Dorsal striatum metabotropic glutamate receptor 8 affects nocifensive responses and rostral ventromedial medulla cell activity in neuropathic pain conditions

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Rats. Furthermore, a decreased level of mGluR8 gene and immunoreactivity withdrawal threshold in sham-operated rats but increased it in SNI rats. -3,4-DCPG did not change mechanical thermal nociception and ON and OFF cell activity in both sham-operated hyperalgesia and mechanical allodynia symptoms (Chudler and Heinricher 1994). Changes of ON and OFF cell activity, expressed on GABAergic terminals, associated with a protein increase was found in the DS of SNI rats. These results suggest that stimulation of mGluR8 inhibits thermostopic responses and mechanical allodynia. These effects were associated with inhibition of ON cells and stimulation of OFF cells within RVM.

Metabotropic glutamate receptor subtype 8; spared nerve injury; dorsal striatum; rostral ventromedial medulla; mechanical allodynia

NEUROPATHIC PAIN, which is often resistant to conventional analgesics (Sindrup and Jensen 1999; Woolf and Mannion 1999), remains a significant clinical problem. After peripheral or central nervous system injury, spinal and brain plastic changes lead to central sensitization and consequent thermal hyperalgesia and mechanical allodynia symptoms (Chudler and Dong 1995; Hagelberg et al. 2004; Jaggi and Singh 2011; Neugebauer 2006). Glutamate plays a key role in persistent activation of nociceptive neurons and hypersensitivity to painful stimuli. An increase in glutamate tone was also demonstrated in a rat model of neuropathic pain (Santangelo et al. 2012). The deleterious consequence of glutamate increase and associated overactivation of postsynaptic receptors is a well-known event in several neuropathologies and can be reversed by inhibiting postsynaptic ionotropic glutamate receptors (iGluRs) or activating presynaptic glutamate autoreceptors (Niswender and Conn 2010). Indeed, group II and III metabotropic glutamate receptors (mGluRs) finely regulate glutamate release since they function as presynaptic autoreceptors (Cartmell and Schoepf 2000; Scanziani et al. 1997). These receptors are widely expressed at spinal, supraspinal, and peripheral sites, where they display different roles in nociception (Chiechio and Nicoletti 2012; Goudet et al. 2009; Varney and Gereau 2002). In this context, the role of individual group III mGluR subtypes in pain is only beginning to emerge concomitantly with the availability of subtype-selective agents.

In our previous studies the stimulation of mGluR subtype 8 (mGluR8) by the selective agonist (S)-3,4-dicarboxyphenylglycine [(S)-3,4-DCPG] (Thomas et al. 2001) in the ventrolateral periaqueductal gray (VL PAG) and central nucleus of the amygdala (CeA) has been shown to inhibit pain and associated changes in the rostral ventromedial medulla (RVM) neuron activity in models of neuropathic and inflammatory pain (Marabese et al. 2007a; Palazzo et al. 2008, 2011). RVM, a relay station in the pain descending system, contains different pain-responding neurons: ON cells that are activated, OFF cells that are inhibited, and neutral cells that are unaffected by nociceptive stimuli (Fields et al. 1983; Heinricher et al. 1989). Systemic or local administration of opioids or cannabinoids, able to inhibit nociception, inhibits ON cells while increasing OFF cells (de Novellis et al. 2005; Fields et al. 1995, 2006; Heinricher and Tortorici 1994). Changes of ON and OFF cell activity are thus predictive of pain response modulation.

There is accumulating evidence indicating that the dorsal striatum (DS), the main input of the basal ganglia, might play a role in pain processing (Barceló et al. 2012). In situ hybridization and immunohistochemistry studies have demonstrated the expression of mGluRs at different levels of the DS (Ferraguti and Shigemoto 2006; Testa et al. 1994; Wang et al. 1997). In particular, group III mGluRs have been reported to be
expressed in the striatum at the presynaptic level on excitatory corticostriatal terminals and GABAergic output fibers (Corti et al. 2002; Kosinski et al. 1999; Testa et al. 1994, 1998). Moreover, previous studies demonstrated that RVM could mediate the action of the striatum; indeed, electrolytic or kainic acid lesion of RVM blocked the inhibition of the nocifensive reflex produced by striatal activation in a model of orofacial pain (Barceló et al. 2012).

In this study we therefore investigated the contribution of DS mGluR8 to pain responses in control conditions and in the condition of neuropathic pain induced by spared nerve injury (SNI) of the sciatic nerve in rats. A selective mGluR8 agonist, (S)-3,4-DCPG, was therefore microinjected in the DS, and mechanical allodynia, latencies to tail flick, and activity of RVM ON and OFF cells in sham-operated and SNI rats were investigated. The effects of AZ12216052 and VU01550401, positive allosteric modulators (PAMs) of mGluR8 and mGluR4 (Duvoisin et al. 2010; Niswender et al. 2008), respectively, on thermoceptive responses and ON and OFF cell activity of the RVM were also evaluated, together with changes of expression of DS mGluR8 in SNI rats.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats (Harlan) weighing 250–280 g were housed three per cage under controlled illumination (12:12-h light-dark cycle; lights on 0600) and standard environmental conditions (ambient temperature 20–22°C, humidity 55–60%) for at least 1 wk before the commencement of experiments. Rat chow and tap water were available ad libitum. All surgery and experimental procedures were done during the light cycle and were approved by the Animal Ethics Committee of The Second University of Naples. Animal care was in compliance with Italian (D.L. 116/92) and European Union (O.J. of E.C. L358/1 18/12/86) regulations on the protection of laboratory animals. All efforts were made to reduce both animal numbers and suffering during the experiments.

**Neuropathic pain induction.** The SNI model of neuropathic pain was induced according to the method used by Decosterd and Woolf (2000). Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The sciatic nerve was exposed at the same level but not ligated. Fourteen days after surgery sham-operated and SNI rats were used for tail flick tests coupled with single-unit extracellular recording or mechanical allodynia experiments or were killed for immunohistochemistry, RT-PCR, and Western blot analysis.

