PAIN® xxx (2013) xxx–xxx

PAIN®

www.elsevier.com/locate/pain

Disturbances in slow-wave sleep are induced by models of bilateral inflammation, neuropathic, and postoperative pain, but not osteoarthritic pain in rats

Laura J. Leys, Katharine L. Chu, Jun Xu, Madhavi Pai, He S. Yang, Holly M. Robb, Michael F. Jarvis, Richard J. Radek, Steve McGaraughty *

Neuroscience Research, AbbVie, Inc., North Chicago, IL, USA

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

ARTICLE INFO

Article history:
Received 7 September 2012
Received in revised form 31 January 2013
Accepted 12 March 2013
Available online xxxx

Keywords:
Sleep
EEG
Chronic pain
Inflammation
Osteoarthritis
Neuropathic
Post-operative

ABSTRACT

Preclinical assessment of pain has typically relied on measuring animal responses to evoked stimulation. Because of inherent limitations of these assays, there is a need to develop measures of animal pain/discomfort that are objective, not experimentally evoked, and mimic the human condition. Patients with chronic pain manifest a variety of co-morbidities, one of which is disturbances in sleep. We used electroencephalography to objectively assess 4 rat models of pain (inflammatory/complete Freund's adjuvant [CFA], neuropathic/chronic constriction injury [CCI], postoperative/skin incision, osteoarthritis/monosodium iodoacetate [MIA]) for the occurrence of sleep disturbances. Four different measures of slow-wave sleep (SWS) were examined: amplitude of 1- to 4-Hz waves, total time spent in SWS, time spent in SWS-1, and time spent in SWS-2. Bilateral injuries were more likely to induce a sleep disturbance than unilateral injuries in the CFA, CCI, and skin incision assays. Sleep disturbances occurred in the deeper stage of SWS, as the amplitude of 1- to 4-Hz waves and time spent in SWS-2 were significantly decreased in all models except the osteoarthritis model. Sleep disturbances lasted for approximately 3 to 14 days, depending on the model, and were resolved despite continued hypersensitivity to evoked stimulation. Morphine, gabapentin, diclofenac, and ABT-102 (TRPV1 antagonist) all improved sleep in the bilateral CFA assay at doses that did not significantly alter SWS in uninjured rats. Preclinical assessment of compounds should follow the path of clinical studies and take into account diverse aspects of the "pain condition." This would include evaluating nociceptive thresholds as well as other endpoints, such as cognition and sleep, that may be affected by the pathological state.

1. Introduction

Pain is difficult to assess in an animal, particularly rodents. As prey, rodents have adapted mechanisms to hide injury or weakness from predators [5]. Preclinical assessment of pain has typically used thermal, mechanical, or chemical stimuli to evoke an animal escape response and measure changes to threshold, latency, or other overt behaviors. How these assays are performed can vary between laboratories and between investigators, and the interpretation of a rodent response can be subjective, thus lending itself to unintentional bias. In addition, although changes to latency and threshold after an experimental manipulation (eg, drug administration) may in fact be due to direct modulation of the nociceptive system, it is also possible that changes to these behavioral endpoints are the result of unintended effects such as shifting of attention or anxiety, altering cardiovascular function or motor activity, and compromised health [19,26,54,58,62]. The translatability of these "evoked" assays to human pain conditions has also been questioned, particularly because the primary reason to seek medical attention is due to spontaneous, non-evoked pain [7,8]. Although there have been recent advances to rodent assessment of behavioral state in models of pain [4,33,40,49,81], there is a continued need to develop measures of discomfort that are both objective and not experimentally evoked.

Patients with chronic pain endure multiple behavioral impairments that affect their quality of life, including sexual dysfunction, depression, diminished appetite, diminished activity, decline in social relationships, lowered cognitive function, and interrupted sleep [11,24,28,48,61,64,74]. The occurrence of these functional impairments along with spontaneous pain are a major, or in some

* Corresponding author. Address: Neuroscience Research, AbbVie, Inc., R4N5, AP9-1, 1 N. Waukegan Road, North Chicago, IL 60064, USA. Tel.: +1 847 935 6826.
E-mail address: Steve.P.McGaraughty@abbvie.com (S. McGaraughty).

0304-3959/$36.00
http://dx.doi.org/10.1016/j.pain.2013.03.019

cases the primary concern of those who experience chronic pain [7,11]. Disturbances in sleep are observed in chronic pain patients with different etiologies, including fibromyalgia, arthritis, inflammation, and nerve damage [22,43,60,69,71,73,79]. Despite the observation of a pain-induced sleep disturbance in patients, there has been only limited use of this endpoint in preclinical studies. These preclinical animal models have had varying levels of success, with results ranging from significant sleep disturbances [3,39,68] to limited or conditional disturbances [32,75], and to no sleep disturbance [35,78].

