The analgesic efficacy of morphine varies with rat strain and experimental pain model

implications for target validation efforts in pain drug discovery

Hestehave, Sara; Abelson, Klas S.P.; Brønnum Pedersen, Tina; Munro, Gordon

ORIGINAL ARTICLE

WILEY EJP
European Journal of Pain

The analgesic efficacy of morphine varies with rat strain and experimental pain model: implications for target validation efforts in pain drug discovery

Sara Hestehave[1,2] | Klas S. P. Abelson[1] | Tina Brønnum Pedersen[2] | Gordon Munro[3]

[1]Department of Experimental Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[2]H. Lundbeck A/S, Valby, Denmark

[3]Department of Neurology, Danish Headache Center, Glostrup Research Institute, Glostrup, Copenhagen, Denmark

Correspondence
Sara Hestehave, Department of Experimental Medicine, Copenhagen N, Denmark.
E-mails: sara.kristensen@sund.ku.dk or hestehave_sara@hotmail.com

Abstract

Background: Translating efficacy of analgesic drugs from animal models to humans remains challenging. Reasons are multifaceted, but lack of sufficiently rigorous preclinical study design criteria and phenotypically relevant models may be partly responsible. To begin to address this fundamental issue, we assessed the analgesic efficacy of morphine in three inbred rat strains (selected based on stress reactivity and affective/pain phenotypes), and outbred Sprague Dawley (SD) rats supplied from two vendors.

Methods: Sensitivity to morphine (0.3–6.0 mg/kg, s.c.) was evaluated in the hot plate test of acute thermal nociception, the Complete Freund's Adjuvant (CFA) model of inflammatory-induced mechanical hyperalgesia, and in a locomotor motility assay in male rats from the following strains; Lewis (LEW), Fischer (F344), Wistar Kyoto (WKY), and SD's from Envigo and Charles River.

Results: F344 and SD rats were similarly sensitive to morphine in hot plate and CFA-induced inflammatory hyperalgesia (Minimum Effective Dose (MED) = 3.0 mg/kg). WKY rats developed a less robust mechanical hypersensitivity after CFA injection, and were less sensitive to morphine in both pain tests (MED = 6.0 mg/kg). LEW rats were completely insensitive to morphine in the hot plate test, in contrast to the reversal of CFA-induced hyperalgesia (MED = 3.0 mg/kg). All strains exhibited a dose-dependent reduction in locomotor activity at 3.0–6.0 mg/kg.

Conclusion: Sensory phenotyping in response to acute thermal and inflammatory-induced pain, and sensitivity to morphine in various inbred and outbred rat strains indicates that different pathophysiological mechanisms are engaged after injury. This could have profound implications for translating preclinical drug discovery efforts into pain patients.

Significance: The choice of rat strain used in preclinical pain research can profoundly affect the outcome of experiments in relation to (a) nociceptive threshold responses, and (b) efficacy to analgesic treatment, in assays of acute and tonic inflammatory nociceptive pain.

1 | INTRODUCTION

An appreciation of pain enables higher organisms to respond appropriately to a spectrum of potentially tissue damaging and even life-threatening stimuli. Accordingly, it involves both learned and innate components, the understanding of which continues to be a source of considerable scientific endeavour by the medical research community.

A recognized pillar of preclinical drug development is the ability to reliably reproduce experimental findings using disease-relevant animal models, both over time, and between laboratories (Andrews et al., 2016). As part of this process, outbred Wistar and Sprague Dawley (SD) rats are the most commonly used rat strains for purposes of experimental testing in medical research (including pain), albeit other outbred/inbred strains and selection lines are occasionally used (Festing, 2014).

Opioid analgesics such as morphine remain a mainstay of treatment for acute moderate to severe pain, despite issues related to side effects including dependence, tolerance and opioid-induced hyperalgesia (Trang et al., 2015). Notably, strain differences in the nociceptive sensitivity to mu-opioid agonists in both mice and rats have been reported (Bulka et al., 2004; Cook, Barrett, Roach, Bowman, & Picker, 2000; Freet, Wheeler, Leuenberger, Mosblech, & Grigson, 2013; Mogil, Chesler, Wilson, Juraska, & Sternberg, 2000; Morgan, Cook, & Picker, 1999; Terner, Barrett, Cook, & Picker, 2003; Terner, Barrett, Lomas, Negus, & Picker, 2006; Terner, Lomas, Smith, Barrett, & Picker, 2003). Accordingly, it might be expected that the development of new pain therapeutics targeting opioid-mediated signalling (e.g., biased ligands, and drugs with improved pharmacokinetic/pharmacodynamic properties which slow entry into the CNS) in rodent models might be influenced by the rat or mouse strain chosen. Moreover, many of the studies detailing strain-dependent analgesic effects of opioids have utilized behavioural assays that for the most part focused on the use of acute nociceptive tests such as tail flick and hot plate, performed in the absence of any overt pathophysiology within peripheral or central pain circuits. From a drug development perspective, this is puzzling given the consensus of expert opinion that improving translation from bench to bedside is a fundamental tenet for facilitating the delivery of improved analgesic treatments to patients (Andrews et al., 2016; Berge, 2011; Clark, 2016; Negus et al., 2006; Rice et al., 2008).

Thus, based on the wealth of knowledge accumulated via opioid drug research and development, in the current series of experiments we chose to use morphine as a prototypical analgesic. Initially, we compared its acute analgesic efficacy in the absence of injury in SD rats obtained from two separate vendors, with three inbred rat strains (Lewis, Fischer F344, Wistar-Kyoto) previously reported to differ in analgesic sensitivity to mu-opioids, stress sensitivity and affective states

(Dhabhar, McEwen, & Spencer, 1993; Pardon et al., 2002; Pare, 1994). Thereafter, the same experimental aim was extended to the same strains in the setting of hindpaw inflammatory hyperalgesia produced by the inflammatory algogen Complete Freund's Adjuvant (CFA).

2 | MATERIALS AND METHODS

2.1 | Animals

For each experiment, a total of 50 male rats per strain (Lewis (LEW), Fischer F344 (F344) and Wistar Kyoto (WKY) from Harlan/Envigo UK; Hsd:SD from Harlan/Envigo, Netherlands; and Crl:CD(SD) from Charles River Laboratories, Germany) were used. They were aged 7 weeks upon arrival, and allowed to acclimatize for 1–2 weeks before experimental testing. For the CFA experiments, which were performed using one strain at a time, an additional 20 Crl:SD rats were included for intra-asssay control purposes. All rats completed the planned experiments except for one Hsd:SD rat which was excluded prior to active inclusion in a CFA experiment due to the presence of a tooth abscess. F344 rats were not tested in the locomotor motility assay due to an abrupt discontinuation of the strain at the supplier, Envigo.

Experimental protocols for the different testing procedures used at H. Lundbeck A/S were approved by The Animal Experiments Inspectorate in Denmark. The entire study was performed in accordance with the Danish legislation (Law no. 474 of May 15th, 2014 and Ministerial Order no. 12 of 07/01/2016) regulating experiments on animals, which are in compliance with the European Directive 2010/63/EU, and in accordance with the International Association for the Study of Pain guidelines (Zimmermann, 1983).

Note that we did not investigate an influence of gender in the following experiments, which has well-documented effects in relation to opioid-mediated analgesia in rodents (Cicero, Nock, & Meyer, 1997; Cook et al., 2000; Mogil et al., 2000; Terner, Barrett et al., 2003; Terner, Lomas et al., 2003), due to group size constraints of our study design.

