Inflammation-Induced Reduction of Spontaneous Activity by Adjuvant: A Novel Model to Study the Effect of Analgesics in Rats

David J. Matson, Daniel C. Broom, Susan R. Carson, James Baldassari, John Kehne, and Daniel N. Cortright

Departments of Pharmacology (D.J.M., D.C.B, S.R.C., J.B., J.K.) and Biochemistry and Molecular Biology (D.N.C), Neurogen Corporation, Branford, Connecticut

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ABSTRACT

The majority of rodent models used to evaluate analgesic drug effects rely on evoked measures of nociceptive thresholds as primary outcomes. These approaches are often time-consuming, requiring extensive habituation sessions and repeated presentations of eliciting stimuli, and are prone to false-positive outcomes due to sedation or tester subjectivity. Here, we describe the reduction of spontaneous activity by adjuvant (RSAA) model as an objective and quantifiable behavioral model of inflammatory pain that can predict the analgesic activity of a variety of agents following single-dose administration. In the RSAA model, activity was measured in nonhabituated rats using standard, photocell-based monitors. Bilateral inflammation of the knee joints by complete Freund’s adjuvant (CFA) reduced the normal level of activity (horizontal locomotion and vertical rearing) by ~60% in a novel environment. This reduction in activity was dose-dependently reversed by ibuprofen, rofecoxib, celecoxib, piroxicam, and dexamethasone, whereas gabapentin and amitriptyline were inactive. Morphine significantly reversed the activity-suppressing effects of CFA, at 1 mg/kg s.c., but at higher doses locomotor activity progressively declined, coincident with the induction of sedation. In contrast to morphine and anti-inflammatory therapies, amphetamine did not affect vertical rearing, even though it increased horizontal locomotion. Thus, unlike standard measures of analgesia such as alteration in thermal or mechanical sensitivity, the RSAA model operationally defines analgesia as a drug-induced increase in spontaneous behavior (vertical rearing in a novel environment). We conclude that the RSAA model is valuable as an objective measure of analgesic efficacy that is not dependent on an evoked stimulus response.

Due to the emotional and subjective nature of pain, the preclinical testing of novel analgesic agents has proven to be a challenging undertaking. The measurement of nociception and subsequent antinociceptive properties of compounds has traditionally been performed in assays that measure an animal’s responsiveness to differing evoked nociceptive stimuli. Such assays have to be amenable to displaying either allodynia or hyperalgesia resulting from inflammatory or neuropathic pain induction, as well as detecting the ability of drugs to reverse this hypersensitivity.

The most widely used analgesia assays assess mechanical and thermal sensitivity. These include mechanical/tactile allodynia measured by von Frey filaments (Chaplan et al., 1994), mechanical hyperalgesia measured by the Randall-Selitto paw pressure test (Randal and Selitto, 1957), and heat hyperalgesia measured by the Hargreaves radiant heat assay (Hargreaves et al., 1988). These standard rodent hypersensitivity models may be potentially biased by rater subjectivity (e.g., von Frey test) or require exposure to a manually applied noxious stimulus (e.g., paw pressure test, radiant heat assay), and all rely on an evoked measure of sensitivity. Furthermore, although these models can successfully predict how certain classes of compounds will behave in the clinic, they tend to be labor-intensive and time-consuming, with multiple habituation and baseline sessions required to ensure that test animals give consistent responses. Finally, due to their subjective nature, these assays, particularly the von Frey and paw pressure tests, also require the testers to be blinded to the grouping and randomization of drug and control animals.

New models have been developed in an attempt to overcome the drawbacks of traditional pain models. Recently, the weight bearing assay has been developed as a model of noci-
ception (Whiteside et al., 2004; Pomonis et al., 2005). This assay involves measuring the weight distribution that the test animal places on both hindpaws, normally with one hindpaw being challenged with an inflammatory or neuropathic injury. The weight bearing assay itself is nonevoked and therefore has the advantage of being an unstimulated measure of hypersensitivity. However, the assay is still subject to tester interpretation, requires multiple habituation and baseline sessions, and can produce false-positive results due to nonspecific side effects such as sedation or muscle relaxation. Another effort to improve nociceptive testing focuses on the assessment of gait to quantify changes in inflammatory (Coulthard et al., 2002, 2003) and neuropathic (Vrinten and Hamers, 2003) hyperalgesia. Like the weight-bearing method, the advantage of gait assessment centers on the unevoked nature of the endpoint being measured. However, the predictive utility of this model is currently unknown because it has yet to be validated with standard analgesic agents such as opioids or nonsteroidal anti-inflammatory drugs. The only drug administration paradigms tested by this approach are infusions of muscimol and bicuculline (Simjee et al., 2004). Finally, other approaches being developed to address problems with evoked stimuli have been focusing on cognitive processing; for example, the measurement of nonselective, nonsustained attention in rats with chronic visceral inflammation (Millecamps et al., 2004), and operant escape from a cold stimulus (Vierck et al., 2005). Although these models exhibit analgesic pharmacology comparable with clinical observations, the test animals require extensive habituation to the testing apparatus; therefore, both are laborious assays.

