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The elusive rat model of conditioned placebo analgesia

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ABSTRACT

Recent research on human placebo analgesia has suggested the need for rodent models to further elucidate the neural substrates of the placebo effect. This series of 3 experiments therefore was performed in an attempt to develop a model of placebo analgesia in rats. In each study, female Sprague-Dawley rats received an L5 spinal nerve ligation to induce a neuropathic pain condition. Each rat then underwent a 4-day conditioning procedure in which an active analgesic drug or its vehicle (unconditioned stimulus) was associated with the following cues (conditioned stimuli): novel testing room (environmental), vanilla scent cue (olfactory), dim incandescent lighting (visual), restraint procedure/injection (tactile), and time of day and injection-test latency (temporal). The analgesics for each experiment were as follows: Experiment 1 used 90 mg/kg gabapentin, experiment 2 used 3 mg/kg loperamide hydrochloride, and experiment 3 used 6 mg/kg morphine sulfate. On the following test day, half of the animals received the opposite treatment, resulting in 4 conditioning manipulations: drug/drug, drug/vehicle, vehicle/drug, and vehicle/vehicle. Nociceptive thresholds were assessed with the mechanical paw withdrawal threshold test each day after the conditioning procedure. In all 3 experiments, no significant differences were detected on test day between control and placebo groups, indicating a lack of a conditioned placebo analgesic response. Our results contrast with prior research that implies the existence of a reliable and robust response to placebo treatment. We conclude that placebo analgesia in rats is not particularly robust and that it is difficult to achieve using conventional procedures and proper experimental design.

1. Introduction

Recent research on placebo analgesia has revealed a need for animal models to elucidate the neural mechanisms of the effect. The current theoretical approaches to the placebo response are expectancy theory and conditioning theory. Expectancy theory suggests that placebo analgesia is a function of the expectation of treatment efficacy [49], whereas conditioning theory contends that placebo analgesia is a classically conditioned response [60,64] that can be influenced by prior experience with active analgesics [3,7]. Because conditioning can induce expectation, these 2 theories are not mutually exclusive, and therefore both contribute to our understanding of placebo responses [49,61].

Nearly all of the research seeking to study the neural underpinnings of placebo analgesia has been conducted with human subjects using functional magnetic resonance imaging (fMRI), which has a limited ability to elucidate mechanisms at the molecular and cellular levels. This has led some researchers to call for the development of animal models of the placebo effect [43,48]. The theories of placebo analgesia suggest that a rat model of the placebo effect could use simple classical conditioning paradigms to elicit the expectation of an analgesic effect.

There have been a number of studies published in the last 50 years that have reported a range of nonanalgesic placebo effects in rats, including the induction of learning deficits [25], decreased locomotor activity [27,51], immobility in a forced swim test [12], hyperactivity [53], and immunosuppression [1]. Recent research on rodent placebo effects is sparse, but there have been 2 reports of conditioned analgesic responses in mice [21,22] and 2 in rats [47,66]. One of the rat publications [47] was praised as "a significant advance" in the field of placebo research [2]. However, each model has drawbacks, which include the required use of an unconventional strain of hairless rats and/or the lack of replication by other laboratories. Thus, the need for a more robust and easily reproducible rat model of placebo analgesia persists.

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We therefore sought to develop a rat model of placebo analgesia based on conditioning experiments, the study of pain, and studies of the placebo effect in humans. Three studies were conducted, each with a nearly identical procedure except for the pharmaceutical intervention, and all of which revealed critical information regarding placebo research in the rat.

2. Methods

Adult female Sprague-Dawley rats from the University of Texas at Arlington vivarium, weighing approximately 200 to 300 g, were used in each experiment (experiment 1, n = 37; experiment 2, n = 41; experiment 3, n = 40). Animals were housed in groups of 3 to 5 and maintained on a 12:12 hour light/dark cycle with free access to food and water throughout the study. All procedures were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee and were conducted in accordance with the guidelines put forth by the International Association for the Study of Pain [67] as well as the Committee for Research and Ethical Issues of IASP.

Female rats were used as per the recommendations of the Sex, Gender, and Pain Special Interest Group of the International Association for the Study of Pain (IASP) [19]. Although we acknowledge certain sex differences in adipose tissue [39], pharmacokinetics [44,45], immune responses [4], as well as enhanced morphine efficacy in male versus female rats [62], there is direct evidence that the estrus cycle of female rats does not modulate behavioral assessments of pain in rodents [62]. Therefore, the use of exclusively female rats should not confound the present study. Also, the IASP special interest group report asserts that, since the most common human pain sufferers are women, the ideal model subject is a female animal [19]. Given that this study sought to develop a rodent model of a human pain-related phenomenon and that estrous cycle does not modulate behavioral measures of pain in rats, it seemed appropriate to use female animals for this study.