2.2 | Housing

All animals were pair-housed in transparent Techniplast polycarbonate macrolone type III-high open cages (42.5*26.6*18.5 cm) from Scanbur, Denmark, with aspen chips (Tapvei, Estonia) used for bedding. Each cage was provided with environmental enrichment consisting of aspen wood chewing blocks (S-Bricks from Tapvei, Estonia), paper-wool shavings (LBS Biotechnology, UK) for nesting material, and red Rat Retreats[TM] (Bio-Serv, Flemington, US) for hiding, and cages were changed twice a week, but never on the days of testing. Food (Altromin 1324, Brogaarden, Denmark) and water were available ad libitum with water

changed on a weekly basis. The light–dark cycle was 12:12 hr with lights on from 06.00 hr; the room temperature was set to 20°C ± 2°C; and the relative humidity was 55% ± 10%. The animals were acclimatized to the surroundings for at least 1 week after arrival from the vendor, following which they were moved to the testing-laboratory at least 3 days prior to baseline testing, unless specified otherwise. The animals then remained in the testing room throughout the entire study.

3 | EXPERIMENTAL PROCEDURE

3.1 | Hot plate experiments

We used a hot plate (Ugo Basile Srl 7280, Gemonio, Italy) with a pre-set plate temperature of 52.5°C as recommended for rats (Bannon & Malmberg, 2007). As soon as the rat was placed onto the hot plate the time taken (s) for it to respond by, for example licking, shaking or stepping of the hindpaws (Bannon & Malmberg, 2007), was measured by the observer (Hestehave, Munro, Pedersen, & Abelson, 2017), at which point the rat was immediately removed from the hot plate. A cut-off time of 60 s was used to minimize the possibility of cutaneous tissue damage.

3.2 | CFA-induced inflammatory hyperalgesia

CFA-induced inflammatory hyperalgesia was induced in rats as described previously (Kristensen et al., 2017). Individual rats received a subcutaneous (s.c.) injection of CFA (CFA; Sigma Aldrich, mixed 1:1 in DPBS, 100 µl total volume per rat) into the plantar surface of the left hindpaw, while gently immobilized in a restraining box. They were then immediately returned to their home cage for 24 hrs.

Prior to CFA injection, reflex nociceptive responses to cutaneous mechanical stimulation were assessed for each rat (pre-CFA baseline response). The investigator gently restrained the rat and then applied progressively increasing mechanical pressure to the mid-plantar region of the left hindpaw using an electronic version of the Randall Selitto device (IITC, Chicago, IL). Pressure application was discontinued when the rat attempted to make a reflex hindpaw withdrawal (which in some instances was accompanied by vocalization) and the paw pressure threshold (g) was recorded. A cut-off threshold of 450 g was used to minimize the possibility of cutaneous tissue damage. Two additional measures were obtained from adjacent regions of the hindpaw, using approximately 20–30 s intervals between each measure. The average of the three measurements was designated as the mean paw pressure threshold. This process was repeated 24 hrs after CFA injection (post-CFA baseline) to obtain an index of evoked mechanical hyperalgesia (Munro, Erichsen, Rae, & Mirza, 2011), and then again 60 min after injection of either vehicle or morphine (post-treatment response).

3.3 | Motility assay

Rats were placed individually in transparent macrolone type III cages with bedding but no enrichment, similar to their home cages, for automated recording of locomotor activity during the light phase. Each cage was then placed within a holding rack which consisted of four sets of equally spaced infrared light sources and photocells located 4 cm above the bottom of the cage. The rats were then allowed to move freely within the cage for 60 min whereby horizontal movement resulted in the infrared light beams being interrupted (Sams-Dodd, 1998). The process was recorded continuously using custom-designed automated hardware and software (Ellegaard Systems A/S, Denmark), with locomotor activity expressed as an activity count obtained during the first 15 min of the test.

3.4 | Processing of plasma samples for corticosterone measurements

For both hot plate and CFA experiments, trunk blood was collected 60 min after testing, into EDTA-tubes. These were then centrifuged and plasma collected and stored at −20°C until analysis. Plasma corticosterone concentration was quantified using a commercial ELISA kit for corticosterone (Corticosterone ELISA, EIA-4164, DRG Instruments GmbH, Germany) according to the manufacturer's instructions.

3.5 | Study design

3.5.1 | Hot plate experiments (Studies 1 and 2)

An initial study (Study 1) compared analgesic efficacy of morphine in SD rats sourced from two different vendors, Charles River (Crl:SD) and Harlan (Hsd:SD). Firstly, 50 male Crl:SD rats were randomly allocated to groups ($n = 10$ group) to receive a s.c. injection of either morphine (0.3, 1.0, 3.0, 6.0 mg/kg) or vehicle (saline). The same protocol was used subsequently in an experiment involving 50 male Hsd:SD rats, with an additional Crl:SD group ($n = 10$) treated with morphine (3.0 mg/kg) included for purposes of intra-assay comparison. All hot plate testing was performed between 9.00 and 13.00 hr.

Although routinely used for experimental purposes due to an innate 'depressive-like' phenotype (Pare, 1994), the inbred Wistar Kyoto (WKY) rat also shows distinct changes in hypothalamo-pituitary-adrenal (HPA) axis function in response to acute and chronic stress paradigms (Pardon et al., 2002). Similarly, the inbred histocompatible Lewis (LEW) and Fischer (F344) strains are often used for experimental purposes based on reports that LEW rats display a relatively hypoactive stress response compared to stress hyper-responsive F344 rats (Dhabhar et al., 1993).

Accordingly, a subsequent study (Study 2) was subdivided into two experiments designed to assess (a) the effects of repeated hot plate testing on baseline nociceptive sensitivity of inbred and outbred strains, and (b) the analgesic efficacy of morphine in inbred strains. Due to the number of rats involved in this study, it was blocked into 5 identical cohorts of animals comprised of representatives from each inbred and outbred strain which all arrived at our facility on the same day. Three baseline hot plate tests were performed, each separated by 2 days. Two days after the third baseline measure, the analgesic efficacy of morphine was assessed. The three inbred strains (WKY, LEW and F344) were randomly allocated to five groups ($n = 10$ per group) for each strain to receive either morphine (0.3, 1.0, 3.0, 6.0 mg/kg) or vehicle (saline). Outbred strains (Crl:SD and Hsd:SD) were randomly allocated to two groups ($n = 10$ per group) for each vendor-supplied strain to receive morphine (3.0 mg/kg) or vehicle. The assessment of antinociceptive effects of morphine was carried out using a block design including two rats from each group for each strain in five consecutive cohorts (meaning 38 animals per cohort), and one cohort of antinociceptive testing was performed per day, from 08.00 to 11.00 hr each day in the exact same manner. At 60 min after testing, animals receiving vehicle or 3.0 mg/kg morphine were euthanized by decapitation, and trunk blood was collected for corticosterone measurements. The remaining test-subjects were euthanized with 80% CO_2 and 20% O_2 for the first 30 s, with a gradual increase to 100% CO_2 for 7 min.