The aim of the present study was to develop a pharmacologically relevant measure of nociception that minimizes the subjectivity of many nociceptive tests, the false-positive results associated with sedation, and the need for multiple habituation and training sessions. Therefore, we have devised and characterized the reduction of spontaneous activity by adjuvant (RSAA) model in rats given bilateral knee joint injections of complete Freund’s adjuvant (CFA).

Materials and Methods

Animals. Adult male Sprague-Dawley rats (Charles River, Kingston, NY) weighing 320 to 340 g at the time of testing were used. All animals were housed in groups of four on solid bottom Plexiglas cages (47 × 20 × 21 cm) with bedding material and had free access to food and water in a temperature- and humidity-controlled room on a 12-/12-h light/dark cycle. Experimental protocols used complied with the Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and were approved by Neurogen Corporation’s Institutional Animal Care and Use Committee.

Induction of Inflammation. Animals were anesthetized under isoflurane (3%) anesthesia. CFA (Sigma, St. Louis, MO), or 0.9% sterile saline solution for controls, was then injected unilaterally or bilaterally into the tibia-femur joint(s). The tibia-femur joint(s) were not manipulated after injection. A D-B tuberculin slip-tip 1-ml syringe with a 26-G PrecisionGlide beveled needle was used to deliver a 50-μg/50-μl dose of CFA, unless stated otherwise. Carrageenan/kaolin, which has been previously shown to induce inflammation and hyperalgesia upon injection into the tibia-tarsal joint (Sluka and Westlund, 1993; Yu et al., 2002), was also tested in the RSAA model. In brief, a suspension of 2% carrageenan and 3% kaolin or 0.9% sterile saline solution for controls was injected bilaterally (0.15 ml, pH 7.4) into the tibia-femur joints and manually articulated for 5 min. Animals were then allowed to recover for 1 h before being returned to their home cages in the vivarium for the 48 h before testing.

Drug Treatments. CFA was injected either fully concentrated at 1 mg/ml or diluted with incomplete Freund’s adjuvant (Difco, Detroit, MI) to achieve concentrations lower than 1 mg/ml. Carrageenan Lambda (2%) and kaolin (5%; Sigma) were suspended in 0.9% saline. Morphine sulfate (Sigma), administered in a dose range shown to be efficacious (Chan et al., 1999), was dissolved in 0.9% saline and dosed s.c. 1 h before testing. Amphetamine (Sigma), administered in a method previously shown to be effective in increasing activity (Mazurski and Beninger, 1988), was dissolved in 0.9% saline and dosed i.p. 10 min before testing. Amitriptyline (Sigma), administered at a dose previously shown to be efficacious (Nagakura et al., 2003), was administered p.o. 45 min before testing. Rofecoxib (Toronto Research Chemicals, ON, Canada), administered in a method previously shown to be efficacious (Chan et al., 1999), was administered p.o., 1 h before testing. Piroxicam (Sigma), administered in a method previously shown to be efficacious (Francischi et al., 2002), was administered s.c., 3 h before testing. Ibuprofen, dexa-methasone (both from Sigma), celecoxib, and gabapentin (both from Toronto Research Chemicals) were administered p.o., 3 h before testing. These compounds were dosed in a method previously demonstrated to be efficacious in models of analgesia (Masferrer et al., 1994; Field et al., 2002; Harris et al., 2004; Jones et al., 2005). Subcutaneous and i.p. injections were administered at a volume of 1 ml/kg, whereas oral gavages were administered at a volume of 10 ml/kg. For oral and s.c. administration drugs were sonicated and suspended in distilled water containing vitamin E o-tocopheryl polyethylene glycol 1000 succinate (2%) (Eastman Chemicals, Anglesley, UK) unless otherwise stated.