**Surgical preparation for intra-DS microinjection.** For direct intra-DS microinjections, sham-operated and SNI rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and a 26-gauge, 12-mm-long stainless steel guide cannula was stereotaxically lowered until its tip was 0.8 mm above the DS by applying coordinates from the atlas of Paxinos and Watson (1986) (AP: 0.20 mm and L: 2.8 mm from bregma, V: 3.2 mm below the dura). The cannula was anchored with dental cement to a stainless steel screw in the skull. We used a David Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the animal positioned on a homeothermic temperature control blanket (Harvard Apparatus, Edenbridge, UK). Direct intra-DS administration of drugs or respective vehicle [artificial cerebrospinal fluid (ACSF), composition in mM: 125 NaCl, 2.5 KCl, 1.18 MgCl2, and 1.26 CaCl2] was conducted with a stainless steel cannula connected by a polyethylene tube to a SGE 1-μl syringe inserted through the guide cannula and extended 0.8 mm beyond the tip of the guide cannula to reach the DS. Volumes of 200 nl of drug solution or vehicle were injected into the DS over a period of 60 s, and the injection cannula was gently removed 2 min later.

**RVM extracellular recordings associated to tail flick.** Twenty to twenty-four hours after the guide cannula implantation (day 13) and at day 14 after sciatic nerve surgery (day 0), anesthesia was induced with pentobarbital (50 mg/kg ip) and maintained with a continuous infusion of propofol (5–10 mg·kg⁻¹·h⁻¹ iv) so that tail flicks were elicited with a constant latency of 4–5 s. A thermal stimulus was elicited by a radiant heat source of a tail flick unit (Ugo Basile, Varese, Italy), focused on the rat tail 3–5 cm from the tip. The intensity of the radiant heat source was adjusted to 50 mW (corresponding to 50 mJ/s) at the beginning of each experiment in order to elicit a constant tail flick latency. Tail flicks were elicited every 5 min for at least 15 min prior to microinjection of the drug or its vehicle into the DS. A glass-insulated tungsten filament electrode (3–5 MΩ) (FHC) was lowered into the RVM with the following stereotaxic coordinates: 2.8–3.3 mm caudal to lambda, 0.4–0.9 mm lateral, and 8.9–10.7 mm deep from the surface of the brain (Fig. 1). RVM noxious stimuli-responsive neurons were identified by the characteristic OFF cell pause and ON cell burst of activity immediately prior to tail flick.
response (Fields et al. 2006). Neutral cells, which did not show any significant change in activity associated with the tail withdrawal, were also encountered during cell searching procedure but were not recorded in this study. The recorded signals were amplified and displayed on both an analog and a digital storage oscilloscope to ensure that the unit under study was unambiguously discriminated throughout the experiment. Signals were sampled by a CED 1401 interface [Cambridge Electronic Design (CED)] and analyzed by Spike2 window software (CED, version 4) to create peristimulus time histograms online and to store and analyze digital records of single-unit activity off-line. The configuration, shape, and height of the recorded action potentials were monitored and recorded continuously with Spike2 software for online and off-line analyses. Once an ON or OFF cell was identified from its background activity, we optimized spike size before all treatments. This study only included neurons whose spike configuration remained constant and could be clearly discriminated from the background activity throughout the entire experiment. By doing this, we were able to determine the activity of only one neuron. In each rat, the activity of only a single neuron was recorded before and after vehicle or drug administration. Ongoing and tail flick-related activity of the OFF cells was recorded before and after the DS microinjection of drugs or vehicle in sham-operated and SNI rats 14 days after the surgical procedure for neuropathic pain induction. For each ON and OFF neuron the ongoing activity was obtained by averaging the firing rate (spikes/s) for 50 s before the tail flick trials (carried out every 5 min). The latency to the onset of the pause (time between onset of heat application and first action potential) and the duration of the tail flick-related pause (time elapsing between pause onset and first action potential following tail flick) of the OFF cells were also quantified. Moreover, the tail flick-related burst (peak height of burst in spikes/s calculated by subtracting the ongoing activity to the burst activity) and the onset of the ON cell burst (time elapsing between onset of heat application and increase in frequency rate, which was at least twofold higher than its baseline) were quantified for the ON cells. At the end of the experiment, a 200-nl volume of neutral red (0.1%) was injected into the DS 30 min before the rats were killed with a lethal dose of pentobarbital. Rats were then perfused intracardially with 20 ml phosphate-buffered saline (PBS) followed by 20 ml 10% formalin solution in PBS. Brains were removed and immersed in a saturated formalin solution for 2 days. After fixation, the microinjection and recording sites were identified (Fig. 1). The injection sites were ascertained by inspecting two consecutive sections (40 µm), one stained with cresyl violet to identify the DS and the other unstained to determine dye spreading. The recording site was marked with a 20-µA DC current applied for 20 s immediately prior to the end of the electrophysiological recordings. Only the data from microinjection and drug diffusion sites located within the DS and those from the recording sites in RVM neurons are included in the results.

Mechanical allodynia. Mechanical allodynia was evaluated 14 days after SNI or sham surgery with the Dynamic Plantar Aesthesiometer (Ugo Basile). Rats were allowed to move freely in one of the two compartments of the enclosure, positioned on the metal mesh surface, and allowed to adapt to the testing environment before any measurement was taken. After a 30-min habituation period, the mechanical stimulus, a steel rod (2 mm) pushed with ascending force (0–30 g in 10 s), was delivered to the plantar surface of the hind paw of the rat from below the floor of the test chamber by an automated testing device. When the animal withdrew its hind paw, the mechanical stimulus was automatically withdrawn and the force was recorded to the nearest 0.1 g. Mechanical withdrawal threshold (MWT) was measured in grams for 1 h before treatment and for 2 h after vehicle or drug administration by an experimenter blind to the treatments. A single trial at each time point (15 min) was performed on the hind paw ipsilateral to the sham or SNI surgery for each rat. Nociceptive responses for mechanical sensitivity were expressed as means ± SE in grams. Groups of five rats per treatment were used, with each animal being used for one treatment only.