3.5.2 | CFA-induced inflammatory hyperalgesia experiments (Studies 3 and 4)

For the first part of the study (Study 3), in a first experiment, only Crl:SD rats were tested. Thereafter, in a second experiment Hsd:SD rats were tested together with a single group of Crl:SD rats treated with morphine (3.0 mg/kg, $n = 10$). Subsequently, for Study 4, individual experiments were performed for each inbred strain (LEW, F344 and WKY) with two groups of Crl:SD rats (3.0 mg/kg morphine, vehicle, both $n = 10$) included for purposes of intra-assay comparison. For each primary strain, rats were randomly allocated to groups ($n = 10$ group) to receive a s.c. injection of either morphine (0.3, 1.0, 3.0, 6.0 mg/kg) or vehicle (saline). The magnitude of inflammatory hyperalgesia for each rat was calculated according to the following equation; [Hyperalgesia = ((pre-CFA baseline−post-CFA baseline)/pre-CFA baseline)*100]. All CFA experiments were performed between 7.00 and 11.00 hr during the light phase.

At the end of the experiment, 60 min after testing, all rats receiving vehicle or 3.0 mg/kg morphine were euthanized by decapitation, trunk blood was collected for corticosterone measurement, and both hindpaws were amputated at the exact same anatomical location by the ankle joint (just above the tarsal bones). The hindpaws were then weighed with the difference between the inflamed versus non-inflamed hindpaws used as a surrogate index of hindpaw oedema, enabling comparison with previously published data from our laboratory (Kristensen et al., 2017). The magnitude of paw oedema/inflammation for each rat was evaluated according to the following equation: [% Oedema = ((Inflamed paw weight−contralateral paw weight)/contralateral paw weight)*100]). The remaining test-subjects were euthanized with 80% CO_2 and 20% O_2 for the first 30 s, with a gradual increase to 100% CO_2 for 7 min.

3.5.3 | Exploratory motility

All animals arrived at the facilities on the same day and after acclimatization moved to the testing room the day prior to the actual test. The locomotor activity testing was divided across 5 consecutive experiments comprised of cohorts of animals from each strain (LEW, WKY, Hsd:SD, Crl:SD) and treatment group, and were performed over 2 days. Rats from each strain were randomly allocated to five different groups ($n = 10$ group) to receive a s.c. injection of either morphine (0.3, 1.0, 3.0, 6.0 mg/kg) or vehicle (saline). All motility experiments were conducted between 7.00 and 13.00 hr during the light phase. At the end of the experiment, animals were reused in another unrelated study (unpublished).

3.6 | Drugs and administration

Morphine hydrochloride was obtained from Nomeco (Denmark). It was diluted in sterile isotonic saline so that the concentration corresponded with a s.c. injection of 5 ml/kg body weight regardless of treatment group. To perform the s.c. injection each rat was placed in the lap of the same experienced experimenter (S.H.), and loosely wrapped in cotton material to form a restraining cone shape around the head and trunk to prevent the rat from turning back on itself (Turner, Pekow, Vasbinder, & Brabb, 2011).The s.c. injection was then administered in the free skin of the back and the rat gently placed back in their home-cage until testing. All doses are expressed as mg/kg body weight of salt. Animals were randomly allocated to treatment groups. Drug treatments were blinded for the experimenter performing the behavioural tests.

3.7 | Data analysis and statistics

Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, CA, USA) for most analysis, but ANCOVA's were performed in IBM SPSS Statistics, version 24 (IBM Corp.). Although no specific power analysis was performed for the current pharmacology experiments, appropriate group sizes had previously been

estimated in Sigmaplot as a function of the desired effect size (approximately 50% reversal to cut-off point), using a significance level of 5%, a power of 80% and a SD of approximately 4 obtained in a pilot study using similar pain assays (Festing & Altman, 2002; Kristensen et al., 2017). Statistical comparisons of treatment effects (morphine vs vehicle) between groups of the same strain were made using one-way ANOVA, with Bonferroni's post test to compare between strain-specific groups. Crl:SD groups were included in some trials as an intra-assay control, and are highlighted in the grey area of the corresponding graphs; however, the control Crl:SD data were not included in the one-way ANOVA in order to avoid influencing the overall effects of the strain. Accordingly, statistical comparison between the assay-control Crl:SD vehicle and morphine 3.0 mg/kg groups were made using unpaired *t*-tests. The overall strain and drug effects were also analysed using two-way ANCOVA (strain*treatment, covariate) with either pre-CFA baseline or the average of the three hot plate baselines as covariate. Two-way Repeated Measures (RM) ANOVA was used to assess the effects of strain and time (or CFA injection) for the development of hyperalgesia in response to CFA, and for effects of repeated testing in the hot plate baselines, (strain * time) followed by Bonferroni post test (Figure 2a). Mauchly's Test of Sphericity was performed in SPSS for univariate RM ANOVA's, and Greenhouse Geisser correction was applied when the assumption was violated (Figure 1b). For CFA experiments, the magnitude of inflammatory hyperalgesia and paw oedema/inflammation after CFA-injection for the individual animals was analysed by use of one-way ANOVA and Bonferroni post comparison test (Figure 2b and c, respectively). Two-way ANOVA was used to assess the overall effect of morphine on locomotor activity. $p < 0.05$ was considered statistically significant.

The Minimum Effective Dose (MED) was determined as the lowest dose of morphine required to elicit a response significantly different from the vehicle response, while the Maximum Possible Effect (%MPE) was calculated for the highest dose of morphine (6.0 mg/kg) by the following equation; % MPE = (post-drug latency−vehicle average)*100/(cutoff−vehicle average).

4 | RESULTS

4.1 | Influence of strain on acute nociceptive responses at baseline and after inflammatory injury

As a precursor to assessing the analgesic efficacy of morphine in each rat strain, we needed to establish whether there were strain-dependent differences in hindpaw nociceptive reflexes to cutaneous thermal or mechanical stimulation. Thereafter, based on differences in stress reactivity between the included strains we hypothesized that they might
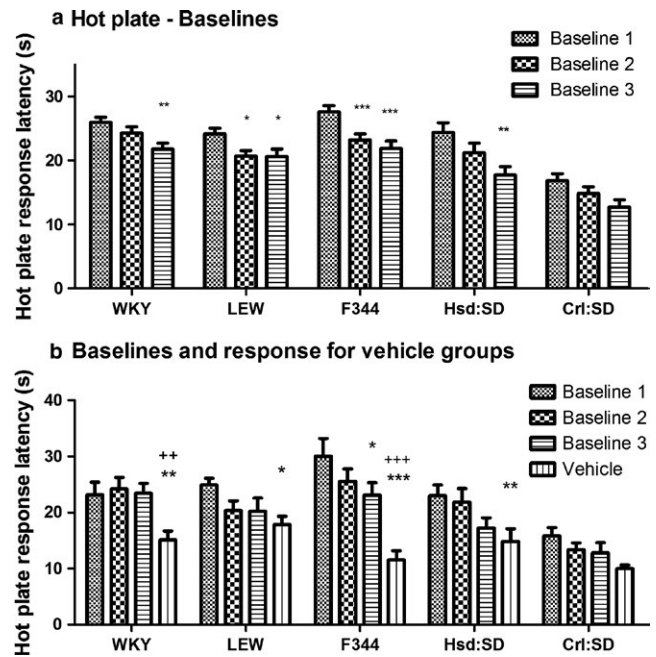