Mobility Measurement. Spontaneous activity was measured using a computerized Digiscan-16 Animal Activity Monitor (model 1300JC/CCDigi, version 2.4; Omntech Electronics, Columbus, OH) equipped with 48 infrared photocell emitters and detectors (2.5 cm between sensors). Horizontal activity (locomotion) was detected by 16 photo sensors on the front and back walls as well as 16 photo sensors on both side walls. Vertical activity (rearing) was measured by 16 photo sensors on the side walls located 13.5 cm above the cage floor. Each box (41.25 × 41.25 × 30 cm) was constructed of Plexiglas, and the floor was covered with a thin layer of beta chips. Multiple test chambers were contained within a sound-isolated testing room, and the rats were tested in the presence of white noise (62 dB) and red light (60 W) and assessed for 15 min. Total distance traveled (centimeters) and vertical activity (number of rears) were recorded for analysis. Versanasmx software version 4.0-125E (Accuscan Instruments, Columbus, OH) was used for activity measure recording and analysis and for the determination of stereotypy.

Hot-Plate Testing. Latency to hindpaw licking or jumping was measured on a 50°C hot-plate (Hotplate Analgesia Meter; Columbus Instruments, Columbus OH). In brief, the testing apparatus surface was 25.5 × 25.5 cm, with four 30.5-cm Plexiglas walls. The testing surface was maintained at 50°C via an internal thermostat. Rats underwent baseline testing on day 1 then were dosed 24 h later with drug or vehicle before testing. After the pretreatment time had elapsed, all rats were retested. For each test measurement, latency to hindpaw licking or jumping was recorded in three trials by an observer blinded to drug treatment. A 5-min interval was imposed between trials. The individual scores were calculated as the mean latency of the three trials. A 90-s cut-off time was imposed to prevent tissue damage.

Statistical Analysis. Data are presented as means ± S.E.M. of 8 to 10 animals per dose group. Overall analysis of the RSAA and mobility measurement data were performed with a one-way analysis of variance. Follow-up group comparisons were determined using Fisher’s least significant difference, with statistical significance set at p = 0.05 or lower. η² was used to determine effect size with values of 0.01, 0.06, and 0.14, representing small, medium, and large ef-
fects. Due to many animals reaching the predetermined cut-off, analyses of hot-plate data were performed using the Kruskal-Wallis one-way analysis of variance with follow-up multiple comparisons. Minimal effective dose (MED) was defined as the lowest dose that produced a statistically significant effect.

**Results**

A comparison between bilateral injections of CFA and carrageenan/kaolin was conducted to determine which substance produced the best window to detect analgesia. The CFA group was injected bilaterally with 150 μg of CFA/150 μl/knee. The carrageenan/kaolin (CK) group was injected bilaterally with 2% carrageenan/3% kaolin/150 μl/knee. Saline was injected bilaterally at a volume of 150 μl/knee as a control. A significant decrease in vertical activity and total distance was exhibited by the CFA-injected group compared with the CK- and saline-injected groups (Fig. 1). Likewise, the CK-injected group showed a significant decrease in vertical activity, compared with the saline-injected group, but did not differ significantly from control in total distance traveled. For vertical activity, the CFA-injected group was statistically lower than both the saline control group and the CK-injected group, whereas the CK-injected group was significantly lower than both the saline control group and the CFA-injected group (Fig. 1A). For total distance traveled, the CFA-injected group was significantly lower than both the saline control group and the CK-injected group, although the CK-injected group was not statistically significant from control ($F_{2,27} = 10.419, p < 0.001, \omega^2 = 0.386$) (Fig. 1B). Stereotypy (data not shown) did not increase but rather showed a significant decrease with carrageenan/kaolin- and CFA-induced injury in proportion to the observed decreases in horizontal activity and vertical rears (Fig. 1). Based on these results, CFA was chosen for further study.