Treatments. Single-unit extracellular recordings in the RVM associated to tail flick were carried out in anesthetized SNI and sham-operated rats. Mechanical allodynia was measured in awake SNI and sham-operated rats. Both experiments were carried out 14 days after sciatic nerve surgery. mGluR8 agonist (S)-3,4-DCPG, AZ12216052, a selective PAM for mGluR8, or VU0155041, a selective PAM for mGluR4, or respective vehicle was microinjected into the DS in sham-operated and SNI rats in a volume of 0.2 µl (S)-3,4-DCPG in combination with LY341495, a group II mGluR antagonist with a selectivity for mGluR4 over mGluR7, and LY341495 alone were microinjected only in SNI rats. In electrophysiology associated to tail flick experiments groups of 10 rats per treatment were used in order to have at least 5 recordings for ON and OFF cells with each animal being used for a single cell recording. Rats received a single intra-DS microinjection of drugs or vehicle 14 days after surgery and were divided as follows: 1) sham-operated and SNI rats receiving intra-DS microinjection of vehicle, 2) sham-operated and SNI rats receiving intra-DS microinjection of (S)-3,4-DCPG (2 nmol for sham-operated rats, 1 and 2 nmol for SNI rats), 3) SNI rats receiving intra-DS microinjection of (S)-3,4-DCPG (2 nmol) in combination with LY341495 (10 µmol), 4) SNI rats receiving intra-DS microinjection of LY341495 (10 µmol), 5) sham-operated and SNI rats receiving intra-DS microinjection of AZ12216052 (20 nmol), 6) sham-operated and SNI rats receiving intra-DS microinjection of VU0155041 (40 nmol), and 7) from the awake rats tested for mechanical allodynia, a group of SNI rats receiving intra-DS microinjection of (S)-3,4-DCPG (2 nmol) in combination with (RS)-α-methylserine- O-phosphate (MSOP), a group III mGluR-selective antagonist.

Motor coordination. Motor coordination was tested with the accelerating rotarod (model 47700, Ugo Basile), in which rats were required to walk against the motion of a rotating drum with the speed accelerating from 4 to 40 rpm/min over 300 s. The time on the rod from the start of acceleration until the animal fell from the drum onto the counter-trip plate was recorded. A 300-s cutoff was used. One training period per day was performed for 2 days before experiments in which ACSF or (S)-3,4-DCPG (2 nmol) was microinjected into DS. On the day of testing, sham-operated and SNI rats were tested before 15, 30, 45, 60, and 90 min after intra-DS ACSF (n = 5) or (S)-3,4-DCPG (n = 5). Time spent on the rod was taken as the mean ± SE in seconds in the consecutive attempts by an experimenter who was blind to the treatment.

RNA extraction and RT-PCR. Total RNA was extracted from homogenized DS (contralateral and ipsilateral to SNI) with an RNA TRI Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s protocol. The extracted RNA was subjected to DNase I treatment at 37°C for 30 min. The total RNA concentration was determined by UV spectrophotometer. The mRNA levels of the genes under analysis were measured by RT-PCR amplification, as previously reported (Galdieri et al. 1999). RT minus controls were carried out in order to check potential genomic DNA contamination. These RT minus controls were performed without using the reverse transcriptase enzyme in the reaction mix. Sequences for the mouse mRNAs from GenBank (DNASTAR, Madison, WI) were used to design primer pairs for RT-PCR (OLIGO 4.05 software, National Biosciences, Plymouth, MN). Each RT-PCR was repeated at least four times so as to achieve optimal reproducibility of data. A semiquantitative analysis of mRNA levels was carried out with the Gel Doc 2000 UV System (Bio-Rad, Hercules, CA). The measured mRNA levels were normalized with three housekeeping genes: hypoxanthine-guanine phosphoribosyltransferase (HPRT), 18S ribosomal RNA (18S), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The HPRT, 18S, and GAPDH housekeeping genes were chosen because their gene expression did not change in different experimental conditions including SNI-induced neuropathic pain (Bangaru et al. 2012; Maione et al. 2002; Piller et al. 2013; Siniscalco et al. 2007, 2010). The gene expression values are expressed as arbitrary units ± SE. Amplifica-
tions of the gene of interest and HPRT, 18S, and GAPDH were performed simultaneously.

**Western blotting.** For the protein extraction, the striatum (contra and ipsi of SNI) was minced into small pieces with a blender and then was suspended in lysis buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue. Tris·HCl, pH 6.8, containing 6 M urea, 50 μM Na₂VO₄, 50 μM PMSF; Sigma Chemical, St. Louis, MO). The total protein concentration was determined by the method described by Bradford (1976). Each sample was loaded, electrophoresed in a 8% polyacrylamide gel, and electroblotted onto a nitrocellulose membrane. Primary antibodies to detect mGluR₈ were used according to the manufacturer’s instructions at 1:500 dilution (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreactive signals were detected with a horseradish peroxidase-conjugated secondary antibody and reacted with an ECL system (Amersham Pharmacia, Uppsala, Sweden). Protein levels were normalized with respect to the signal obtained with three housekeeping proteins: anti-β-actin monoclonal antibody (Sigma Chemical; 1:1,000 dilution), β-tubulin polyclonal antibody (Santa Cruz Biotechnology; 1:1,000 dilution), and GAPDH monoclonal antibody (Sigma; 1:20,000 dilution).

**Immunohistochemistry.** Sham-operated and SNI rats were anesthetized with pentobarbital (50 mg/kg ip) and transcardially perfused with saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed, postfixed for 3 h in the perfusion fixative, cryoprotected for 72 h in 10%, 20%, and 30% sucrose in 0.1 M phosphate buffer, and frozen in O.C.T. embedding compound. Transverse sections (15 μm) were cut with a cryostat, and those containing the whole striatum were thaw-mounted onto glass slides. Sections were subsequently incubated for 1 day at room temperature in a humid chamber with the respective polyclonal antibodies (all diluted in specific block solution). All sections were processed for goat anti-vesicular glutamate transporter-1 (VGluT1) (Santa Cruz Biotechnology; 1:100), goat anti-vesicular GABA transporter (VGAT) (SySy, Goettingen, Germany; 1:100), rabbit anti-mGluR₈ (Santa Cruz Biotechnology; 1:100), rabbit anti-synaptophysin (SySy; 1:200), and mouse anti-parvalbumin (Millipore, Billerica, MA; 1:500). After incubation, sections were washed and incubated for 3 h with secondary antibody solution (donkey anti-goat or donkey anti-rabbit IgG-conjugated Alexa Fluor 488 and 568; Molecular Probes; 1:1,000). Slides were washed, coverslipped with Vectashield mounting medium (Vector Laboratories), and visualized under a Leica fluorescence microscope. Negative control using secondary antibodies alone did not reveal any positive staining.