FIGURE 1 Hot plate testing in naïve rats. Development of response latency between testing sessions. (a) Effects of repeated hot plate testing on baseline response latency (s) in naïve rats from each strain. Group sizes; $n = 50$ for LEW, F344 and WKY, $n = 20$ for Hsd:SD and Crl:SD. Data are presented as mean ± *SEM*. Statistical comparison: two-way RM ANOVA, with Bonferroni's post test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs strain-specific Baseline 1. (b) Effects of vehicle injection as a novel stressor on response latency and comparison with prior baseline latency responses. All groups $n = 10$ rats. Data are presented as mean ± *SEM*. Two-way RM ANOVA with Bonferroni's post test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus Baseline 1; ++$p < 0.01$, +++$p < 0.001$ versus Baseline 3

respond differently to repeated nociceptive testing even in the absence of injury. Overall, two-way RM ANOVA revealed statistically significant effects of both time (F [2, 370] = 5.90, $p < 0.0001$) and strain (F [4, 370] = 14.22, $p < 0.0001$) (Figure 1a) on thermal response latency. Moreover, Figure 1a shows this effect was present in each strain tested (WKY; F [2, 149] = 5.872, $p = 0.0039$. LEW; F [2, 149] = 5.845, $p = 0.0040$. F344; F [2, 149] = 11.48, $p < 0.0001$. HSD:SD; F [2, 59] = 10.80, $p = 0.0002$, one-way RM ANOVA), albeit slightly less prominently in Crl:SD rats (F [2, 59] = 4.814, $p = 0.0137$). Notably, Crl:SD rats differed from the other strains in two main respects: (a) they were the only strain in which baseline responses did not vary significantly between tests (b) they were consistently more sensitive to noxious thermal stimulation of the hindpaws compared with the other strains.

We further explored the issue of stress sensitivity by comparing the impact of vehicle injection on the response latency in each strain measured on the day of the pharmacological experiment with morphine (Figure 1b). Notice
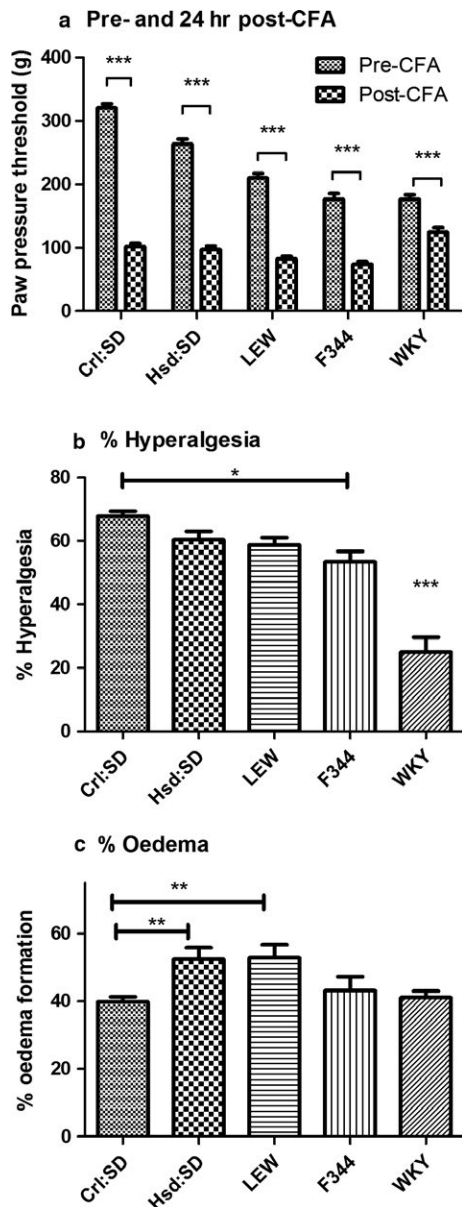
FIGURE 2 Strain differences in response to CFA injection. (a) Paw pressure thresholds (g) prior to and 24 hrs after hindpaw CFA injection. All groups $n = 50$, except Hsd:SD, $n = 49$. Data are presented as mean $\pm$ *SEM*. Two-way RM ANOVA with Bonferroni's post test. ***$p < 0.001$ Pre- versus Post-CFA. (b) Presence of inflammatory-induced hyperalgesia (% hyperalgesia) 24 hrs after hindpaw CFA injection. All groups $n = 50$, except Hsd:SD, $n = 49$. *$p < 0.05$, ***$p < 0.001$. (c) Hindpaw inflammatory response (% oedema formation) induced by CFA injection measured as the weight difference between the inflamed paw versus the non-inflamed paw. All groups $n = 20$ except Crl:SD, $n = 70$. Data are presented as mean $\pm$ *SEM*. One-way ANOVA, with Bonferroni's post test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

that Figure 1b only includes testing of the 10 animals of each strain receiving vehicle treatment, while Figure 1a includes baseline tests of all groups prior treatment ($n = 50$ for inbred strains, $n = 20$ for SD-strains). These data

violated the assumption of sphericity (Mauchly's Test of Sphericity, SPSS), and the results have therefore been corrected by use of Greenhouse-Geisser. Overall, we observed a further decrease in response latency with repeated testing (Time: F [2.645, 119] = 27.946, $p < 0.0001$. Strain: F [4, 45] = 8.028, $p < 0.0001$. Interaction: F [10.579, 119] = 2.599, $p = 0.0038$, two-way RM ANOVA). Moreover, even when accounting for the effect of repeated baseline testing Figure 1b shows that in the stress-hyperresponsive strains F344 and WKY, the vehicle response was significantly lower ($p < 0.001$ and $p < 0.01$, respectively, two-way RM ANOVA, Bonferroni post test) than the final baseline response. Once again, the Crl:SD strain exhibited a more stable response across both baseline testing and vehicle injection (Figure 1b).

Next, to simulate facets of injury-induced pathophysiology, we injected rats with the inflammatory algogen CFA. Figure 2a shows that corresponding pre-CFA and post-CFA baselines differed significantly in all strains (*F*-value for time [1, 244] = 1148, $p < 0.0001$, two-way RM ANOVA), indicating the presence of inflammatory-induced hyperalgesia. Similarly, two-way RM ANOVA also revealed a significant effect of strain (F [4, 244] = 48.40, $p < 0.0001$), and a strain*time-interaction, indicating that the CFA-injection/time had different effects on the various strains (F [4, 244] = 51.58, $p < 0.0001$). This allowed a comparison of pre- and post-CFA paw pressure thresholds between strains to be performed. Accordingly, the pre-CFA paw pressure threshold was significantly higher in Crl:SD than all the other strains ($p < 0.001$, Bonferroni post test). Conversely, after induction of inflammation, the post-CFA paw pressure threshold was significantly higher in WKY rats than all the other strains ($p < 0.05$–0.001).

Subsequent normalization of the post-CFA response to the pre-CFA response enabled the manifestation of the inflammatory pain response to be visualized as "% hyperalgesia" for each strain (Figure 2b). Again, we observed a significant effect of strain (F [4, 244] = 28.85, $p < 0.0001$, one-way ANOVA). Of note, WKY rats displayed only a modest hyperalgesic response ($25.0 \pm 4.6\%$) compared with the other strains (Crl:SD; 67.9 ± 1.5, Hsd:SD; 60.4 ± 2.7, LEW; 58.8 ± 2.2, F344; $53.4 \pm 3.4\%$), all $p < 0.001$ versus WKY, one-way ANOVA, Bonferroni post test).

