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ASSESSMENT OF AVOIDANCE BEHAVIORS IN MOUSE MODELS OF MUSCLE PAIN

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Abstract—Pain encompasses both a sensory as well as an affective dimension and these are differentially processed in the cortex. Animal models typically use reflexive behaviors to test nociceptive responses; these are thought to reflect the sensory dimension of pain. While several behavioral tests are available for examining the affective dimension of pain it is unclear if these are appropriate in animal models of muscle pain. We therefore tested the utility of existing paradigms as well as new avoidance paradigms in animal models of muscle pain in mice. Specifically we used an escape-avoidance test to noxious mechanical stimuli, a learned avoidance test to noxious mechanical stimuli, and avoidance of physical activity. We used three animal models of muscle pain: carrageenan-induced inflammation, non-inflammatory muscle pain, and exercise-enhanced pain. In the carrageenan model of inflammation mice developed escape-avoidance behaviors to mechanical stimuli, learned avoidance to mechanical stimulation and avoidance of physical activity – these models are associated with unilateral hyperalgesia. When both muscles were inflamed, escape-avoidance behaviors did not develop suggesting that equivalent bilateral pain behaviors cannot be tested with an escape-avoidance test. In the non-inflammatory muscle pain model mice did not show significant changes in escape-avoidance behaviors or learned avoidance, but did avoid physical activity. In the exercise-enhanced pain model, there were no changes in escape-avoidance, learned avoidance of noxious or physical activity In conclusion, we developed several testing protocols that assess supraspinal processing of pain behaviors in models of muscle pain and that are most sensitive in animals with unilateral hyperalgesia. © **2013 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: escape-avoidance, inflammation, carrageenan, hyperalgesia, analgesia, pain.

INTRODUCTION

Animal models of muscle pain are commonly used to gain a better understanding of underlying mechanisms of pain processing. Pain encompasses both a sensory experience as well as an affective experience. Imaging studies in human subjects suggest that the sensory experience of pain is processed in the somatosensory cortex and the affective experience is processed in the cingulate cortex (Rainville et al., 1997; Hofbauer et al., 2001). As such, behavioral tests that assess both the sensory and the emotional aspects of pain are critical to understanding the whole pain experience. The majority of available nociceptive tests depend on the measurement of withdrawal thresholds, which are reflexive responses. Nociceptive withdrawal reflex behaviors are under supraspinal control but can occur in the absence of supraspinal input (for review see Ossipov et al., 2006). Higher order pain behaviors such as escape-avoidance and conditioned place preference (CPP) involve the anterior cingulate cortex (LaGraize et al., 2004; Qu et al., 2011), a region involved in the emotional component of pain (Devinsky et al., 1995).

There has been a growing trend toward using behavioral tests that assess higher order cortical processing of pain; these involve having the animal choose to avoid or escape a pain-inducing behavior. The two most commonly used tests for assessing higher order pain-related behaviors in animals are the escape-avoidance test and CPP tests (Fuchs, 2000; LaBuda and Fuchs, 2000; LaGraize et al., 2004; Ding et al., 2005; Pedersen and Blackburn-Munro, 2006; Betourne et al., 2008; van der Kam et al., 2008; Baastrup et al., 2011; Qu et al., 2011; Fuchs and McNabb, 2012; He et al., 2012; McNabb et al., 2012). In the escape-avoidance test, the animal chooses to avoid a noxious stimulus by moving to another chamber of the box. Lesions of the cingulate cortex block the development of escape-avoidance pain behaviors (Johansen et al., 2001; LaGraize et al., 2004; Qu et al., 2011; Uhelski et al., 2012). In contrast, lesions of the somatosensory cortex have no effect on the escape-avoidance task, but they do attenuate withdrawal reflex responses (Uhelski et al., 2012). Thus, the escape-avoidance test is useful to examine the affective or emotional component of pain.

Higher order processing using the escape-avoidance paradigm has been tested in animal models of neuropathic and inflammatory pain, primarily in rats (LaBuda and Fuchs, 2000; LaGraize et al., 2004; Pedersen and Blackburn-Munro, 2006; van der Kam

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Abbreviations: ANOVA, analysis of variance; CPP, conditioned place preference.

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et al., 2008; Baastrup et al., 2011; Qu et al., 2011; McNabb et al., 2012). The purpose of the current study was to develop higher order behavioral tests that would be useful for mice with both acute and chronic muscle pain. Specifically, we examined the utility of escape-avoidance, learned avoidance, and physical activity avoidance in an acute inflammatory muscle pain model that presents with unilateral decreases in withdrawal thresholds, in a model of non-inflammatory muscle pain that presents with bilateral decreases in withdrawal thresholds and a model of exercise-enhanced pain that also results in bilateral decreases in withdrawal thresholds.