**Drugs.** (S)-3,4-DCPG, MSOP, LY341495, and VU0155041 were purchased from Tocris Bioscience (Bristol, UK) and were dissolved in ACSF (vehicle) on the day of the experiment. AZ12216052 was purchased from AxonMedchem (Groningen, The Netherlands). The doses of (S)-3,4-DCPG, VU0155041, LY341495, and AZ12216052 were chosen according to EC₅₀/IC₅₀ and according to our and other in vivo studies using brain local microinjections that have been shown to change pain behavior (Chi et al. 2006; Dong et al. 2012; Duvoisin et al. 2006).

Fig. 2. Effect of intra-dorsal striatum (DS) microinjections of vehicle [artificial cerebrospinal fluid (ACSF)] or (S)-3,4-dicarboxyphenylglycine [(S)-3,4-DCPG, 1 and 2 nmol] (A); vehicle or (S)-3,4-DCPG (2 nmol) alone or in combination with LY341495 (LY, 10 μmol) (B); and vehicle or AZ12216052 (AZ, 20 nmol; C) on tail flick latencies in sham-operated and spared nerve injury (SNI) rats 14 days after SNI. Vehicle and drugs were administered at time 0. Each point represents the mean ± SE of 10 rats/group. Statistically significant difference: *vs. sham operation/vehicle, °vs. SNI/vehicle, #vs. SNI/(S)-3,4-DCPG (2 nmol). P values < 0.05 were considered statistically significant.

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RESULTS

Effect of intra-DS (S)-3,4-DCPG, AZ2216052, or VU0155041 on tail flick latencies in sham-operated and SNI rats. Tail flicks were elicited every 5 min for 15 min prior to microinjection of drugs or respective vehicle into the DS. In sham-operated rats tail flick latencies before any treatment were 5.2 ± 0.26 s (n = 10). In SNI rats tail flick latencies were significantly lower compared with sham-operated rats (2.9 ± 0.7 s, P < 0.05, n = 10). Intra-DS microinjection of injection did not change tail flick latency in sham-operated and SNI rats [5.0 ± 0.23 s (n = 10) and 3.1 ± 0.4 s (n = 10), respectively] compared with pretreatment values. (S)-3,4-DCPG (2 nmol) significantly increased tail flick latency to 5.88 ± 0.5 s (P < 0.05, n = 10) in SNI but not sham-operated rats (Fig. 2A). This effect lasted for the whole period of observation. Intra-DS microinjection of the lowest dose of (S)-3,4-DCPG (1 nmol) significantly changed the nocifensive response in SNI rats at 15 min after drug microinjection (4.36 ± 0.6 s, P < 0.05, n = 10; Fig. 2A). The effect of (S)-3,4-DCPG (2 nmol) observed in SNI rats was antagonized by LY341495 (10 μmol), a group II mGluR antagonist with a selectivity for mGluR8 over mGluR4/7 (Fig. 2B). LY341495 (10 μmol) per se did not change the tail flick latency in SNI rats (not shown).

Intra-DS microinjection of AZ2216052 (20 nmol), a selective mGluR8 PAM, significantly increased tail flick latency to 4.99 ± 0.4 s (P < 0.05, n = 10) in SNI but not sham-operated rats (Fig. 2C). Intra-DS microinjection of VU0155041 (40 nmol) did not change the nocifensive response in sham-operated and SNI rats (not shown).

Effect of intra-DS (S)-3,4-DCPG on ongoing activity of RVM ON and OFF cell in sham-operated and SNI rats. Neurons identified as ON cells by a burst of activity just before tail flick responses were spontaneously active in 33.3% of cases and inactive in the remaining cases. ON cells with spontaneous activity were chosen to better characterize the activity of this ON cell subgroup and to consider postdrug changes in their spontaneous activity. In sham-operated rats the population of spontaneous active ON cells had a mean frequency of 7.4 ± 0.9 spikes/s. In SNI rats the spontaneous activity of the ON cells was significantly increased (14.1 ± 1.2 spikes/s, P < 0.05, n = 5). Microinjection of vehicle did not change the spontaneous activity of the ON cells [7.6 ± 0.68 spikes/s (n = 5) and 14.8 ± 1.3 spikes/s (n = 8) in sham-operated and SNI rats, respectively] (Fig. 3A). The highest dose of (S)-3,4-DCPG (2 nmol) did not change the spontaneous activity of ON cells in sham-operated rats (Fig. 3A). Intra-DS
microinjection of (S)-3,4-DCPG (1 and 2 nmol) caused a decrease in the spontaneous firing activity of ON cells [9.0 ± 0.6 spikes/s (P < 0.05, n = 5) and 0.9 ± 0.5 spikes/s (P < 0.05, n = 5), respectively] in SNI rats. The decrease of ON cell ongoing activity produced by (S)-3,4-DCPG (2 nmol) was observed after 15 min and remained significant for the whole period of recording. This last effect was antagonized by LY341495 (10 μmol) (Fig. 3C). LY341495 (10 μmol) per se did not change the ON cell spontaneous activity in SNI rats (Fig. 3C). The population of cells identified as OFF cells were spontaneously active, and they had a mean frequency of spontaneous activity of 8.3 ± 1.0 (n = 5) in sham-operated animals. In SNI rats the spontaneous activity was significantly reduced (4.6 ± 0.9 spikes/s, P < 0.05, n = 5) compared with the sham-operated rats. Microinjection of vehicle did not change the spontaneous activity of OFF cells [8.1 ± 0.8 spikes/s (n = 5) and 4.0 ± 0.5 spikes/s (n = 5)] in sham-operated and SNI rats, respectively (Fig. 3B). The higher dose of (S)-3,4-DCPG (2 nmol) did not change the spontaneous activity of the OFF cells in sham-operated rats (Fig. 3B). Intra-DS microinjections of (S)-3,4-DCPG (1 and 2 nmol) caused an increase in the spontaneous firing activity of OFF cells in SNI rats that was already significant after 15 min and lasted until 60 min [9.4 ± 0.9 spikes/s (P < 0.05, n = 5) and 20.1 ± 1 spikes/s (P < 0.05; n = 5)], respectively (Fig. 3B). The effect of (S)-3,4-DCPG (2 nmol) on the OFF cell spontaneous activity in SNI rats was also antagonized by LY341495 (10 μmol) (Fig. 3D). LY341495 (10 μmol) per se did not change the OFF cell spontaneous activity in SNI rats (Fig. 3D).