Finally, an index of the inflammatory response per se, was obtained by measuring the % paw oedema present at the end of the experiment in rats from the morphine 3 mg/kg and vehicle-treated groups. Although Figure 2c shows there was a significant effect of strain on the % paw oedema response at this time (F [4, 145] = 5.49, $p < 0.0004$, one-way ANOVA) this did not appear to correlate with the robustness of hyperalgesia present (Figure 2b). Notably, the largest response was obtained in Hsd:SD and LEW strains ($p < 0.01$ vs. Crl:SD, one-way ANOVA and Bonferroni
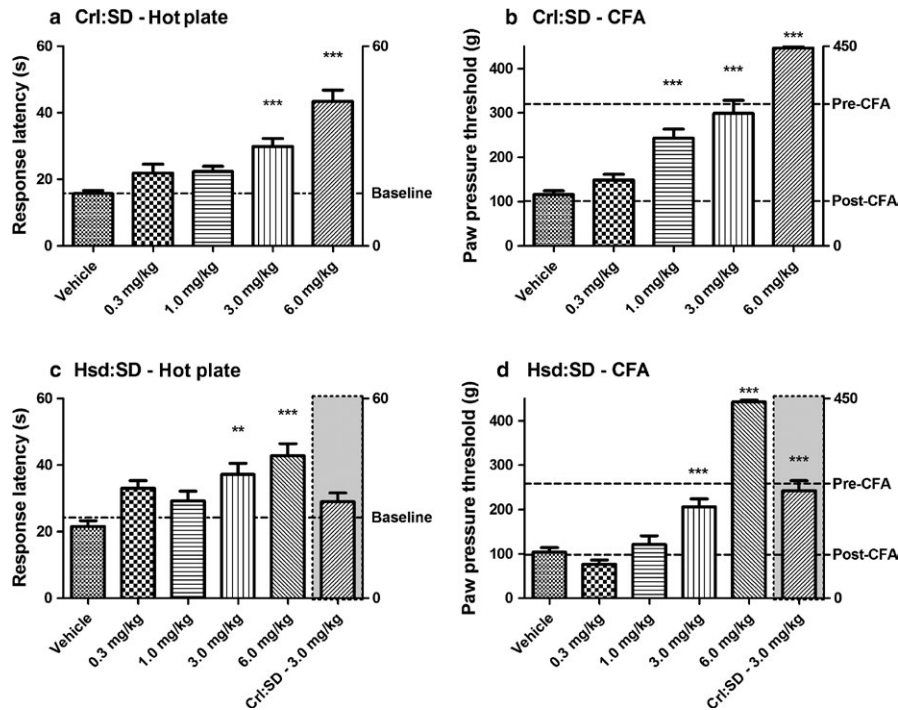
FIGURE 3 Effects of morphine on acute nociceptive pain and CFA-induced hyperalgesia in SD rats obtained from two different vendors. Animals were administered either morphine (0.3–6.0 mg/kg, s.c.) or vehicle prior to measuring (a, c) the response latency to thermal stimulation (52.5°C) in the hot plate test, or (b, d) effects on paw pressure thresholds (g) using an electronic Randall Selitto device as an index of mechanical hypersensitivity. Data are presented as mean ± *SEM*. The dotted lines in (a, c) indicate pre-injection baselines across the Crl:SD and Hsd:SD groups, and in (b, d) baselines across Crl:SD and Hsd:SD groups before ("Pre-CFA") and 24 hrs after injection ("Post-CFA") of CFA. As indicated in 3a, then the Crl:SD-control strain exhibits a lower baseline/vehicle-response, and comparable response latencies after 3.0 mg/kg for Crl:SD in figure a and c. All groups *n* = 10 rats. Note that the Hsd:SD vehicle and baseline responses in (c) are higher than the corresponding responses for Crl:SD rats in (a). This might have potentially masked a significant analgesic effect of 3.0 mg/kg morphine in Crl:SD in (c). One-way ANOVA, Bonferroni's post test. **$p < 0.01$, ***$p < 0.001$ versus vehicle

post test) with no difference in % paw oedema between WKY and Crl:SD strains.

4.2 | Acute analgesic effects of morphine in the hot plate test

Dose-dependent antinociceptive effects of morphine (0.3–6.0 mg/kg, s.c.) treatment were clearly apparent in 4 out of 5 of the tested strains, albeit the MED and %MPE varied (Figures 3–5, and Table 1); note that the data presented in these figures reflect experiments performed in hot plate studies 1 and 2. Accordingly, a two way ANCOVA of the data obtained in Study 2 which utilized a block design to comprise rats from all strains demonstrated significant effects of both treatment (F [4, 170] = 42.443, $p < 0.0005$) and strain (F [4, 170] = 4.373, $p < 0.002$), and a significant interaction between treatment and strain (F [4, 170] = 5.606, $p < 0.0005$). The largest effect size was for treatment (partial eta squared = 0.5), with F344, Crl:SD and Hsd:SD strains all possessing a morphine MED = 3.0 mg/kg ($p < 0.001$ (F344 and Crl:SD), $p < 0.01–0.001$ (Hsd:SD)

vs. vehicle, one-way ANOVA, Bonferroni's post test) as indicated in Figures 3a and c & 4c. In WKY rats, a higher dose of 6.0 mg/kg was required to produce a significant antinociceptive effect ($p < 0.001$ vs. vehicle), (Figure 5a). In contrast, LEW rats were insensitive to the antinociceptive effects of morphine (F [4,45] = 2.25, $p = 0.0784$, one-way ANOVA), (Figure 4a).

4.3 | Anti-hyperalgesic effects of morphine in rats with CFA-induced inflammation