EXPERIMENTAL PROCEDURES

Animals

All experiments in mice (male, C57BL/6) were approved by the Animal Care and Use Committee at the University of Iowa and are in accordance with the National Institute of Health. Mice were acclimated to their environment for 1 week before any experimental procedures were initiated. All mice were housed five per cage and kept in a temperature controlled environment with available food and water.

Models of muscle pain

Muscle inflammation. To generate acute inflammation mice were anesthetized with isoflurane (4%) and injected with 20 μl of carrageenan (3%, sterile saline) into the left gastrocnemius muscle as previously described (Radhakrishnan et al., 2003; Yokoyama et al., 2007a). Controls were injected with 20 μl of pH 7.2 saline into the left gastrocnemius muscle. An additional group was tested with bilateral carrageenan injection and compared to controls with bilateral pH 7.2 saline injections.

Repeated acid injections. To generate non-inflammatory hyperalgesia, mice received two injections of pH 4.0 saline 5 days apart into the left gastrocnemius muscle as previously described (Sluka et al., 2001; Sluka et al., 2003; Yokoyama et al., 2007a). Mice were anesthetized with isoflurane (4%) and 20 μl of pH 4.0 saline was injected each time. Controls were injected with 20 μl of pH 7.2 saline.

Exercise-enhanced pain. To generate exercise-enhanced pain mice were given two injections of 20 μl of pH 5.0 saline into the left gastrocnemius muscle 5 days apart in combination with a 2-h run in a running wheel immediately before the second injection of pH 5.0, as previously described (Yokoyama et al., 2007b; Sluka et al., 2012). Controls were exercised similarly but were injected with 20 μl of pH 7.2 saline. Mice were acclimated to the running wheel for 2 days, 10 min each time, three times a day. To ensure the mice continued running in the wheel, the top of the cage was tapped when mice stopped running for more than five seconds.

Behavioral protocols

Escape-avoidance experiment. We used a modified escape-avoidance paradigm as previously published (LaBuda and Fuchs, 2000). The testing box was made of Plexiglas with dimensions 16 cm × 7 cm × 13 cm and placed on top of a wire mesh screen. The box was divided into two chambers. So that there was no preference in animals without injury one chamber was white with vertical black lines while the other chamber was solid white. Mice were randomly placed on either the left or the right side of the box to start and an equal number of animals in each group started on either the left or the right side of the box. During a subsequent test, the side of the box was switched to the opposite side. During behavioral testing, the mice were allowed to move unrestricted to either side of the box for 30 min. Mechanical stimulation was initiated with a 0.4 mN von Frey filament to the plantar surface of either the right or left hind paw. The right side was stimulated when the animal was on one side of the box and the left side was stimulated when the animal was on the opposite side of the box. Stimuli were given to the hind-paw once per second. All three models of muscle pain were tested in the escape-avoidance protocol. Measurements were taken before, 24 h, and 1 week after initiation of the model. The time the animal spent on each side of the box was recorded using a stopwatch. Four different groups of experiments were performed (1) unilateral carrageenan ($n = 6$) compared to unilateral saline control ($n = 6$), (2) bilateral carrageenan injection ($n = 6$) compared to bilateral saline injections ($n = 6$), (3) 2, pH 4.0 injections ($n = 10$) compared to 2, pH 7.2 saline control injections ($n = 10$), (4) 2, pH 5.0 injections plus exercise ($n = 10$) compared to 2, pH 7.2 saline control injections plus exercise ($n = 10$).

Learned avoidance experiment. To determine whether mice learn to avoid a painful stimulus, the following protocol was tested in a separate group of animals. The pain model was induced and 24 h after injection, mice were stimulated with a 0.4 mN von Frey filament at a rate of once per second. When mice were on one side of the box, their left hindpaw was stimulated. When mice were on the opposite side of the box, their right hindpaw was stimulated. An hour later, mice were tested again in escape-avoidance box without mechanical stimulation of the paw, and the time they spent on each side of the box was calculated. Mice were randomly placed on either the left or the right side of the box to start and an equal number of animals in each group started on either the left or the right side of the box. Three different groups of experiments were performed (1) unilateral carrageenan ($n = 6$) compared to unilateral saline control ($n = 6$), (2) 2, pH 4.0 injections ($n = 6$) compared to 2, pH 7.2 saline control injections ($n = 6$), (3) 2, pH 5.0 injections plus exercise ($n = 6$) compared to 2, pH 7.2 saline control injections plus exercise ($n = 6$).