Effect of intra-DS AZ2216052 or VU0155041 on ongoing activity of RVM ON and OFF cell in sham-operated and SNI rats. Intra-DS microinjection of AZ2216052 (20 nmol) did not change the ON cell ongoing activity in the sham-operated rats (Fig. 4A). However, it caused a decrease in the spontaneous firing of the ON cells (8.6 ± 0.8 spikes/s, P < 0.05, n = 5) in SNI rats (Fig. 4A). AZ2216052 (20 nmol) also significantly increased the OFF cell spontaneous activity in SNI rats (15.8 ± 1.2 spikes/s, P < 0.05, n = 5), but it was devoid of activity in the sham-operated rats (Fig. 4B). Intra-DS microinjection of VU0155041 (40 nmol) did not change the ON cell ongoing activity in sham-operated and SNI rats (Fig. 4C) or the OFF cell spontaneous activity in sham-operated and SNI rats (Fig. 4D).

Effect of intra-DS (S)-3,4-DCPG on tail flick-related ON and OFF cell activity in sham-operated and SNI rats. The ON cells had a tail flick-induced burst of firing of 6.1 ± 0.9 spikes/s and 8 ± 0.4 spikes/s in sham-operated and SNI rats, respectively. The onset of the burst was 2,650 ± 40 ms and 1,680 ± 90 ms in sham-operated and SNI rats, respectively. Thus the mean of the frequency of the ON cell burst and the onset of burst in the sham-operated rats did not differ from those of healthy rats (6.9 ± 1.0 spikes/s and 2,200 ± 33 ms, n = 5), whereas the mean frequency of the ON cell burst was significantly increased and the onset of the burst was decreased in SNI rats compared with both sham-operated and healthy rats [P < 0.05 (n = 5) and P < 0.05 (n = 5)]. The OFF cells had a pause of 7.0 ± 1.5 s and 16.9 ± 2.5 s and an onset of pause of 2,500 ± 45 ms and 1,230 ± 80 ms in sham-operated and SNI rats, respectively. The duration and the onset of the OFF cell pause in the sham-operated rats did not differ from those in healthy rats (6.24 ± 2.4 s and 2,370 ± 26 ms), whereas the pause of OFF cells was significantly increased and the onset of the pause was significantly decreased in SNI rats compared with both sham-operated and healthy rats [P < 0.05 (n = 5) and P < 0.05 (n = 5), respectively]. Microinjections of vehicle in sham-operated and SNI rats did not change the tail flick-induced ON cell burst [5.6 ± 1.0 spikes/s (n = 5) and 7.89 ± 0.3 spikes/s (n = 8), respectively] (Fig. 5A), the onset of ON pause was significantly decreased in SNI rats compared with both sham-operated and healthy rats [P < 0.05 (n = 5) and P < 0.05 (n = 5)]. The OFF cells had a mean pause duration of 2,900 ± 200 ms and 3,400 ± 200 ms in sham-operated and SNI rats, respectively. The mean of the frequency of the OFF cell pause was significantly increased in SNI rats compared with both sham-operated and healthy rats [P < 0.05 (n = 5)]. The OFF cells had a pause duration of 2,900 ± 200 ms and 3,400 ± 200 ms in sham-operated and SNI rats, respectively. The mean of the frequency of the OFF cell pause was significantly increased in SNI rats compared with both sham-operated and healthy rats [P < 0.05 (n = 5)]. The OFF cells had a pause duration of 2,900 ± 200 ms and 3,400 ± 200 ms in sham-operated and SNI rats, respectively. The mean of the frequency of the OFF cell pause was significantly increased in SNI rats compared with both sham-operated and healthy rats [P < 0.05 (n = 5)]. The OFF cells had a pause duration of 2,900 ± 200 ms and 3,400 ± 200 ms in sham-operated and SNI rats, respectively. The mean of the frequency of the OFF cell pause was significantly increased in SNI rats compared with both sham-operated and healthy rats [P < 0.05 (n = 5)].

Fig. 4. Effects of intra-DS microinjections of vehicle or AZ2216052 (20 nmol; A and B) or vehicle or VU0155041 (VU, 40 nmol; C and D) on the spontaneous firing of RVM ON or OFF cells in sham and SNI rats at 14 days after sciatic nerve surgery. Vehicle and drugs were administered at time 0. Each point represents the mean ± SE of 5 neurons. Statistically significant difference: *vs. sham/vehicle, †vs. SNI/vehicle. P values < 0.05 were considered statistically significant.
cell burst [2,240 ± 28 ms (n = 5) and 1,700 ± 200 ms (n = 5), respectively] (Fig. 5C), the OFF cell pause [6.48 ± 1.35 s (n = 6) and 15.8 ± 2.5 s (n = 5), respectively] (Fig. 5B) and the onset of the OFF cell pause [2,520 ± 78 ms (n = 6) and 1,420 ± 100 ms (n = 5), respectively] (Fig. 5D). Intra-DS microinjections of (S)-3,4-DCPG (1 and 2 nmol) caused a decrease in both ON cell burst [1.6 ± 1.0 spikes/s (P < 0.05, n = 5) and 0.3 ± 0.6 spikes/s (P < 0.05, n = 5), respectively] (Fig. 5A) and OFF cell pause [8.8 ± 1.8 s (P < 0.05, n = 5) and 3.95 ± 1.85 s (P < 0.05, n = 5), respectively] (Fig. 5B) and an increase in both the onset of the ON cell burst [2,350 ± 180 ms (n = 5) and 2,400 ± 108 ms (n = 5), respectively] (Fig. 5C) and the onset of the OFF cell pause [2,400 ± 230 ms (n = 5) and 2,250 ± 140 ms (n = 5), respectively] (Fig. 5D) in SNI rats. The higher dose of (S)-3,4-DCPG did not change the tail flick-related activity in sham-operated rats (not shown).