Figures 3–5 show that s.c. administration of morphine (0.3–6.0 mg/kg) produced a robust dose-dependent reversal of CFA-induced hyperalgesia (F [4, 223] = 307.84, $p < 0.0005$, two way ANCOVA with pre-CFA baseline as covariate) with a large effect size for treatment (partial eta squared = 0.847). There was also a significant effect of strain (F [4, 223] = 13.295, $p < 0.0005$, two way ANCOVA), and a significant interaction effect of treatment*strain (F [16, 223] = 2.671, $p < 0.001$), but with smaller effect sizes than the effect of morphine (partial eta squared = strain: 0.193, and
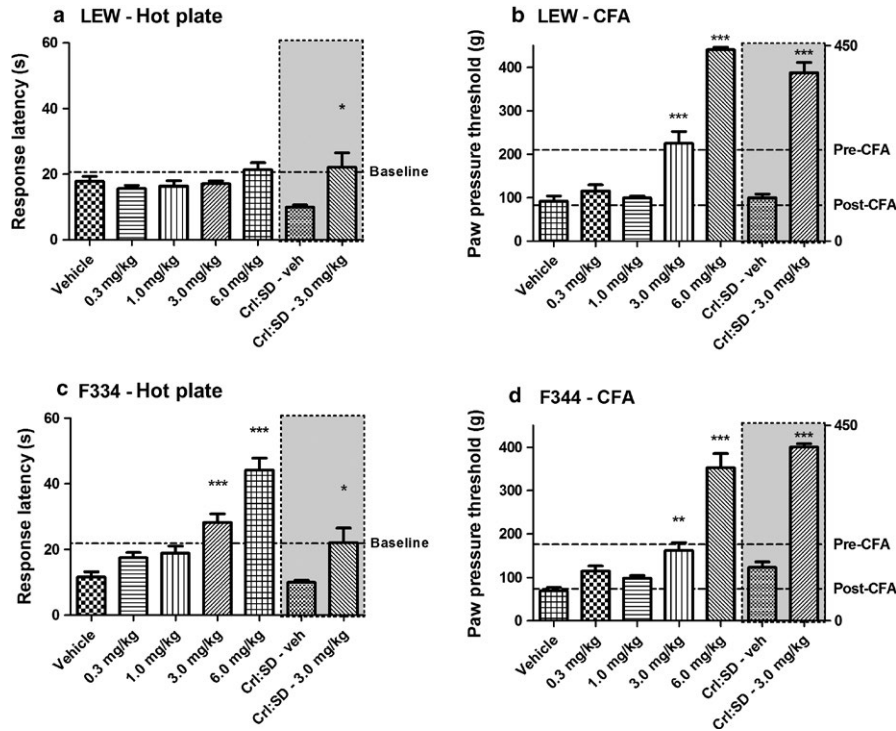
FIGURE 4 Effects of morphine on acute nociceptive pain and CFA-induced hyperalgesia in Lewis and Fischer (F344) rats. Animals were administered either morphine (0.3–6.0 mg/kg, s.c.) or vehicle 60 min prior to measuring (a, c) the response latency to thermal stimulation (52.5°C) in the hot plate test, or (b, d) effects on paw pressure thresholds (g) using an electronic Randall Selitto device as an index of mechanical hypersensitivity. Data are presented as mean $\pm$ *SEM*. The dotted lines in (a, c) indicate pre-injection baselines across the LEW or F344 groups, and in (b, d) baseline across LEW or F344 groups before ("Pre-CFA") and 24 hrs after injection ("Post-CFA") of CFA. All groups $n = 10$ rats. One-way ANOVA, Bonferroni's post test for F344 and LEW groups, Unpaired *t*-test for Crl:SD-groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle
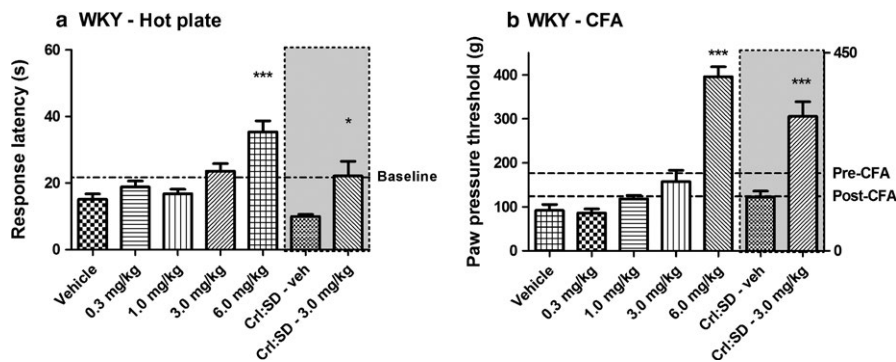

FIGURE 5 Effects of morphine on acute nociceptive pain and CFA-induced hyperalgesia in Wistar Kyoto rats. Animals were administered either morphine (0.3–6.0 mg/kg, s.c.) or vehicle 60 min prior to measuring (a) the response latency to thermal stimulation (52.5°C) in the hot plate test, or (b) effects on the paw pressure threshold (g) using an electronic Randall Selitto device as an index of mechanical hypersensitivity. Data are presented as mean $\pm$ *SEM*. The dotted lines in (a) indicate pre-injection baselines across WKY groups, and in (b) baseline across WKY groups before ("Pre-CFA") and 24 hrs after injection ("Post-CFA") of CFA. All groups $n = 10$ rats. One-way ANOVA, Bonferroni's post test for WKY groups, Unpaired *t*-test for Crl:SD-groups. *$p < 0.05$, ***$p < 0.001$ versus vehicle

treatment*strain; 0.161). Moreover, at the level of each individual experiment, significant effects of morphine treatment were detected in all strains (LEW: $F [4,45] = 95.30$, $p < 0.0001$, WKY: $F [4,45] = 56.40$, $p < 0.0001$, F344: $F [4,45] = 40.43$, $p < 0.0001$, Hsd:SD: $F [4,45] = 122.1$, $p < 0.0001$, Crl:SD: $F [4,45] = 58.08$, $p < 0.0001$, one-way ANOVAs), with a rank

order of potency ranging from MED = 1–6 mg/kg (Crl:SD > Hsd:SD = LEW = F344 > WKY) (Table 1).

Importantly, administration of 3 mg/kg morphine to Crl:SD rats for inter-assay control purposes produced robust antihyperalgesia in each experiment ($p < 0.0001$ vs. strain-specific vehicle, Student's *t*-test) (grey area of graphs

TABLE 1 Comparative efficacy of morphine in different rat strains

	Hot plate		CFA		LMA		CFA-parameters (%)	
Strain	MED	%MPE	MED	%MPE	MED	%MPE	Hyperalgesia	Paw oedema
Crl:SD	3.0	62.7 ± 7.5	1.0	98.9 ± 0.8	3.0	91.1 ± 2.2	67.9 ± 1.5	38.9 ± 1.5
Hsd:SD	3.0	55.4 ± 9.4	3.0	97.9 ± 1.2	3.0	89.7 ± 3.6	60.4 ± 2.7	52.4 ± 3.5
LEW	>6.0	8.4 ± 5.1	3.0	97.5 ± 1.6	3.0	84.2 ± 3.8	58.8 ± 2.2	52.8 ± 4.0
F344	3.0	67.6 ± 7.3	3.0	74.5 ± 8.4	–	–	53.4 ± 3.4	43.2 ± 4.0
WKY	6.0	45.2 ± 7.3	6.0	84.9 ± 6.2	3.0	94.5 ± 2.7	25.0 ± 4.6	41.1 ± 1.9

Data are presented as MED (Minimum Effective Dose) or as % MPE (Maximum Possible Effect) = (post-drug value − vehicle average)*100/(assay cut-off value − vehicle average) and calculated for the highest dose of morphine (6.0 mg/kg) in each case. The assay cut-off values were LMA = 0, CFA = 450 and hot plate = 60. mean ± *SEM*. CFA, CFA-induced inflammation; LMA, Locomotor activity. Note that LMA was not measured for the F344-strain due to supply issues from the vendor.

Figures 3–5). We would have preferred to have used a block design with individual CFA experiments split into treatment groups containing smaller numbers of rats from each strain. However, pragmatic reasons dictated the experimental design employed herein since we established early on that the vendors could not guarantee availability of test subjects simultaneously for such a purpose.

4.4 | Plasma corticosterone levels in hot plate and CFA experiments

Glucocorticoids are released in response to stress, and as potent inhibitors of inflammatory processes (Tonelli, Webster, Rapp, & Sternberg, 2001) can be used as adjuvants to reduce opioid use in various pain conditions. Accordingly, we wanted to investigate if any putative strain differences in the antinociceptive or anti-hyperalgesic efficacies of morphine might be linked to glucocorticoid activity. Note that a limitation to the corticosterone measurements presented here is that they were obtained 120 min after injection, in contrast to behavioural measures which were obtained 60 min after injection (Study 2 & 4). Thus, it is possible that any stress associated with behavioural handling might have inadvertently impacted upon corticosterone measurements.