A CFA dose-response curve was generated over two experiments. CFA was administered either unilaterally or bilaterally into the knee joint(s) to induce inflammation (Fig. 2). For unilateral injection, 150 μg/150 μl of CFA was injected into the left knee joint. Bilateral injection groups were dosed at 0.1 μg/50 μl, 1 μg/50 μl, 10 μg/50 μl, 50 μg/50 μl, 100 μg/100 μl, and 150 μg/150 μl of CFA per knee. To determine whether the injections alone might produce an effect in the RSAA model, bilateral injections of saline were used as an injection control. Likewise, to determine the effect the CFA vehicle in this model, bilateral injections of incomplete adjuvant were used as vehicle controls. Although CFA reduced behavior on both activity measures recorded, vertical activity was the most sensitive and reliable measure in the RSAA model. Therefore, vertical activity was the focus of this and subsequent studies. As shown in Fig. 2A, the noninjected control level of vertical rearing was significantly reduced by a uni-

![Fig. 1. The effect of CFA (150 μg/150 μl/knee) or carrageenan (2%)/kaolin (3%) (CK, 150 μl/knee) on vertical activity (A) and total distance (B) in a novel open field. The CFA-injected group significantly reduced behavior on both measures compared with both the saline- and CK-injected groups. The CK-injected group moderately reduced behavior in the vertical activity measure only. Bars, means ± S.E.M. (n = 10/group). *p < 0.05 or ***p < 0.001 compared with the saline-injected group. ++++p < 0.001 compared with carrageenan/kaolin group.](image)

![Fig. 2. The effect of CFA at high (A) and low (B) doses on vertical rearing. CFA doses of 50 μg/50 μl/bilaterally and higher produced a significant reduction in rears compared with the noninjected group, the 150 μg/150 μl/unilaterally (A) or the incomplete adjuvant (B) group (50 μl, bilaterally). CFA doses of 10 μg/50 μl/bilaterally and lower are not significantly different from the incomplete adjuvant group. Bars, mean rears ± S.E.M. (n = 10/group). *p < 0.05; **p < 0.01; ***p < 0.001 compared with the saline or noninjected groups. ###p < 0.001 compared with the 150 μg of CFA unilateral injection group. ++++p < 0.001 compared with the incomplete adjuvant group.](image)
lateral dose of 150 μg of CFA and bilateral doses of 50, 100, and 150 μg of CFA ($F_{6,53} = 34.583, p < 0.001, \omega^2 = 0.737$). Furthermore, bilateral dosing of 50, 100, and 150 μg of CFA produced significantly lower vertical rearing than the unilateral dose of 150 μg of CFA. The saline-injected group was not statistically different from the noninjected control group.

In the second experiment, CFA was injected bilaterally at 0.1, 1, 10, and 50 μg (Fig. 2B). Incomplete adjuvant was also injected to determine the effect of the CFA vehicle alone. As shown in Fig. 2B, the noninjected control level of vertical rearing was significantly reduced by 50 μl of incomplete adjuvant as well as the 0.1, 1, 10, and 50-μg doses of CFA. Only the 50-μg dose of CFA was significantly lower than the incomplete adjuvant group ($F_{5,54} = 10.220, p < 0.001, \omega^2 = 0.434$). Since a bilateral injection of 50 μg/50 μl of CFA provided the best window for determining drug effects while minimizing the dosage of CFA used, each subsequent CFA group received this dose to produce inflammation. Animals receiving bilateral injections of CFA exhibited normal grooming and feeding behaviors over the 48-hour time period of the experiment. No adverse effect on animal health was observed.

