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BRAIN RESEARCH

Research Report

Evaluating underlying neuronal activity associated with escape/avoidance behavior in response to noxious stimulation in adult rats

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ABSTRACT

The place escape/avoidance paradigm (PEAP) is a behavioral test designed to quantify the level of unpleasantness evoked by painful stimuli by assessing the willingness of a subject to escape/avoid a preferred area when it is associated with noxious stimulation. Previous studies have demonstrated that escape/avoidance behavior is dependent on activity in the anterior cingulate cortex (ACC), a region of the limbic system involved in processing the emotional component of pain in humans and animals. Analysis of c-Fos expression in the ACC confirmed that the escape/avoidance response to noxious stimuli corresponds to changes in neural activation in this region. Behavioral tests such as the PEAP may be more sensitive to changes in supraspinal pain processing and could contribute to the development of novel analgesics in the future.

1. Introduction

Many of the behavioral tests used to assess experimentally-induced pain conditions rely on reflexive behaviors such as tail flick or paw withdrawal that may not be influenced by activity above the spinal or brainstem level. Since brain areas involved in higher cognitive processes are also known to be involved in pain processing, it is important to evaluate behaviors in animals that are influenced by activity in these areas in order to properly assess animal models and novel treatments for chronic pain conditions. Recently, a growing number of studies have presented behavioral assessments designed to evaluate behaviors that are more complex than reflexive withdrawal responses, such as passive avoidance and conditioned place preference/avoidance. These rely on conditioning a subject to avoid a certain area or behavior when it is paired with noxious stimulation, whether from thermal or mechanical stimulation, previous experience with formalin pain, or other sources of noxious stimulation (Johansen et al., 2001; LaBuda and Fuchs, 2000; Vierck et al., 2002). The involvement of higher cognitive processes, including learning, memory and motivation, in the outcome of these assessments distinguishes them from traditional, reflex-based assessments of sensory pain processing.

The place escape/avoidance paradigm (PEAP) is a behavioral test that quantifies the level of unpleasantness evoked by

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painful stimuli and has been assessed using both inflammatory and neuropathic pain models in rats of both sexes and various strains and ages (Ansah et al., 2010; Baastrup et al., 2010, 2011; LaBuda and Fuchs, 2000; LaGraize et al., 2004; Uhelski and Fuchs, 2010; Wilson et al., 2008). Previous studies in our laboratory have demonstrated that escape/avoidance behavior is dependent on activity in the anterior cingulate cortex (ACC), a region of the limbic system involved in processing the emotional component of pain in humans and animals (Devinsky et al., 1995; Porro et al., 1998; Tolle et al., 1999). Various manipulations (including lesions and microinjections of GABA agonists and morphine) targeting this region attenuated escape/avoidance behavior in rats following ligation of the L5 spinal nerve, while hypersensitivity to mechanical stimuli remained intact (LaGraize and Fuchs, 2007a, 2007b; LaGraize et al., 2004, 2006).

While these and other studies have established the role of cortical brain regions such as the ACC in the processing of the emotional response to pain, the underlying cellular activity that occurs in conjunction with escape/avoidance behavior has not been established. Therefore, the purpose of the current study was to examine the relationship between performance in the place escape/avoidance paradigm and activation of the ACC as measured by c-Fos expression. Adult male Sprague–Dawley rats were assessed in the PEAP and sacrificed after the completion of behavioral testing. Following perfusion, brains were removed, prepared and sliced for immunohistochemistry analysis to quantify c-Fos expression in the ACC. This factor is released following action potentials, and increased expression of c-Fos mRNA indicates recent neuronal activity. Previous studies have performed analyses of c-Fos expression in response to noxious stimuli, but most of these were limited to the spinal cord, with some examinations of brainstem and midbrain regions (Buritova et al., 1996; Day et al., 2001; Harris, 1998; Ono et al., 2009; Villarreal et al., 2003). No studies have previously examined the c-Fos immunoreactivity of the ACC to determine whether cellular activation is positively correlated with escape/avoidance behavior following noxious stimulation.