Figure 6a shows that morphine treatment had no effect on plasma corticosterone levels in the different strains, albeit significant effects of strain per se were observed (F [4,90] = 7.728, $p < 0.0001$; two-way ANOVA). Accordingly, Bonferroni's post hoc tests detected strain differences between F344 vehicle-treated rats compared WKY ($p < 0.01$), Hsd:SD ($p < 0.001$) and Crl:SD rats ($p < 0.05$). Similarly, vehicle-treated LEW showed significantly higher plasma concentrations than Hsd:SD ($p < 0.01$). The only difference between morphine-groups was found between F344 and Hsd:SD ($p < 0.01$). For the CFA study shown in Figure 6b only results from histocompatible LEW and F344 are presented (due to the widely acknowledged impact of the immune system on inflammatory and associated stress reactivity), together with Crl:SD rats
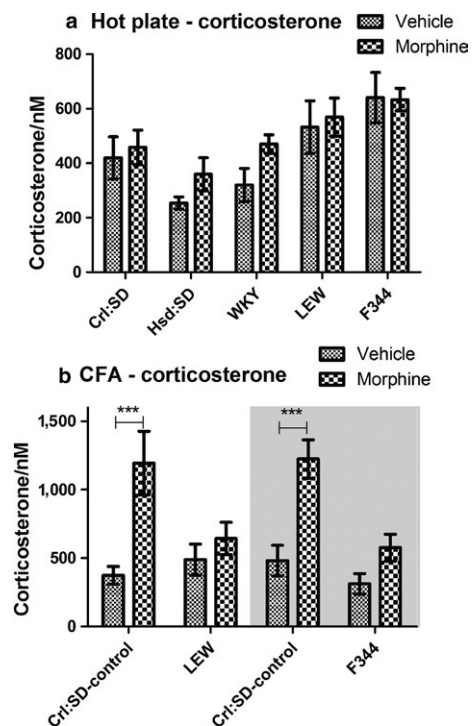

FIGURE 6 Plasma corticosterone levels in hot plate and CFA experiments. Plasma corticosterone was measured at 1 hr after nociceptive testing, and 2 hrs after s.c. injection of either 3 mg/kg morphine or vehicle. (a) Plasma corticosterone levels (nM = nmol/L) in hot plate experiments including groups from all strains. Comparing vehicle groups, F344 was significantly higher than WKY ($p < 0.01$), Hsd:SD ($p < 0.001$) and Crl:SD ($p < 0.05$), and LEW was significantly higher than Hsd:SD ($p < 0.01$), two-way ANOVA with Bonferroni's post test. Following morphine treatment, only Hsd:SD was significantly lower than F344 ($p < 0.01$). (b) Plasma corticosterone levels in CFA experiments comparing LEW and F344 rats with Crl:SD rats included for inter-assay control purposes. All groups $n = 10$ rats. Data are presented as mean ± *SEM*. Two-way ANOVA and Bonferroni's post test revealed significant difference between vehicle and morphine-treated groups of the same strain. ****p < 0.001* versus vehicle. Additional post tests also revealed that morphine-treated Crl:SD were significantly higher than LEW and F344 ($p < 0.01$). No differences were detected between vehicle groups

which were included for assay-control purposes. Morphine treatment resulted in elevated plasma levels of corticosterone (F [1,72] = 29.51, $p < 0.0001$) and interacted significantly with strain (F [3,72] = 3.367, $p = 0.0232$, two-way ANOVA) in CFA rats, indicating that the effect was not similar across the different strains. Notably, Bonferroni's post test indicated that this effect of morphine only occurred in Crl:SD rats ($p < 0.001$ vs. vehicle). Comparison between strains showed no differences between vehicle-treated groups, but morphine-treated Crl:SD-controls were significantly higher than morphine-treated LEW and F344 (both $p < 0.01$, Bonferroni's post test).

4.5 | Effects of morphine on locomotor activity

As indicated in Figure 7a–d, s.c. administration of morphine (0.3–6.0 mg/kg) produced a dose-dependent inhibition of activity in all strains included in the experiment (LEW: F [4,45] = 45.63, $p = <0.0001$; WKY: F [4,45] = 30.81, $p = <0.0001$; Hsd:SD: F [4,45] = 43.07, $p = <0.0001$; Crl:SD: F [4,45] = 25.46, $p = <0.0001$, one-way ANOVA) when measured over the first 15 min of the assay procedure. Notably, morphine was equally potent in each strain (MED = 3.0 mg/kg, Table 1). Thus, any putative sedative or motor-impairing effects of morphine are unlikely to have been a primary confounding issue explaining the strain-dependent analgesic efficacy of morphine observed in the hot plate or CFA experiments.

5 | DISCUSSION

We have consolidated a number of important experimental parameters (e.g., investigator, vendor, rat strain, pain assays) which can impact upon the pharmacological outcome for a candidate analgesic in the preclinical setting (Chesler, Wilson, Lariviere, Rodriguez-Zas, & Mogil, 2002; Lacroix-Fralish & Mogil, 2009; Lariviere & Mogil, 2010; Lewejohann et al., 2006; Sorge et al., 2014). Our findings revealed strain- and vendor-dependent differences in nociceptive thresholds and sensitivity to morphine, prior to and after inflammatory injury. We believe these observations have important implications for translating preclinical drug discovery efforts into pain patients, as they highlight a limited translational value of in vivo data sets when derived from a single animal strain or single pain assay alone.

5.1 | Nociceptive thresholds prior to and after acute injection or inflammatory injury

For the strains included here, baseline nociceptive thresholds varied considerably depending on the stimulus modality. Noxious thermal stimulation of the hindpaws revealed that Crl:SD rats were most sensitive at the first test (Crl:SD>Hsd:SD=WKY=LEW=F334). Repeated testing then produced an expected decline in baseline response latency, albeit it varied between strains. Subsequently, when the final session of baseline nociceptive testing was followed by an acute injection of vehicle, sensitizing effects of this
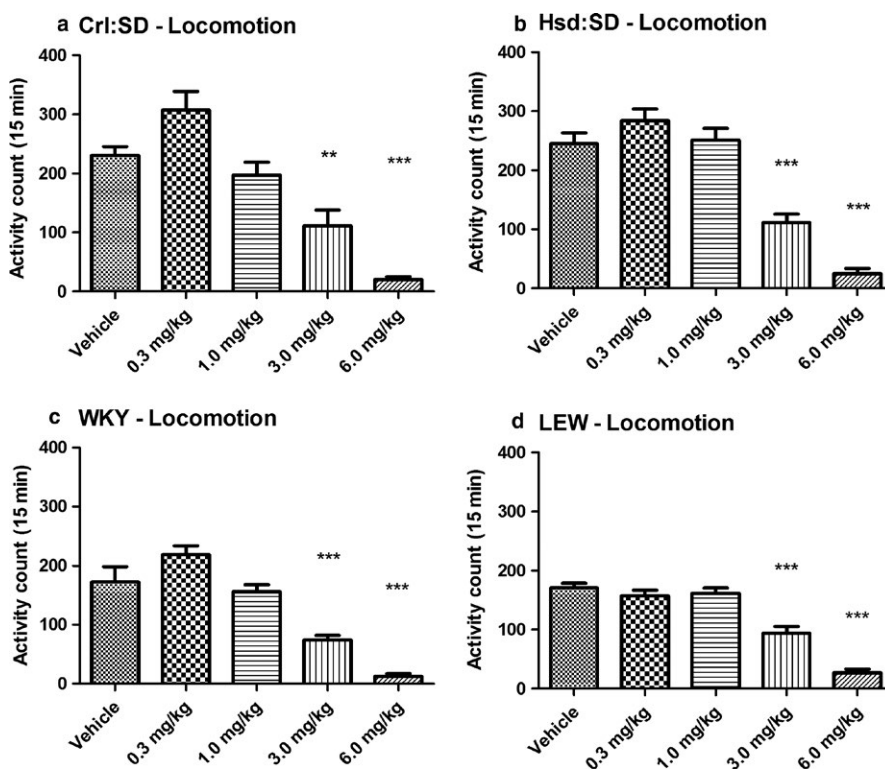