Figure 6 shows representative ratemeter records showing the ongoing and tail flick-related activity of ON (Fig. 6, A, C, and E) and OFF (Fig. 6, B, D, and F) cells before and after (S)-3,4-DCPG in sham-operated (Fig. 6, A and B) and SNI (Fig. 6, C, D, and E) rats. The effect of (S)-3,4-DCPG (2 nmol) on the burst of firing and the onset of the burst of the ON cells as well as the pause duration and onset of pause of OFF cells was antagonized by LY341495 (10 μmol) in SNI rats (Fig. 5).

Fig. 5. Effects of intra-DS microinjections of vehicle or (S)-3,4-DCPG (1 and 2 nmol) on tail flick-evoked ON cell burst of firing (A) and onset of the burst (C) or tail flick-evoked OFF cell pause (B) and onset of the pause (D) in sham-operated and SNI rats at 14 days after sciatic nerve surgery. Vehicle and drugs were administered at time 0. Each point represents the mean ± SE of 5–10 neurons/group. Statistically significant difference: *vs. sham/vehicle, ºvs. SNI/vehicle, †vs. SNI/DRCG (2 nmol). P values < 0.05 were considered statistically significant.

Effect of intra-DS AZ2216052 or VU0155041 on tail flick-related ON and OFF cell activity in sham-operated and SNI rats. Intra-DS microinjections of AZ2216052 (20 nmol) caused a significant decrease in both ON cell burst (3.5 ± 0.4 spikes/s, P < 0.05, n = 5) and OFF cell pause (5 ± 0.3 s, P < 0.05, n = 5) (Fig. 7, A and B). Moreover, AZ2216052 (20 nmol) significantly increased the onset of the ON cell burst and of the OFF cell pause [2,272 ± 189 ms (n = 5) and 2,329 ± 113 ms (n = 5), respectively] and the onset of the OFF cell pause [2,296 ± 245 ms (n = 5) and 2,181 ± 129 ms (n = 5), respectively] in SNI rats. Intra-DS microinjections of VU0155041 (40 nmol) did not affect the tail flick-related of both ON and OFF cell activity in sham-operated or SNI rats (Fig. 7, C and D).

Effect of intra-DS (S)-3,4-DCPG on mechanical allodynia in sham-operated and SNI rats. The MWT in the sham-operated rats was 29.1 ± 5 g before any treatment. Microinjection of vehicle did not change the MWT in the sham-operated rats (Fig. 8A). The intra-DS microinjection of (S)-3,4-DCPG (2 nmol) did not show significant changes in MWT in the sham-operated rats (1 nmol not shown). SNI of the sciatic nerve resulted in a significant decrease in MWT (11 ± 7 g, P < 0.05, n = 8) in the ipsilateral paw of rats 14 days after surgery (Fig. 8), while there were no significant changes on the contralateral paw.
The intra-DS microinjection of \((S)-3,4\)-DCPG (1 and 2 nmol) induced a significant increase in MWT \([26.2 \pm 0.8 \text{ g} (P < 0.05, n = 10) \text{ and } 24.7 \pm 2.0 \text{ g} (P < 0.05, n = 10)]\) vs. \(12 \pm 0.7 \text{ g} \text{ and } 28 \pm 0.6 \text{ g} (P < 0.05, n = 10)\); \(25 \pm 1.46 \text{ g} (P < 0.05, n = 8) \text{ vs. } 11 \pm 7 \text{ g} \text{, respectively}\) (Fig. 8A). MSOP (100 nmol) microinjected in combination with \((S)-3,4\)-DCPG (2 nmol) antagonized the effects of the latter on mechanical withdrawal latency in the SNI rats (Fig. 8B). MSOP alone did not change the MWT in the SNI rats (not shown). No overt behavioral changes were observed in this study after intra-DS administration of the drugs used in freely moving, unanesthetized rats. Rats remained alert and generally active throughout the experiment.

**Rotarod.** In the rotarod test, neither vehicle (228 \pm 15 s) or \((S)-3,4\)-DCPG (1 nmol) (238 \pm 10 s) affected motor performance compared with pretreatment latency (240 \pm 12 and 233 \pm 10 s, respectively) when administered in DS of sham-operated rats.

**Expression and localization of mGluR8 receptor.** The semi-quantitative analysis of mRNA levels in the contralateral DS of SNI rats, measured by RT-PCR amplification, showed a significant decrease of mGluR8 gene expression \((HPRT 1.9 \pm 0.21, 18S 0.65 \pm 0.20, \text{ and GAPDH } 0.60 \pm 0.22; \text{ mean } \pm \text{ SE arbitrary units, } P < 0.05, n = 5)\) compared with the contralateral DS of sham-operated rats \((HPRT 3.00 \pm 0.30, 18S 1.30 \pm 0.11, \text{ and GAPDH } 1.25 \pm 0.10)\) (Fig. 9, A and B). No changes of mGluR8 gene expression were detected in the ipsilateral DS of SNI rats compared with the ipsilateral DS of sham-operated rats. Western blot analysis showed a significant increase of mGluR8 protein level in contralateral DS of SNI rats \((HPRT 0.51 \pm 0.05, 18S 0.55 \pm 0.03, \text{ and GAPDH } 0.28 \pm 0.05)\) compared with the contralateral DS of sham-operated rats \((HPRT 0.15 \pm 0.03, 18S 0.15 \pm 0.02, \text{ and GAPDH } 0.40 \pm 0.04)\). A significant decrease of the mGluR8 protein level was also found in the ipsilateral DS of the SNI rats vs. the ipsilateral DS of the sham-operated rats \((HPRT 0.31 \pm 0.03, 18S 0.33 \pm 0.02, \text{ and GAPDH } 0.56 \pm 0.04)\).
Finally, immunofluorescence showed mGluR8 localization in the DS on the VGAT-expressing neurons, rather than VGLUT1. The localization mGluR8-positive profile was counterstained with the presynaptic marker synaptophysin (Fig. 10, left). Moreover, a double staining with mGluR8 and parvalbumin did not reveal significant expression of mGluR8 on parvalbumin-positive GABAergic interneurons (Fig. 10, right).

