American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 15, No 2 (February), 2014: pp 121-128
Available online at www.jpain.org and www.sciencedirect.com

Original Reports

Suppression of Voluntary Wheel Running in Rats Is Dependent on the Site of Inflammation: Evidence for Voluntary Running as a Measure of Hind Paw-Evoked Pain

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Abstract: **Decreased voluntary wheel running has recently been proposed as a preclinical pain measure for inflammatory pain, but whether this reflects pain evoked by use of the affected limbs is unknown. To assess the role of inflammation site as a determinant of this measure, complete Freund's adjuvant (CFA), formalin, or equivolume vehicle was subcutaneously injected into the plantar surface of the hind paws (bilateral) or L1 dorsum dermatome (leaving paws unaffected) of male Sprague Dawley rats. CFA-induced hind paw mechanical allodynia ($P < .001$) did not correlate with reduced voluntary wheel running. Intraplantar formalin did not attenuate voluntary running, despite eliciting robust licking/writhing/flinching behavior and hind paw mechanical allodynia ($P < .001$). Subcutaneous L1 dorsum dermatome formalin, but not CFA, induced licking/writhing/flinching behavior ($P < .001$), but neither induced hind paw mechanical allodynia or attenuated voluntary running. That voluntary running is decreased by hind paw CFA, but not by L1 dorsum CFA, implies that the behavior is a measure of CFA-induced pain evoked by use of the affected limbs rather than supraspinal pain processing that is independent of inflammation site. Furthermore, the results suggest that interpretation of voluntary wheel running data cannot simply be explained by correlation with mechanical allodynia.**

Perspective: ***Whether decreased voluntary running is dependent on inflammation site is unknown. We show that intraplantar, but not L1 dorsum, CFA suppressed voluntary running and formalin-induced licking/writhing/flinching behavior but had no effect on voluntary running. These data suggest that suppressed voluntary running by CFA likely reflects pain evoked by use of the affected limbs.***

Key words: *Peripheral inflammation, allodynia, pain assessment, spontaneous pain, reflex.*

Over the past decade, the preclinical study of pain has come under considerable scrutiny because of limited success in translating basic science into clinical therapies. One particular issue raised is the narrow range of outcome measures applied in preclinical pain models.[24,25,27,36] Whereas clinical studies examine spontaneous pain (although the pathophysiological accuracy of this terminology has recently been challenged[3]), paroxysmal pain, and evoked pain, preclinical studies have almost exclusively depended on reflex measures.[27] The sole reliance on reflex measures has been criticized, as these are unlikely to capture the full spectrum of systemic adaptations that underlie chronic pain.[25,36] As a result, alternative measures have been developed that may reflect supraspinal pain processing, such as conditioned place aversion[15] and the mouse grimace scale.[17] Recently, decreased running activity has been observed in inflammatory, acetic acid, and osteoarthritis pain models, partially correlating with other indicators of inflammation.[7,21,30] However, these studies have not identified whether attenuated voluntary running activity is reflective of pain evoked

Received July 2, 2013; Revised September 23, 2013; Accepted October 7, 2013.
This work was supported by National Institutes of Health Grants DA024044, DE107782, and DA023132. P.M.G. is a National Health and Medical Research Council C.J. Martin Fellow (ID: 1054091).
The authors have no conflicts of interest to declare.
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1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2013.10.001

in the affected paw because of the motion and weight-bearing activity of running, or of supraspinal pain processing that is independent of inflammation site.

To assess the role of the inflammation site as a determinant of decreased voluntary running, we compared voluntary running wheel activity between rats that had received subcutaneous injections of formalin or complete Freund's adjuvant (CFA) into either the hind paws (bilateral) or L1 dorsum dermatome. CFA or formalin injections into the dorsal lumbar surface of the back, within the L5 dermatome, are sufficient to induce hind paw sensitivity.[14] Hence, injections into the L1 dermatome would likely induce nociceptive hypersensitivity at the injection site but would leave the plantar surface of the hind and fore paws unaffected because the spinal projections of the L1 dermatome afferents and the forepaw and hind paw afferents do not overlap (unlike the L5 dermatome). Thus, nociceptive activation of L1 dermatome afferents is less likely to produce paw sensitization. CFA was examined here because it has been the subject of previous investigation of voluntary wheel running in mice.[7] Formalin was employed as a comparator because the underlying mechanisms of formalin-induced allodynia substantially differ from those of CFA.[10,19] These differences, such as paw edema,[19] may influence running ability.

