain (Sluka and Westlund, 1993). Time-dependent behavioral alterations also benefit from descending inputs from supraspinal regions such as the RVM, which both inhibits and facilitates nociception. The balance between these opposing descending inputs varies according to the time elapsed since inflammatory pain onset (Vanegas and Schaible, 2004). Therefore, IL mGluR5 activation might yield opposing descending modulatory effects at different stages of inflammatory pain; further observations, however, would require a longitudinal study of molecular, functional and behavioral impact of inflammatory pain in the IL.

To the best of our knowledge, direct projections from the IL to the spinal cord have not been described. We hypothesized that a downstream pain facilitatory area, such as the DRt, mediated at least partially the observed behavioral hyperalgesia. The assessment of DRt neuron response to electric IL stimulation suggests that although responses with a latency shorter than 9.5 ms could not be appropriately assessed in the present conditions, according to recently described criteria for corticofugal projections some of the studied projections from the IL to the DRt might be oligo- or even monosynaptic (Doig et al., 2014). Furthermore, transient block of the DRt with LIDO or inhibition of synaptic signaling with GABA agonists was able to prevent the IL/mGluR5-induced behavioral hyperalgesia in both SHAM and ARTH animals. The interpretation of this finding however is complicated by the antinociceptive action induced by LIDO, MUSC or GABA alone in the DRt of both SHAM and ARTH animals. In contrast, single-cell electrophysiological recordings in the DRt indicate that IL administration of CHPG increased spontaneous and heat-evoked activity of DRt neurons in SHAM but not in ARTH animals. Since activation of mGluR5 in the IL induces pronociceptive behavior in both experimental groups, we hypothesize the existence of another supraspinal area besides the DRt that relays the pronociceptive effect originating in the IL of ARTH animals. Moreover, it is possible that the DRt promotes nociception in parallel with this second relay also in healthy controls.

Our earlier behavioral results indicate that IL MPEP administration induced antinociception in ARTH animals and had no effect in SHAM animals (David-Pereira et al., 2016). This behavioral finding contrasts with the present electrophysiological result showing that IL MPEP failed to influence the discharge of DRt neurons in ARTH as well as SHAM animals. The discrepancy in the effect of IL MPEP on spinally organized behavior versus the discharge of DRt neurons in the ARTH group supports the hypothesis that

unlike in SHAM animals, the DRt of ARTH animals may not be the only or the critical relay for the descending pronociceptive effect induced by the IL mGluR5, but another parallel pathway exerts a key role in the descending pronociceptive effect in the ARTH group.

Anatomical evidence on descending projections from the DRt to spinal cord laminae I and IV–V (Lima and Coimbra, 1988; Tavares and Lima, 1994; Almeida et al., 1995; Villanueva et al., 1995) and electrophysiological evidence showing that stimulating the DRt with glutamate increases WDR responses to noxious sciatic nerve stimulation (Dugast et al., 2003), indicate that the modulation of nociception by the DRt relies, at least in part, in the modulation of spinal WDR cell activity. Therefore, it is not surprising that our data indicate that IL/mGluR5 activation increases spontaneous activity of spinal WDR cells in SHAM but not ARTH animals. Additionally, the CHPG-induced facilitation of heat-evoked responses was significantly weaker in WDR neurons of the ARTH than SHAM group. However, as observed earlier with RVM ON-cell discharge in animals with peripheral nerve injury (Carlson et al., 2007), there is also the possibility that the noxious stimulation-induced responses of spinal WDR cells of ARTH animals were influenced by the ceiling effect. In line with this proposal, spontaneous activity of WDR neurons was significantly higher in the ARTH than SHAM group.

An interesting observation is that CHPG administration in the IL of SHAM animals increased both spontaneous and heat-evoked activity in WDR neurons, whereas only heat-evoked activity was increased in NS neurons. WDR neurons are considered important for perception of prolonged pain (Coghill et al., 1993; Fallis, 2006); functionally, there is evidence that WDR cells can be important for sensory/discriminative aspects of pain as, for instance, intensity-encoding deep-laminae WDR neurons heavily project through the spinothalamic tract (STT) to brain regions more commonly associated with the sensory-discriminative aspects of pain, such as the SI (Millan, 2002). On the other hand, NS neurons are considered to be involved in the phasic aspects of pain, such as the signaling of a new noxious stimulus and the activation of autonomic responses to a new challenge (Coghill et al., 1993; Fallis, 2006). In parallel with WDR cells, NS neurons from superficial dorsal horn laminae project through the STT to supraspinal regions associated with the emotional/cognitive dimensions of pain, such as the PFC (Millan, 2002). Although this distribution is not an absolute distinction between WDR and NS neuronal function, it is likely to have implications for pain development and its treatment.