To investigate the potential of the RSAA model to predict analgesic activity, a number of nonsteroidal anti-inflammatory drugs were used in an effort to reverse the decrease in spontaneous activity. Celecoxib, a cyclooxygenase (COX)-2 inhibitor that is 7-fold more potent at COX-2 than COX-1 (Riendeau et al., 2001), was dosed orally at 0.03, 0.1, 0.3, 1, and 3 mg/kg (3-h pretreatment). These doses of celecoxib have been shown previously to produce analgesia in other rodent models of pain (Harris et al., 2004). The number of vertical rears in the CFA/vehicle group was significantly lower than those in the noninjected/vehicle control group. Celecoxib, at doses of 0.03, 0.01, and 0.3 mg/kg, did not produce statistically significant increases in vertical rears compared with the CFA/vehicle group, although these groups were also not significantly different from the noninjected/vehicle group. However, at doses of 1 and 3 mg/kg, celecoxib significantly increased vertical rears compared with the CFA/vehicle group ($F_{6,49} = 2.83, p = 0.019, \omega^2 = 0.136$) (Fig. 3A). A dose of 1 mg/kg was determined to be the MED in the RSAA model. Rofecoxib, a COX-2-selective inhibitor that is 35-fold more potent at COX-2 than COX-1 (Riendeau et al., 2001), was administered orally at 1, 3, 10, and 30 mg/kg (1 h pretreatment), significantly increased vertical rearing compared with CFA/vehicle controls ($F_{4,48} = 4.116, p = 0.002, \omega^2 = 0.211$) (Fig. 3B). Rofecoxib, at these doses, was demonstrated to be analgesic in other models of inflammatory pain (Chan et al., 1999). A dose of 1 mg/kg was determined to be the MED in the RSAA model. Piroxicam, which is 12.5-fold more potent at COX-1 than COX-2 (Riendeau et al., 2001), produced a significant increase in vertical rearing compared with the CFA/vehicle group at 10 and 30 mg/kg ($F_{6,63} = 10.568, p < 0.001, \omega^2 = 0.451$). Piroxicam at these doses was previously determined to be analgesic in other models of inflammatory pain (Francischi et al., 2002). A dose of 10 mg/kg was determined to be the MED in the RSAA model. Finally, ibuprofen (IB), which is 5-fold more potent at COX-1 than COX-2 (Riendeau et al., 2001), produced a significant increase in vertical rearing compared with the CFA/vehicle group at 1, 3, 10, and 30 mg/kg ($F_{6,63} = 16.559, p < 0.001, \omega^2 = 0.571$) (Fig. 4B). At doses comparable with those shown here, IB has also been demonstrated previously to be analgesic in other models of inflammatory pain (Jones et al., 2005). A dose of 1 mg/kg was determined to be the MED in the RSAA model. Likewise, statistically significant efficacy was previously demonstrated with piroxicam at a dose of 3 mg/kg p.o. (Francischi et al., 2002).

Dexamethasone was dosed orally at 0.003, 0.01, 0.03, 0.1, and 0.3 mg/kg (3 h pretreatment), doses that have previously been shown to decrease inflammation (Masferrer et al., 1994), to evaluate the effect of a steroidal anti-inflammatory drug in the RSAA model (Fig. 5). IB was dosed orally at 10 mg/kg as a positive control. Although ineffective at doses of 0.003 and 0.01 mg/kg, dexamethasone significantly increased rearing compared with the CFA/vehicle group at doses of 0.03, 0.1, and 0.3 mg/kg. The maximal effect seen with dexamethasone was comparable with that seen with 10 mg/kg IB, and both groups still displayed significantly lower vertical activity than the noninjected/vehicle group ($F_{7,74} = 5.066, p < 0.001, \omega^2 = 0.262$). A dose of 0.03 mg/kg was determined to be the MED in the RSAA model.

Atypical analogues were also assessed in the RSAA model. Gabapentin (Fig. 6) was dosed orally at 300 mg/kg (3 h pretreatment), a dose that has been shown as efficacious in models of acute and neuropathic pain (Field et al., 2002; Fernihough et al., 2004). Gabapentin exhibited a significant decrease in vertical rears compared with the noninjected/vehicle group at 10 and 30 mg/kg ($F_{6,54} = 7.621, p < 0.001, \omega^2 = 0.385$). Gabapentin at 0.03 mg/kg produced no significant increase in vertical rearing compared with the noninjected/vehicle group ($F_{6,54} = 0.068, p = 0.704, \omega^2 = 0.001$). However, at 1 and 3 mg/kg, gabapentin produced a significant increase in vertical rearing compared with the noninjected/vehicle group ($F_{6,54} = 6.496, p < 0.001, \omega^2 = 0.302$) (Fig. 6B). At doses comparable with those shown here, gabapentin has also been demonstrated previously to be analgesic in other models of inflammatory pain (Jones et al., 2005). Gabapentin at 1 and 3 mg/kg produced a significant increase in vertical rearing compared with the noninjected/vehicle group ($F_{6,54} = 9.594, p < 0.001, \omega^2 = 0.406$). Gabapentin was also effective at doses of 0.03 and 0.1 mg/kg, although these groups still exhibited significantly lower vertical rearing compared with the noninjected/vehicle group ($F_{6,54} = 7.621, p < 0.001, \omega^2 = 0.385$). A dose of 0.03 mg/kg was determined to be the MED in the RSAA model.
Compared with the noninjected group. Compared with CFA/vehicle group.

Compared with the noninjected/vehicle group. 0.001 compared with CFA/vehicle group.