**DISCUSSION**

The first finding of the study was that intra-DS mGluR8 stimulation by (S)-3,4-DCPG, a selective mGluR8 agonist (Thomas et al. 2001), increased thermal threshold and MWT in the SNI rats. However, intra-DS (S)-3,4-DCPG failed to change pain responses in sham-operated rats.

mGluR8 stimulation has already been evaluated in the VL PAG and CeA, where it reduced pain responses in normal and chronic pain conditions (Marabese et al. 2007a, 2007b; Palazzo et al. 2008, 2011) and proved to be associated with GABA decrease and glutamate increase (Marabese et al. 2005, 2007a; Palazzo et al. 2011). Within the nucleus tractus solitarii (NTS), mGluR8 stimulation produced instead a pain facilitatory effect on cardiac nociception (Liu et al. 2012). These different pain responses may be related to the mGluR8 location on different neuron terminals within supraspinal sites modulating pain perception differently.

mGluR8 proved to be expressed on presynaptic sites of asymmetric and symmetric synapses within the PAG (Marabese et al. 2005). Since the GABAergic neural population constitutes ~50% of total neural elements (the majority are active tonic interneurons) of the PAG controlling its intrinsic antinociceptive activity (Reichling and Basbaum 1991; Reynolds 1969), mGluR8 stimulation would induce an inhibition of GABAergic tone and consequent PAG disinhibition, which is consistent with the antinociceptive effect (Marabese et al. 2005, 2007a, 2007b). Within the CeA, mGluR8 proved to be expressed on GABAergic terminals, and its stimulation reduced pain responses and GABA release (together with glutamate and serotonin increase) (Palazzo et al. 2008, 2011). In the NTS, mGluR8 stimulation activated the descending facilitatory pathway and enhanced the nociceptive transmission at the spinal cord level (Liu et al. 2012).

Beside pain responses, in the present study we also investigated whether mGluR8 receptor stimulation within the DS was able to affect the activity of the ON and OFF cells of RVM. Among the several forebrain or brain stem nuclei, the RVM is also under the influence of the DS. There are no direct projections from DS to the RVM. The convergence point among DS and the RVM seems to lie in the medullary dorsal reticular nucleus (DRt). DRt receives projections from globus pallidus and substantia nigra pars reticulata (which in turn receive...
projections from the DS) and sends inputs to the RVM (Leite-Almeida et al. 2006), as illustrated in Fig. 11. Thus the DRt is strategically positioned among the extrapyramidal and pain descending systems, indicating a possible involvement in the motor reaction to pain. It has been also recently reported that RVM mediates the analgesic action of the striatum: lesion of RVM blocked the inhibition control of striatum on orofacial pain (Barceló et al. 2012). Evidence that stimulation of striatum leads to pain reflex inhibition (Belforte et al. 2001) through RVM, however, has been scarcely investigated so far (Barceló et al. 2012). Within the RVM, ON cells are activated by nociceptive stimuli and have a pain facilitatory effect (Fields et al. 1983), whereas OFF cells are inactivated and have an inhibitory effect on nociception (Fields et al. 1983, 2006; Heinricher and Tortorici 1994; Kincaid et al. 2006; Neubert et al. 2004). We found in this study, as already shown in others (Gonçalves et al. 2007; Ossipov and Porreca 2006; Palazzo et al. 2011, 2012; Pertovaara 2000; Porreca et al. 2001, 2002), that RVM cell activity changed in neuropathic pain conditions: the ongoing and tail flick-evoked activity of OFF cells decreased and the pause and onset of the pause of the OFF cells increased and decreased, respectively. Thus it appears that ON and OFF cell activity in the RVM undergoes functional phenotypic changes after SNI that lead to ON cell hyperactivity and OFF cell hypoactivity. The microinjection of (S)-3,4-DCPG into the DS increased the ongoing activity of the OFF cells and inhibited that of the ON cells in rats with SNI. (S)-3,4-DCPG also reduced tail flick-induced ON cell burst and OFF cell pause and increased the onset of both ON cell burst and OFF cell pause in the same animals. A delayed onset of the ON cell burst, as well as the reduction of the burst of firing, proved to be critical events in the occurrence of antinociception (Foo and Mason 2003; Heinricher et al. 1989; Jinks et al. 2004). Moreover, an inhibition of pain transmission correlates with an increase of spontaneous OFF cell activity and a reduction of its pause as well as a delay of the pause onset (Heinricher and Tortorici 1994). Thus, interestingly, mGluR8 stimulation in the DS modified the ongoing and tail flick-evoked activity of RVM ON and OFF cells, consistent with the antinociceptive effect produced by DS mGluR8 stimulation in the tail flick test and in mechanical allodynia.