Earlier studies have demonstrated IL neurons that encode nociception (Zhang et al., 2004). Moreover, the mPFC, including the IL, has been implicated in multiple other roles ranging from anxiety modulation (Bi et al., 2013) to decision-making (Ji et al., 2010). Together these earlier and the present findings raise a hypothesis that the activation of mGluR5 in the IL is among mechanisms contributing to the interaction of the emotional/cognitive state and pain through descending control of spinal pain-relay neurons.

It should be noted that when interpreting the IL/mGluR5-induced changes in the ongoing discharge rates of spinal dorsal horn as well as DRt neurons in terms of behavior, a limitation for the interpretations is that the analysis of pain behavior was based on heat-evoked behavioral responses and not on ongoing pain behavior assessed e.g. by drug-induced conditioned place-avoidance. Additionally, while the anesthesia level was kept as stable as possible, it cannot be excluded that anesthesia or a change in its level may have had an influence on neuronal responses. Importantly, however, since the anesthesia procedure was identical in all experimental conditions, the possible effects of anesthesia or a change in its level cannot explain the differences between the effects induced by IL administration of VEH vs mGluR5 agonist, differences between SHAM vs ARTH rats, or differences among different neuronal populations (DRt vs spinal dorsal horn WDR and NS neurons).

TRPV1 is tonically active and its ablation has been shown to prevent the development of nociceptive behaviors such as that evoked by thermal or chemical stimulation (Caterina et al., 1997, 2000). In the present study, when TRPV1 antagonist AMG 9810 was administered alone, it had no effect upon the PWL of SHAM or ARTH animals. The dose of AMG 9810 was chosen based on earlier results showing that when it was used intrathecally, it reversed mechanical and thermal hyperalgesia in a rat model of inflammatory pain (Yu et al., 2008). The currently used dose of AMG 9810 was not sufficient to alter baseline latencies in SHAM controls, or in the ARTH group four weeks after the induction of K/C monoarthritis when the arthritis was no longer accompanied by secondary hyperalgesia. Interestingly, expression of TRPV1 in different dorsal root ganglia cell types is variable depending on the time after chronic inflammatory pain induction (Yu et al., 2008), and, for instance, in the CFA model, TRPV1 expression peaks 14 days after induction, while at 28 days it returns to control levels (Luo et al., 2004). Concomitantly, hot plate latency responses decrease in those animals, with the lowest values registered 14 days after induction (Luo et al., 2004). Based on these earlier findings it is possible that the late time point of testing in the present ARTH group may attest for the lack of effect by a TRPV1 antagonist alone on heat nociception along with the lack of heat hyperalgesia.

While the spinally administered TRPV1 antagonist failed to influence the baseline PWL, it did prevent the IL mGluR5-mediated pronociception in both SHAM and ARTH groups. This finding indicates that spinal TRPV1 is mediating the IL mGluR5-induced pronociception at the spinal cord level in ARTH as well as SHAM condition. Although until recently spinal TRPV1 was thought to be expressed only on central terminals of primary afferent nerve fibers, some studies showed the expression of TRPV1 on GABAergic interneurons in the superficial laminae of the spinal dorsal horn (Valtschanoff et al., 2001; Ferrini et al., 2010; Kim et al., 2012). Activation of spinal TRPV1 has been linked to increased excitability of spinal dorsal horn neurons, leading to mechanical allodynia in neuropathic pain models

(Cui et al., 2006). Taking this evidence into account, one interesting possibility is that the IL mGluR5-induced pronociception involves activation of TRPV1 on spinal interneurons. However, intrathecal drug administration affects indiscriminately the target receptor, and therefore further studies are needed to distinguish between the functional roles of TRPV1 expressed on central endings of peripheral nerve terminals and spinal interneurons.

In healthy and monoarthritic animals, mGluR5 activation in the IL facilitated spinally organized pain behavior as revealed by a decrease in the heat-evoked paw withdrawal latency. In healthy controls, this descending pronociceptive effect was accompanied by an increase in the heat-evoked discharge rate of medullary DRt neurons and spinal dorsal horn WDR and NS neurons. In experimental monoarthritis, the IL/mGluR5-induced descending facilitation of the heat-evoked responses was absent in medullary DRt neurons but still present in spinal dorsal horn WDR and NS neurons. Together these findings suggest the DRt is a relay in the descending pronociceptive pathway activated by IL/mGluR5 in healthy controls, but an additional descending pronociceptive pathway, which does not relay in the DRt, is likely to be recruited in experimental monoarthritis (in parallel, it may be involved also in healthy controls). Interestingly, one or both of these descending pronociceptive pathways target, at least partly, spinal TRPV1 as indicated by the loss of the IL/mGluR5-induced pronociceptive effect following pharmacological block of spinal TRPV1 in both healthy and arthritic animals.

AUTHOR CONTRIBUTIONS

ADP, AP and FPR developed the concept and designed experiments. ADP performed and analyzed all of the experiments. BS was involved in part of the electrophysiological experiments. HW assisted in the surgical procedures. ADP, AP and FPR wrote the paper. AA revised the manuscript. All authors discussed and revised the manuscript.

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