2.1. Surgical procedure to induce neuropathic pain

On day 1, all animals received a unilateral left-side L5 spinal nerve ligation (SNL) as described previously [28]. Animals were anesthetized with 3% isoflurane for induction and 2% for maintenance. Depth of anesthesia was confirmed by the absence of the eye blink reflex as well as the withdrawal reflex to pinch stimulation of the hind paws. The animal's health was monitored throughout anesthesia with periodic checks of breathing rate and reflexive responses. The incision area was shaved and then cleaned with povidone-iodine. The first incision was 1.5 to 2 inches in length and was 2 to 3 millimeters lateral and to the left of the spinal cord. Muscle tissue was removed to expose the overlying transverse process, which was also removed. The L5 spinal nerve was exposed and tightly ligated using 6-0 silk thread. Povidone-iodine was applied once again to the wound before suturing the internal tissue with 4-0 silk thread and closing the overlaying skin with surgical staples. Animals were allowed to recover for 3 full days (days 2–4) before any behavioral tests were conducted. Postoperative signs of infection or discomfort were closely monitored during the recovery phase as well as during behavioral testing. The L5 spinal nerve ligation model of neuropathic pain was chosen for these studies because the resulting hypersensitivity to mechanical stimulation is long-lasting, relatively stable over time, responsive to treatment with gabapentin, loperamide, and morphine [20,28,34], and there is no evidence that the model negatively affects cognition [57].

2.2. Drug preparation

The primary difference among the studies was the type of analgesic treatment used: experiment 1 used gabapentin, experiment 2 used loperamide, and experiment 3 used morphine. Gabapentin was mixed at a concentration of 90 mg/mL in .9% normal saline solution and administered subcutaneously (s.c.) at a dosage of 90 mg/kg. Loperamide hydrochloride, a peripherally acting μ-opioid receptor agonist, has been shown in double-blind placebo-controlled trials to alleviate abdominal pain in irritable bowel syndrome in humans [13,38], but has only recently been investigated in animals for its antinociceptive properties in experimental pain [20]. Loperamide was prepared in a 20% solution of 2-hydroxypropyl-β-cyclodextrin (CDEX) at a concentration of 3 mg/mL and administered s.c. at a dosage of 3 mg/kg. Morphine sulfate was mixed in 0.9% normal saline solution at a concentration of 3 mg/mL and administered s.c. at a dosage of 6 mg/kg as per prior research.

In all 3 experiments, the concentration, dosage, and route of administration for the pharmaceuticals was chosen based on prior evidence of reliable attenuation of sensory pain thresholds as measured by the Mechanical Paw Withdrawal Threshold test [20,33,34]. All vehicle treatments were administered in the same volume as active drug to maximize similarity between the experience of control and experimental treatments.

In each experiment, the time from injection until testing was tailored to the pharmacodynamics of each drug in order to ensure that the testing took place when the analgesic effect was maximal. In the gabapentin experiment, testing took place 60 minutes after injection, whereas in the loperamide and morphine experiments, testing took place 30 minutes after injection [5,20,34].

Previous studies have shown that the selected doses of gabapentin and loperamide do not produce sedative effects. Gabapentin 50 mg/kg and 100 mg/kg did not elicit a change in exploratory locomotion relative to vehicle [50]. The ED50 for loperamide-induced piloerection (>160 mg/kg), loss of righting reflex (>160 mg/kg), ataxia (80 mg/kg), and hypotonia (89 mg/kg) are considerably higher than the dose used in the present research [37].

Although there is evidence of acute behavioral sedation 30 minutes after a single s.c. dose of 6 mg/kg morphine in male rats [50], female rats show significantly weaker sedation after a dose of 10 mg/kg [9]. Another study reported that 7 mg/kg administered intraperitoneally produced no sedation in female rats [26]. In addition, repeated dosing paradigms have shown that, after the initial dose, subsequent doses retain their analgesic efficacy, yet produce no sedative effect [40]. Consequently, previous experimenters have used repeated dosing to eliminate the sedative effect of morphine to selectively test its analgesic effect [14,23,52]. We have used the same approach in our experiments by testing the primary outcome measure after a fifth dose of morphine, which is well after the development of tolerance to sedation. Given that our experiments used females in a repeated dosing paradigm, it is reasonable to conclude that morphine sedation should not have affected responding on the test day.

2.3. Measurement of tactile allodynia

To assess mechanical hypersensitivity, a mechanical paw withdrawal threshold (MPWT) test was conducted before surgery (baseline), after the surgical recovery period (day 5 pre), and on each day after the treatment injection (day 5 post–day 9). To derive MPWT scores, animals were placed in a Plexiglas chamber (20 × 10.5 × 40.5 cm) and habituated for 10 minutes. The chamber

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