(5)-3,4-DCPG has already been shown to inhibit RVM ON cell and enhance OFF cell activity consistently with mGluR8-induced antinociception in SNI rats when locally microinjected into the VL PAG or CeA (Marabese et al. 2007b; Palazzo et al. 2011). A critical finding of this study was, however, that administration of (5)-3,4-DCPG in the DS was devoid of effect in sham-operated rats. Indeed, intra-DS administration of (5)-3,4-DCPG did not change thermal nociception, MWT, and the ongoing and tail flick-evoked ON and OFF cell activity in sham-operated animals. Evidence that group III mGluR stimulation inhibits pain in different pathological pain states of various etiologies but not in normal conditions has been already reported (Chen and Pan 2005; Goudet et al. 2008; Neugebauer 2006; Palazzo et al. 2008, 2011, 2013; Zhang et al. 2009). In particular, we have found that intra-CeA (5)-3,4-DCPG failed to change pain and affective behavior, neurotransmitter release, and RVM cell activity in physiological conditions (Palazzo et al. 2008, 2011). Accordingly, (5)-3,4-DCPG did not affect baseline synaptic transmission in hippocampal slices (Ayala et al. 2008) and failed to modify anxiety-like behavior when administered in the basolateral amygdala (Stachowicz et al. 2005). Therefore the analgesic effect of mGluR8 activation requires some neuroplasticity generated by pathological conditions, including chronic pain, which may change mGluR8 sensitivity. Indeed, in our previous study the expression of mGluR8 proved to be upregulated in the CeA after peripheral carrageenan administration (Palazzo et al. 2011). Apart from a change of mGluR8 expression, other factors have to be taken into consideration to justify why mGluR8 stimulation reduces pain responses and RVM cell activity only in neuropathic conditions: 1) the control of glutamate level by mGluR8, which is critical for maintaining normal sensory transmission, may be increased in pathological conditions associated with glutamatergic overactivity (Dougherty et al. 1992; Leem et al. 1996; Santangelo et al. 2012; Töllö et al. 1996); 2) the neuron terminals expressing mGluR8 may be normally inactive and be activated by nerve injury; and 3) the downstream effectors associated with mGluR8 may be overexpressed such that even if a decrease of receptor expression is detected an improved regulation of mGluR8 signal can result. The analysis of mGluR8 expression carried out in this study showed that mGluR8 mRNA level was downregulated in the DS of SNI rats in contralateral, but not ipsilateral, DS. Intriguingly, the protein levels showed an opposite trend in the contralateral DS of SNI. Indeed, while mGluR8 protein levels proved to be increased in the contralateral side, a reduction was observed in the ipsilateral DS, as also evidenced...
by immunofluorescence outcomes. These findings could explain, at least in part, why (S)-3,4-DCPG exerts an antinociceptive effect, also in terms of RVM ON and OFF cell activity in SNI but not sham-operated animals. However, it is still critical to explain the different mGluR8 mRNA and protein changes in neuropathic conditions that deserve further investigations.

When MSOP, a group III mGluR antagonist, was administered alone it did not change mechanical allodynia, notwithstanding mGluR8 expression changes in SNI animals. However, it is still critical to explain the different mGluR8 mRNA and protein changes in neuropathic conditions that deserve further investigations.

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both thermal threshold increase and RVM ON and OFF cell activity changes. Moreover, we carried out experiments with AZ12216052 and VU0155041, two selective mGluR8 and mGluR4 PAMs, respectively. While AZ12216052 was able to increase the tail flick latency and to decrease and increase the ON and OFF cell activity in SNI rats, as (S)-3,4-DCPG did, VU0155041 was not. These further experiments altogether underline the role of mGluR8 in modulating pain responses and

![Image of mGluR8 and synaptophysin colocalization](image_url)

Fig. 10. Colocalization of mGluR8 (green) and the presynaptic marker synaptophysin (red). Left column shows counterstaining of the mGluR8-positive profiles and synaptophysin, although several synaptophysin-positive profiles were negative to mGluR8. Right column shows that mGluR8-positive profiles do not colocalize with parvalbumin-positive GABAergic interneurons (red). Some mGluR8 counterstained with the parvalbumin-positive profiles (not shown). Scale bars, 100 μm (main images) and 50 μm (insets).

![Image of DS connections](image_url)

Fig. 11. A simplified scheme illustrating the DS connections to the RVM via the dorsal reticular nucleus area. Dorsal reticular nuclei receive projections from globus pallidus and substantia nigra pars reticulata (SNr), which in turn receive projection from DS. The effect of mGluR8 stimulation on RVM ON and OFF cells [which is part of the descending pain pathway that includes periaqueductal gray (PAG)] is far from being direct.
RVM cell activity at the DS level. By contrast, mGluR₄ does not seem to be involved.

In conclusion, mGluR₈ stimulation in the DS of SNI rats 1) inhibited thermoceptive responses in the tail flick test in anesthetized SNI rats, 2) changed RVM cell activity accordingly to behavioral analgesia, and 3) reduced mechanical allodynia in a way that was antagonized by MSOP, a group III mGluR antagonist. mGluR₈ stimulation did not affect motor coordination in the rotarod experiments, RVM activity, and pain responses in sham-operated rats. The effects of (S)-3,4-DCPG stimulation only in the neuropathic pain condition, associated to mGluR₈ gene decrease and protein increase, deserve further study.

**REFERENCES**

[Chen SR, Pan HL. Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 75: 889–907, 2000.]
[Chen SR, Pan HL. Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 75: 889–907, 2000.]
[Chudler FH, Dong WK. Distribution and synaptic localization of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS. Neuroscience 110: 403–420, 2002.]
[De Novellis V, Mariani L, Palazzo E, Vita D, Marabese I, Scaffuro M, Rossi F, Maione S. Periaqueductal grey CB1 cannabinoid and metabotropic glutamate subtype 5 receptors modulate changes in rostral ventromedial medulla neuronal activities induced by subcutaneous formalin in the rat. Neurosci Lett 134: 269–281, 2005.]
[Dong E, Wellman LL, Yang L, Sanford LD. Effects of microinjections of Group II metabotropic glutamate agents into the amygdala on sleep. Brain Res 1452: 85–95, 2012.]
[Dougherty PM, Palecek J, Paleckova V, Sorkin LS, Willis WD. The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. J Neurosci 12: 3025–3041, 1992.]
[Jinks SL, Carstens E, Antognini JF. Isofuranec differentially modulates medullary on and off neurons while suppressing hind-limb motor withdrawal. Anesthesiology 100: 1224–1234, 2004.]

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Ren W, Palazzo E, Maione S, Neugebauer V. Differential effects of mGluR7 and mGluR8 activation on pain-related synaptic activity in the amygdala. Neuropharmacology 8: 1334–1344, 2011.


Testa CM, Friberg IK, Weiss SW, Standaert DG. Immunohistochemical localization of metabotropic glutamate receptors mGluR1a and mGluR2/3 in the rat basal ganglia. J Comp Neurol 390: 5–19, 1998.