**F** CFA/vehicle group (vehicle group but was not significantly different from the CFA/vehicle group ($F_{4,42} = 21.487, p < 0.001, \omega^2 = 0.672$). Furthermore, acute, oral administration of gabapentin did not decrease measured variables in a standard model of spontaneous locomotor activity (data not shown). Likewise, amitriptyline (Fig. 6), dosed orally at 30 mg/kg (45-min pretreatment), exhibited a significant decrease in vertical rears compared with the noninjected control group and was not significantly different from the CFA/vehicle group ($F_{4,42} = 21.487, p < 0.001, \omega^2 = 0.672$). Acute, oral administration of amitriptyline decreased measured variables in a standard model of spontaneous locomotor activity (data not shown). In contrast, a dose of 30 mg/kg amitriptyline has been previously reported to be analgesic in various pain models (Nagakura et al., 2003).

To evaluate the effect of opioid analgesic agents in the RSAA model, morphine was administered at doses previously shown to be efficacious in other models of pain (Chan et al., 1999). These results are displayed in Fig. 7A. As expected, CFA/vehicle administration significantly reduced vertical activity compared with the vehicle controls, and ibuprofen, as the positive control, significantly reversed the CFA-induced deficit (Fig. 7A). Morphine demonstrated an inverted “U”-shaped dose-response curve for vertical activity where only the 1 mg/kg morphine dose group significantly increased vertical rears above the CFA/vehicle level. The 10 mg/kg morphine group displayed no difference in vertical activity compared with the CFA/vehicle group ($F_{6,46} = 20.842, p < 0.001, \omega^2 = 0.630$). A dose of 1 mg/kg was determined to be the MED in the RSAA model.

Because opioids produce sedation with increasing doses, the effect of morphine was evaluated in a standard model of locomotor activity without inflammatory injury. Following morphine administration, a clear dose-dependent decrease in behavior was observed in vertical activity (Fig. 7B). Morphine produced a small but significant reduction in vertical activity at 1 mg/kg with more dramatic reductions at higher doses ($F_{5,28} = 48.399, p < 0.001, \omega^2 = 0.815$).

The 50°C hot-plate assay was performed with several doses of morphine to compare the increase in activity shown by lower doses of morphine in the RSAA model with a standard model of antinociception in which morphine is efficacious. Overall statistical analysis determined there was a significant difference between treatment groups [Kruskal-Wallis result, $\chi^2(3, n = 56) = 39.34, p < 0.001$]. Post hoc analysis demonstrated that 3 mg/kg morphine was not significantly different from vehicle, whereas the effect of 5 mg/kg trended toward significance ($p < 0.100$). At a dose of 10 mg/kg, morphine produced significantly increased latencies compared with all other dose groups (Fig. 7C). All three of these morphine doses were previously shown to reduce vertical rearing (Fig. 7B).
To assess the potential of producing false-positive results in the RSAA model, amphetamine, a stimulant with no clinically useful analgesic properties, was tested at a relevant dose (Mazurski and Beninger, 1988). Amphetamine was dosed i.p. at 1 mg/kg (10-min pretreatment) with IB, dosed orally at 30 mg/kg, as a positive analgesic control. All groups exhibited a decrease in vertical rears compared with the noninjected control (Fig. 8A). Only IB significantly increased vertical rears compared with the CFA/vehicle group ($F_{3,34} = 11.884$, $p < 0.001$; $+ + +$, $p < 0.001$ compared with the noninjected/vehicle or vehicle group. $+ +$, $p < 0.001$ compared with CFA/vehicle group).

Of the compounds tested in the RSAA model, only celecoxib and rofecoxib were able to increase vertical rearing back to noninjected control levels (Fig. 3, A and B). IB, despite multiple replications and although demonstrating a significant increase compared with the CFA/vehicle controls, was never able to completely reverse the deficit in vertical rearing back to noninjected control levels (Fig. 4B). Likewise, dexamethasone (Fig. 5) and morphine (Fig. 7A) were only able to demonstrate a partial reversal of vertical rearing.