2. Results

Data for mean paw withdrawal thresholds (MPWT), behavior in the place escape/avoidance paradigm (PEAP), and c-Fos positive cells in the ACC were evaluated for three groups of rats: subjects injected in the plantar left hind paw with .05 ml of 1% carrageenan in normal saline, subjects injected with normal saline only, and subjects that were briefly restrained but did not receive an injection. The non-injected group was included to ensure that the injection itself did not produce significant changes in ACC activity. No overall group differences were present between the two control groups (saline and non-injected controls); therefore, data for these subjects were collapsed for analyses. One subject from the collapsed control group was excluded due to toenail damage during the experimental procedure and one was excluded from the carrageenan group due to tissue damage during brain extraction. A total of 13 subjects were included in the analyses (n=6 carrageenan and n=7 collapsed control).

2.1. *MPWT*

Thresholds were obtained for both hind paws at baseline and 4 hours post-injection. Thresholds for the right hind paw remained at or near the maximum value and post-injection thresholds did not differ significantly from baseline levels for either group (data not shown). Repeated measures analysis of variance (ANOVA) was used to analyze changes in mean paw withdrawal thresholds from baseline to post-injection for the left paw only. There was a significant main effect for group, $F(1,11)=119.69$, $p<.001$, time, $F(1,11)=371.14$, $p<.001$, and a significant interaction, $F(1,11)=310.08$, $p<.001$. Animals injected with carrageenan demonstrated a significant decrease in MPWT compared to baseline values, while animals in the control group remained at pre-injection threshold levels. See Fig. 1.

2.2. *PEAP*

For the place escape/avoidance paradigm (PEAP), the percent of time spent on the light side of the chamber (where subjects could avoid stimulation of the left hind paw that occurred on the dark side of the chamber) was calculated for the thirty-minute testing period. Data for each subject were collapsed into three time points (1–10, 11–20 and 21–30 min) for analyses. Repeated measures ANOVA revealed a significant main effect for group, $F(1,11)=4.91$, $p<.05$, but no main effect for time, $F(2,22)<1$, n.s., or group by time interaction, $F(2,22)=2.73$, n.s. Post-hoc analyses (Fisher's LSD) revealed that carrageenan-injected animals spent significantly more time on the light side of the chamber overall, and this effect was significant for the second and third time points (see Fig. 2). This pattern is similar to that seen in previous experiments with this paradigm (LaBuda and Fuchs, 2000), where animals tend to explore both sides of the chamber during the first 5–10 min, then settle on a preferred side for the latter part of the testing period.

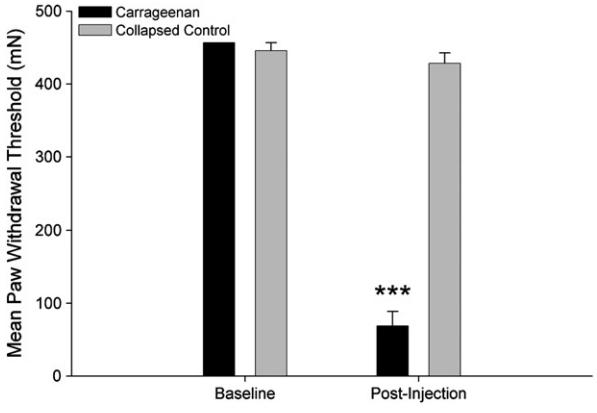

Fig. 1 – Changes in mean paw withdrawal thresholds (MPWT) produced by carrageenan inflammation. Analysis of baseline and post-injection assessments revealed a significant decrease in paw withdrawal thresholds for subjects injected with carrageenan only, confirming that the inflammatory condition induced by carrageenan was associated with mechanical hypersensitivity. *** $p<.001$.