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The Orofacial Formalin Test in the Mouse: A Behavioral Model for Studying Physiology and Modulation of Trigeminal Nociception

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Abstract: **The aim of the current study was to adapt the orofacial formalin pain model previously developed in rats for use in mice and to characterize as fully as possible the behavioral changes in this species. The effects of subcutaneous injection of different formalin concentrations (.5%, 1%, 2%, 4%, and 8%) were examined on the face-rubbing response. In mice, formalin injection into the upper lip induced sustained face-rubbing episodes with vigorous face-wash strokes directed to the perinasal area. A positive linear relationship between formalin concentration and amplitude of the rubbing activity was observed during the first and second phase of the test with concentration up to 4%. With the highest concentration used (8%), the amplitude of both phases had plateaued. Systemic administration of morphine and paracetamol induced a dose-dependent inhibition of the rubbing behavior during the second phase. Although both paracetamol and morphine inhibited the first phase, a dose-dependent inhibition was found only for morphine. The ED50 value (95% confidence interval) for suppressing the rubbing response during the first phase was 2.45 mg/kg (1.90-3.08 mg/kg) for morphine. The ED50 values for suppressing the rubbing response during the second phase were 3.52 mg/kg (2.85-4.63 mg/kg) for morphine and 100.66 mg/kg (77.98-139.05 mg/kg) for paracetamol. Heterosegmental nociceptive stimulation evoked by subcutaneous injection of capsaicin into the back of the animal 10 min before the formalin test produced a dose-dependent inhibition of the second phase of the rubbing response. The ED50 values for suppressing the rubbing response during the first and second phases were 9.04 μg (1.36-65.13 μg) and 0.92 μg (0.28-2.99 μg), respectively. In conclusion, the mouse orofacial formalin test appears to be a reliable model for studying the behavioral encoding of the intensity of nociceptive orofacial stimulation and the counter-irritation phenomenon and for testing analgesic drugs.**

Perspective: ***To further exploit the new opportunities of investigating nociceptive processing at the molecular level with the transgenic "knockout" approach, we require suitable behavioral models in mice. The presented mouse orofacial formalin test appears to be a reliable model for studying the behavioral encoding of the intensity of nociceptive stimulation and the counter-irritation phenomenon and for testing analgesic drugs.***

Key words: *Rubbing behavior, morphine, paracetamol, capsaicin, pain, counter-irritation.*

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The orofacial region is one of the most densely innervated, by the trigeminal nerve, areas of the body, which focuses some of the most common acute pains, ie, those accompanying the pathologic states of the teeth and the related structures. It is also the site of frequent chronic post-herpetic neuralgia, migraine, and referred pains. However, the mechanisms underlying these pains are still poorly understood. In particular, there are relatively few behavioral models in laboratory animals dedicated to the study of nociception in the trigeminal region.[28,29] In addition, only few analgesic trials have been undergone in trigeminal region.

The recent acceleration of basic science studies of pain involving the mouse can largely be attributed to the development of transgenic "knockout" technology in this species only.[40] Indeed, a number of transgenic mouse models that display alterations in nociceptive behavior owing to targeted disruption of a gene encoding for a specific receptor, neurotransmitter, or second messenger molecule have recently been described. However, results of these studies have been variously confirmatory, contradictory, and enlightening compared with conventional investigations.[40] One reason for these discrepancies is the inconsistent application of behavioral essays of nociception to transgenic mice. This likely results from the small number of laboratories with extensive experience performing behavioral assays of nociception in the mouse[40] and from the limited number of models of nociception routinely used in mice. Therefore, to further exploit the new opportunities of investigating nociceptive processing at the molecular level with the transgenic "knockout" approach, we require a more extensive range of suitable behavioral models in mice. We also need to carefully validate behavioral assays of nociception in the mouse, particularly before adapting experimental protocols that were designed for rats. Indeed, the genetic, physiologic, and behavioral differences between rats and mice render such adaptations nontrivial.[40]

In the case of orofacial pain, there is no behavioral nociceptive test currently used in mice. Some years ago, we adapted the formalin test in the rat[14] to assess nociceptive processes in the orofacial region,[8] which has since been used with success.[29] The aim of the current study was to adapt the orofacial formalin pain model for use in mice and to characterize as fully as possible the behavioral changes evoked in this species. In particular, we carefully studied the relationship between the amount of time the mice spent rubbing their lip and the concentration of the formalin solution, investigated the effects of 2 analgesic drugs, morphine and paracetamol, and investigated the effect of concomitant application of a heterotopic noxious stimulus.

Materials and Methods

Animals

Adult male NMRI mice weighing 30-35 g (Charles Rivers, Les Oncins, France) were used in these experiments. They were housed in plastic cages (4 per cage) with soft bedding with free access to food and water and were maintained in climate- (23 ± 1°C) and light-controlled (12/12-h dark/light cycle with light on at 8:00 am) protected units (Iffa-Credo, L'Arbresle, France) for at least 1 week before the experiments. Test sessions took place during the light phase between 11:00 AM and 7:00 PM in a quiet room maintained at 23-24°C. The test box had the dimensions of 30 × 30 × 15 cm with 3 mirrored sides. Each animal was first placed in this box for a 10-min habituation period to minimize stress. The mice did not have access to food or water during the test. Each mouse was used only once and was killed at the end of the experiment by the administration of a lethal dose of pentobarbital. All procedures were performed in accordance with the European Communities Council Directive 86/6609/EEC.

Testing Procedure

Nociceptive Effects of Formalin

Mice were randomly assigned to 6 groups (8 per group) and received a 10-μL subcutaneous injection of diluted formalin or saline into the right upper lip, just lateral to the nose. Solutions were prepared from commercially available stock formalin further diluted in isotonic saline to 0.5%, 1%, 2%, 4%, and 8%. Stock formalin is an aqueous solution of 37% formaldehyde. Formalin was injected subcutaneously through a 27-gauge needle into the center of the right vibrissa pad as quickly as possible, with only minimal animal restraint. Following injection the animals were immediately placed back in the test box for a 45-min observation period. The recording time was divided into 15 blocks of 3 min, and a nociceptive score was determined for each block by measuring the number of seconds that the animals spent grooming the injected area with the ipsilateral fore- or hindpaw. Movements of the ipsilateral forepaw were accompanied by movements of the contralateral forepaw. A videocamera was used to record the grooming response. Analysis of the behavior was made by an investigator who was blinded to the animal's group assignment.[8]

Antinociceptive Effects of Morphine and Paracetamol

From the protocol described in the preceding, formalin concentration of 4% was chosen as a standard noxious stimulus to evaluate the effects of systemic morphine and paracetamol on the rubbing response. Morphine chlorohydrate and paracetamol were purchased from Sigma Chemical Co (St Louis, MO) and were dissolved in saline (0.9% NaCl solution) and 8% dimethyl sulfoxide (DMSO), respectively. Morphine chlorohydrate and paracetamol were administered subcutaneously into the neck 20 min before formalin. Control animals received saline or 8% DMSO at this time. Morphine was administered at doses of 1.0, 2.0, 4.0, and 8.0 mg/kg (8 per group) and paracetamol was administered at doses of 25, 50, 100, 200, and 400 mg/kg (8 per group).