### Discussion

This study describes the use of spontaneous activity in a novel environment, which we have termed RSAA, as a method for measuring the effects of analgesics in rats with inflamed knee joints. In this model, animals are injected with CFA into both tibia-femur joints 48 h before testing. On test day, animals are injected with test compounds, placed into a novel open field and allowed to explore for a total of 15 min. The testing room is dark with a low level of white noise to maximize the rodents' inherent behavior to explore a novel environment. The potential for rater bias is eliminated with the use of computer software to quantify behavioral scores. A further advantage of the RSAA model is that it does not use an evoked stimulus to measure hypersensitivity and relies upon the animal's level of spontaneous exploration to display an analgesic drug effect. Morphine, dexamethasone, ibuprofen, piroxicam, celecoxib, and rofecoxib, which are all analgesic in humans, produce a reversal of the CFA-induced behavior in the RSAA model. These pharmacological data...
suggest that the CFA-induced RSAA test is predictive of analgesic activity in humans.

We chose to use direct injection of proinflammatory agents directly into the tibial-femur joint. This allows focal inflammation to develop while minimizing widespread, systemic activation of the immune system in a convenient time frame. Although unilateral injection of CFA or bilateral carrageenan/kaolin resulted in a statistically significant decrease in locomotor activity, these decreases were judged too small to allow dose titration of analgesics. Bilateral joint injections of CFA were necessary to ensure an adequate reduction in spontaneous locomotor activity and also permitted the use of lower doses of CFA in each animal.

The photocell monitoring system used in this study collects two measures of locomotor activity in all experiments, but we found that vertical activity was the most sensitive to the CFA treatment and displayed the greatest percentage decrease in activity. We hypothesize that this is due to the localized inflammation in the hindlimb tibia-femur joints. In rising to a vertical position, the animal extends and bears all of its weight on these joints, in contrast to two-dimensional exploration, which uses all four limbs to bear weight and move. Hence, an animal suffering painful inflammation of hindleg joints may be less likely to rear up even as it exhibits normal movement in horizontal locomotion.

One limitation of this approach is that the RSAA method is not readily amenable to repeated designs. The driving force for rat activity is the novelty of the environment, which elicits a sufficiently high level of basal activity to allow for a reliable suppression with the CFA stimulus. Previous exposure to this environment results in a substantial decrease in all photocell parameters measured. However, by including sham-injected or noninjected controls in each study, reproducible effects of CFA and reversal by analgesics are seen using a between-subjects design.

The RSAA model proved highly efficient at detecting the behavioral activity of the nonsteroidal anti-inflammatory analgesics ibuprofen, piroxicam, celecoxib, and rofecoxib, and the anti-inflammatory steroid, dexamethasone. The dose-effect relationships for these drugs are similar in this model compared with those obtained in more traditional antihyperalgesia tests (Table 1), such as the carrageenan-induced thermal and mechanical hyperalgesia assays (Gegout et al., 1995; Chan et al., 1999; Harris et al., 2004). These results indicate that RSAA is pharmacologically well validated with clinically useful analgesic agents.

Because RSAA is a subchronic inflammatory pain model, we hypothesized that gabapentin, which exhibited efficacy in a posthysterectomy pain clinical study (Gilron et al., 2005) and gave antihyperalgesic effects in a rat acute arthritis model (Lu and Westlund, 1999), would be efficacious in RSAA. However, neither gabapentin nor amitriptyline mediated a statistically significant increase in activity relative to vehicle controls (Fig. 6). Although both agents are widely used in treating various forms of neuropathic pain, their effectiveness in inflammatory joint pain is equivocal (Lu and Westlund, 1999; Nagakura et al., 2003). Furthermore, this study examines responses 48 h after induction of joint inflammation, possibly at a suboptimal time point for gabapentin to display efficacy. Models in which gabapentin has strong efficacy, such as peripheral nerve trauma models, typically assess drug effects 7 to 14 days postinjury (Field et al., 2002). Therefore, further refinement of this model, such as altering the time frame of testing following CFA injection or administering drug subchronically before testing, may be necessary to observe the analgesic effects of gabapentin and amitriptyline.