The current study investigates 4 rat models of pathological pain using electroencephalography (EEG) to determine the impact on slow-wave sleep (SWS). We wanted to include a diverse spectrum of pain etiologies in this analysis, and thus models of inflammation, neuropathic, osteoarthritis, and postoperative pain were examined for a pain-induced sleep disturbance (PISD). Finally, reference analgesics were evaluated for effectiveness to improve sleep in an inflammation-induced PISD assay.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 400 to 550 g were used for these studies. Animals were approximately 3 months old upon arriving at AbbVie (North Chicago, IL) and were immediately single housed in individually ventilated cages in an American Association for Accreditation of Laboratory Animal Care–approved housing facility at AbbVie. Room temperature and humidity were maintained at 68° to 76°F and 30% to 70%, respectively, with a normal 12-hour light–dark cycle (home cage and laboratory lights on, 06:00-18:00; off 18:00-06:00). Animals were housed on aspen chip bedding (Sanichip, PJ Murphyp, Montville, NJ). To control and maintain animal weight, rats were kept on a maintenance diet of approximately 17 g of Teklad Global 18% protein rodent diet (Harlan, Madison, WI) per day with water available ad libitum. During the study, animals were weighed 5 times per week, and before the study were weighed 3 times per week. All animal handling and experimental protocols were approved by AbbVie's Institutional Animal Care and Use Committee and adhered to the guidelines of the Committee for Research and Ethical Issues of IASP [82].

2.2. Implantation of EEG electrodes

To surgically implant EEG electrodes, rats were anesthetized with isoflurane (4% induction followed by 2–3% maintenance) and placed in a stereotaxic apparatus. Holes were drilled into the skull over the frontal (AP +3.0 mm, ML ±2 mm to bregma) and parietal cortices (AP −3 mm, ML ±4 mm to bregma), the cerebellum (AP −10.0 mm, ML −1 mm to bregma), and over the fontal bone (AP +6 mm, ML +1 to bregma). Wire leads attached to stainless steel screws (Plastics One, Roanoke, VA) served as EEG electrodes, and were threaded into the drill holes to a depth no greater than 1 mm into the skull. This depth has previously been determined not to penetrate the dura or brain tissue. The wire lead from each electrode was inserted into a muti-channel electrode pedestal (Plastics One, Roanoke, VA) and permanently affixed to the skull with dental acrylic. An acute dose of the postoperative analgesic banamine (2.5 mg/kg SC) was administered after the surgery, and rats were allowed 1 to 2 weeks of recovery from the surgical procedure. During this recovery period, animals were handled but EEG recordings were not conducted. It was at least another 2 weeks after implant recovery that the animals underwent procedures for pain modeling.

2.3. Pain models

2.3.1. Inflammatory pain

Inflammation was induced by either a unilateral or bilateral injection of complete Freund's adjuvant (CFA; Sigma, St. Louis, MO) into the plantar surface of the hind paw(s) using a 26-gauge needle. CFA was suspended 1:1 in phosphate-buffered saline and injected at a volume of 150 μL per paw. Control rats did not receive any manipulations.

2.3.2. Neuropathic pain

The neuropathic condition was induced by either a unilateral or bilateral chronic constriction injury (CCI) to the sciatic nerve(s). Rats were anesthetized with isoflurane (4% induction followed by 2% to 3% maintenance), and a 1.5-cm incision was made dorsal to the pelvis. The common sciatic nerve was exposed and isolated from surrounding tissue. Four ligatures (5.0 chromic gut) were placed circumferentially around the sciatic nerve (<1 mm spacing). For the sham group, the common sciatic nerves on both sides were exposed and isolated from surrounding tissue but were not ligated. For both CCI and sham animals, the skin was closed with tissue glue (Gluture, Abbott Laboratories, Abbott Park, IL).

2.3.3. Acute postoperative pain

Acute postoperative pain was induced by performing either unilateral or bilateral skin incision surgery to the hind paw(s). Rats were anesthetized with isoflurane (4% induction followed by 2–3% maintenance) and a 1-cm incision was made through the plantar skin and fascia of the hind paw(s). The plantaris muscle was then elevated and incised longitudinally. The skin incision was closed with 2 mattress sutures (5-0, nylon). Sutures were removed after testing on postoperative day 2. Control rats did not receive any manipulations.

2.3.4. Osteoarthritic pain

Osteoarthritis was induced by injecting monosodium iodoacetate (MIA; Sigma, St. Louis, MO) into both knee joint cavities using a 28-gauge needle. Animals were anesthetized with isoflurane (4% induction followed by 2–3% maintenance) during this procedure. MIA was injected at a dose of 3 mg, dissolved in 50 μL of saline solution. Control rats did not receive any manipulations.

2.4. Behavioral characterization

2.4.1. Tactile allodynia

Tactile allodynia was measured using calibrated von Frey filaments (Stoelting, Wood Dale, IL) in CFA, CCI, skin-incised, and respective control rats. Rats were placed into inverted individual plastic containers (20 × 12.5 × 20 cm) on top of a suspended wire mesh grid, and acclimated to the test chambers for 20 minutes. Filaments with different bending forces (progressively increasing from the lowest force) were presented perpendicularly to the plantar surface of the selected hind paw, and then held in this position for approximately 8 seconds with enough force to cause a slight bend in the filament. Positive responses included an abrupt withdrawal of the hind paw from the stimulus, or flinching behavior immediately after removal of the stimulus. The maximum force applied was 26 g.

2.4.2. Grip force

Compressive grip force (CGF) was determined in MIA-OA and control rats by recording the maximum compressive force exerted on a hind limb strain gauge, using a commercially available grip force measurement system (Columbus Instruments, Columbus, OH). In this assay, each rat was gently restrained and both hind paws were allowed to grasp the wire mesh frame (10 × 12 cm)

Please cite this article in press as: Leys LJ et al. Disturbances in slow-wave sleep are induced by models of bilateral inflammation, neuropathic, and postoperative pain, but not osteoarthritic pain in rats. PAIN® (2013), http://dx.doi.org/10.1016/j.pain.2013.03.019