Methods

Subjects

Pathogen-free male Sprague Dawley rats (300–325 g; Harlan Laboratories, Madison, WI) were single housed with temperature (23 ± .3°C) and light (12:12 light-dark cycle; lights on at 0700 hours) controls. All groups were n = 6. Rats had ad libitum access to water and standard chow and were acclimated to the colony for 1 week before experimentation. Von Frey testing and licking/writhing/flinching behavior assessment occurred during the first 3 hours of lights on, whereas voluntary wheel running was performed during the first hour of lights off. No animals were excluded in this study for any reason. The Institutional Animal Care and Use Committee of the University of Colorado at Boulder approved all procedures.

Formalin or Complete Freund's Adjuvant Injections

Subcutaneous injections of dilute formalin or CFA are commonly used methods to induce persistent or chronic inflammatory nociceptive stimulation in animal studies of pain.[29,34] To determine whether hind paw hypersensitivity was required to decrease voluntary running activity, injections of CFA containing heat-killed *Mycobacterium tuberculosis* (50% in .9%, w/v pyrogen-free saline [1:1 paraffin oil and mannide monooleate-saline emulsion]; Sigma-Aldrich, St. Louis, MO), formalin (4% in .9%, w/v pyrogen-free saline; Sigma-Aldrich), or equivolume vehicle were made bilaterally into the hind paws (100 μL for CFA per injection; 50 μL for formalin per injection) with the needle directed between the toes and the tip placed subcutaneously into the plantar surface, or subcutaneously on the dorsal lumbar surface of the back (200 μL for CFA; 100 μL for formalin) at the region identified by Takahashi et al[32,33] to be within the L1 dermatome. The rats were lightly held in toweling and rapidly injected. Doses and volumes of CFA and formalin represent those commonly reported in pain studies.[4,14,20] All injections were performed on day 0 between 0900 and 1000 hours. Except for all baseline measurements, voluntary wheel running assessment began on the night of injections (night 1), von Frey assessments began the following day (day 1), and licking/writhing/flinching behaviors were recorded for the first hour immediately after injections.

Voluntary Wheel Running

To ensure acquisition of running behavior, all rats were allowed voluntary, unrestricted access to in-cage running wheels for 3 days. From nights 4 to 7 during acquisition, running was restricted to the first hour of the dark cycle by unlocking the wheel at 1900 hours and relocking at 2000 hours, in which stable running during the 3 nights prior to injection was observed. Voluntary wheel running was recorded for the first hour of the dark cycle prior to (0) and up to 7 nights after injection. Wheel revolutions were recorded digitally using Vital View software (Mini Mitter, Bend, OR), and distance was calculated by multiplying number of revolutions by wheel circumference (1.081 m). Running time was calculated by summing the number of minutes in which wheel revolutions were >0.

Von Frey Test for Mechanical Allodynia

Testing was conducted blind with respect to group assignment. Rats received at least three 60-minute habituations to the test environment prior to behavioral testing. The von Frey test[6] was performed at the distal region of the heel in the hind paws, within the region of sciatic innervation as previously described in detail.[5,23] Importantly, this test site was posterior to the formalin/CFA injections site, avoiding possible confounds of tissue damage and hypoalgesia observed previously.[10] Assessments were made prior to (baseline) and on days 1, 2, 3, 4, and 7 postinjection. A logarithmic series of 10 calibrated Semmes-Weinstein monofilaments (von Frey hairs; Stoelting, Wood Dale, IL) were applied randomly to the left versus right hind paws to define the threshold stimulus intensity required to elicit a paw withdrawal response. Log stiffness of the hairs ranged from manufacturer-designated 3.61 (.407 g) to 5.18 (15.136 g) filaments. The behavioral responses were used to calculate absolute threshold (the 50% probability of response) by fitting a Gaussian integral psychometric function using a maximum-likelihood fitting method,[12,35] as described previously.[22,23] This fitting method allows parametric analyses that otherwise would not be statistically appropriate.[22,23] All assessments took place between 0900 and 1100 hours.

Licking/Writhing/Flinching Behaviors

Following injection, rats were observed for pain-evoked behavior. A time-sampling procedure assessed