Morphine also exhibited efficacy in the RSAA paradigm, but with an inverted U-shaped dose-response-effect relationship. This is attributable to the sedating action of morphine, as demonstrated by its action in reducing all measures of spontaneous locomotor activity in non-CFA-injected rats at doses of 3 mg/kg and above. Hence, although CFA-injected rats that received 0.3 to 3 mg/kg morphine s.c. exhibited statistical increases in locomotor activity compared with vehicle controls, higher doses also produced sedation, as evidenced by a decrease in locomotor activity. Sedation complicates the interpretation of classical analgesia tests, which depend on intact motor coordination and activity to respond to noxious stimuli. If an analgesic agent is sedating, then an exquisitely sensitive nociception assay must be used to observe any potential therapeutic window. For example, morphine exhibited overlapping dose-effect relationships in the 50°C hot-plate assay and in the non-CFA spontaneous locomotor activity assay. Taken alone, these results could lead to speculation that sedation played a role in morphine’s effect in the hot-plate assay. However, the RSAA test demonstrated an analgesic effect of morphine at a dose lower than its sedative effect (1 mg/kg s.c.), therefore identifying a thera-

**TABLE 1**

Comparison of efficacy between the RSAA model and literature references

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<th>MED in RSAA</th>
<th>Literature Efficacy</th>
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<td>Amitriptyline</td>
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<tr>
<td>Ibuprofen</td>
<td>1</td>
<td>121; 300</td>
<td>Jones et al. (2005)</td>
</tr>
<tr>
<td>Morphine</td>
<td>1</td>
<td>1.9</td>
<td>Jones et al. (2005)</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>10</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td>Rofecoxib</td>
<td>1</td>
<td>1</td>
<td>Rienodeau et al. (2001)</td>
</tr>
</tbody>
</table>

All values are in milligrams per kilogram. N.D., not determined; N.A., not available.

a Carrageenan-induced thermal hyperalgesia (ED<sub>50</sub>);

b CCI-induced static allodynia (MED).
c von Frey (ED<sub>50</sub>);
d Carrageenan-induced thermal hyperalgesia (ED<sub>50</sub>);
e Capsaicin-induced mechanical allodynia (ED<sub>50</sub>);
f Carrageenan-induced thermal hyperalgesia (ID<sub>50</sub>),
peutic window and indicating a greater sensitivity than the hot-plate assay. The morphine results indicate that the RSA model requires compounds to be nonselecting at analgesic doses to see an analgesic effect. Therefore, unlike almost all other analgesia assays, the RSA model is less likely to produce a false-positive result due to sedating properties of the test drug.

A potential drawback of the RSA model was thought to be false positives with compounds that increase locomotion, such as amphetamine. In standard tests of locomotion, amphetamine at 1 mg/kg i.p. significantly increased both horizontal and vertical exploration (data not shown). The RSA model, however, is able to discern between differences in increased locomotor activity and willingness to rear up on the hindlimbs. Horizontal movement, in which weight is distributed between all four limbs, is increased in the RSA model with administration of a stimulant. Vertical rearing, which transfers all of the animal’s weight to the hindlimbs and requires the joints to be extended, is unchanged from CFA/vehicle levels in the RSA model when assessed with amphetamine. This suggests that an animal’s willingness to increase its level of vertical rearing is dependent on the level of analgesia it is experiencing and not due to drug effects that grossly increase locomotor activity.

In some experiments, we noted that certain test drugs did not produce a complete reversal of the CFA-induced reduction in activity. It is possible that a single dose is not sufficient to reliably give complete efficacy in this model. Longer term treatment with CFA may prove even more of an efficacy challenge for a single, therapeutic dose of these drugs. Previous studies have demonstrated an effect of nonsteroidal anti-inflammatory drugs in CFA-injected rats used chronic dosing starting around the time of CFA administration (Safieh-Garabedian et al., 1995; Broom et al., 2004). The current RSA model attempts to balance the length of the study (to minimize animal discomfort) relative to the more chronic traditional models, as well as observe drug effects when given therapeutically.

The RSA model provides several theoretical and operational advantages over classical analgesia models. The model uses a normal rodent behavior—exploration of a novel environment—to assess the effect of analgesics. An analgesic effect in the RSA model is quantified in terms of a positive, unevoked behavioral response (e.g., increased vertical rearing) rather than a negative, evoked response (e.g., withdrawal from stimulus). The animal is not restrained, and the injection of immunological adjuvant is localized such that systemic inflammatory effects are minimized. The animals require no habituation, and behavioral observation subjectivity is eliminated. Because sedative effects have a negative impact on the observation of analgesia in this model, compounds producing analgesia or antihyperalgesia via sedative-like effects are quantitatively evident. CFA-induced inflammation of the tibial-femur joint resulting in decreased locomotion has some analogy to clinical signs and symptoms of arthritis. Moreover, the observation that known analgesics mediate efficacy in the RSA model at clinically relevant doses suggests that this model can effectively predict therapeutic benefit for treating inflammatory pain in humans.

References