FIGURE 7 Effect of morphine on locomotor activity. The activity count represents the number of horizontal laser beams broken by the rat during the first 15 min of the assay and was measured 45–60 min after injection of either morphine (0.3–6.0 mg/kg, s.c.) or vehicle. All groups $n = 10$ rats. Data are presented as mean ± *SEM*. One-way ANOVA with Bonferroni's post test, significant difference compared with vehicle treated group. **$p < 0.01$, ***$p < 0.001$ versus vehicle

novel stressor on nociceptive thresholds were only seen in the stress-responsive F344 and WKY strains (Dhabhar et al., 1993; Gomez, Lahmame, de Kloet, & Armario, 1996; Sternberg et al., 1989). This latter finding is important in light of various studies reporting strain differences wherein analgesic efficacy of opioids have been inferred from baseline rather than vehicle comparisons (Cook et al., 2000; Herradon, Morales, & Alguacil, 2003; Herradon, Morales, Perez-Garcia, & Alguacil, 2003; Mogil et al., 2000; Morgan et al., 1999; Terner, Barrett et al., 2003; Terner, Lomas et al., 2003; Terner et al., 2006; Vaccarino & Couret, 1995; Vit et al., 2006; Woolfolk & Holtzman, 1995).

A positive correlation between thermal and mechanical stimuli on hindpaw nociceptive responses has been observed in naive male rats representing eight inbred and outbred strains (Shir et al., 2001). Thus, we were surprised that Crl:SD rats were paradoxically least sensitive to Randall Selitto stimulation of the hindpaw (Crl:SD<Hsd:SD<LEW<WKY=F344). Thereafter, following CFA-injection, with the threshold difference considered as an index of mechanical hyperalgesia (Kristensen et al., 2017; Soignier et al., 2011), we observed similar responses in all strains, with the exception of WKY which developed only a modest hyperalgesia. These findings were unexpected based on (a) reports that F344 rats develop more robust hyperalgesia than LEW rats in response to *acute* inflammation induced by CFA (Zhang, Lao, Qiao, & Ruda, 2003), carrageenan (Fecho, Manning, Maixner, & Schmitt, 2007; Fecho & Valtschanoff, 2006; Juif, Anton, & Hanesch, 2012) or formalin (Lariviere, Sattar, & Melzack, 2006) (b) increased expression of formalin-induced second phase nocifensive behaviours in WKY versus SD rats (Burke et al., 2010) (c) the comparable level of CFA-induced oedema in WKY and the other strains in this study. Although handling was transient during Randall Selitto testing, differences in stress-reactivity and affective state of the various strains (Dhabhar et al., 1993; Pardon et al., 2002; Pare, 1994), might theoretically have impacted on nociceptive thresholds as a consequence of stress-induced analgesia (Butler & Finn, 2009; Vit et al., 2006) or learned helplessness (Pare, 1994; Wu & Wang, 2010). Ultimately, sensitivity to nociceptive stimulation at baseline does not generalize to a hyper-responsive pain phenotype after acute inflammatory injury (Liang et al., 2006).

5.2 | Antinociceptive actions of morphine in the hot plate test and analgesic effects in CFA-inflamed rats

Acute analgesic effects of opioids in rodent assays of thermal nociception can vary with genotype, gender, stimulus intensity and potency/pharmacokinetics of the opioid in question (Cook et al., 2000; Morgan et al., 1999; Terner, Barrett et al., 2003; Terner, Lomas et al., 2003). Consistent with our findings, LEW rats have been found to be less sensitive to opioids

compared with Long Evans or SD rats using a tail withdrawal assay (Morgan et al., 1999), as well as histocompatible F344 rats across a number of both nociceptive and non-nociceptive behaviours (e.g., tail withdrawal, conditioned place preference or conditioned taste aversion) (Cook et al., 2000; Davis, Roma, Dominguez, & Riley, 2007; Freet et al., 2013; Morgan et al., 1999; Terner, Barrett et al., 2003; Terner, Lomas et al., 2003; Vaccarino & Couret, 1995). Similarly, WKY rats appear to be less sensitive to morphine than SD rats in acute thermal nociceptive assays (Bhargava, Villar, Rahmani, & Larsen, 1992; Hoffmann, Plesan, & Wiesenfeld-Hallin, 1998). While these observations would simply appear to reflect a general lack of sensitivity to opioids within the LEW and WKY strains, this concept is not supported by our automated locomotor activity data where the mild motor-impairing actions of 3 mg/kg morphine were extremely similar across the strains tested, and in agreement with previous observations reported for SD rats (Munro, 2009). Unfortunately, we were unable to include F344 rats in these experiments due to an abrupt discontinuation of the strain by the vendor. However, a 3 mg/kg dose of morphine would also be expected to impair locomotor activity in F344 rats (Sudakov et al., 1993).

To simulate facets of injury-induced pathophysiology, we injected rats with the inflammatory algogen CFA, which sensitizes peripheral sensory neurons and drives central hyperexcitability changes within dorsal horn pain circuits (Julius and Basbaum, 2001; Marchand, Perretti, & McMahon, 2005; Schafer, Imai, Uhl, & Stein, 1995). As expected morphine reversed inflammatory hyperalgesia in each strain albeit with varying potency (Fecho et al., 2007; Kristensen et al., 2017). Thereafter, comparison with morphine efficacy in hot plate revealed strain-dependent differences in opioid-mediated analgesia following injury. Multiple studies have shown that inflammatory pain and genetics combine to modulate analgesia mediated by opioids in rodents (Chesler et al., 2003; Liang et al., 2006; Mogil, Lichtensteiger, & Wilson, 1998). Here, the clearest increase in both morphine efficacy and potency in response to CFA injection was observed in LEW rats, to the extent that they were essentially indistinguishable from the other strains. Interesting in itself, this type of strain-dependent analgesia has important implications for the uninitiated investigator from a drug discovery perspective in pain.

5.3 | How might analgesic efficacy to morphine manifest variously across different rat strains?

Differences in HPA axis function and stress responsiveness can contribute to varying severity of inflammation (Dhabhar, 2002). Morphine activates the HPA axis (Pechnick, 1993), which in turn via its inherent role in pain modulation and immune function has been proposed to potentiate morphine analgesia (Bodnar & Kest, 2010; Kosten & Ambrosio, 2002;

Mellon & Bayer, 1998; Woolfolk & Holtzman, 1995). However, this is unlikely to have contributed to the acute antinociceptive actions of morphine in the hot plate study due to the transient nature of the thermal stimulus applied, and as supported by the lack of treatment effects on plasma corticosterone levels. We cannot exclude a role of HPA axis activation in potentiation of morphine efficacy and potency in LEW rats after tonic inflammatory injury. However, commensurate changes were not observed in the stress-sensitive F344 strain, and the corticosterone levels for morphine-treated LEW and F344 rats were markedly less than measured in Crl:SD rats. Accordingly, any putative contribution of strain-dependent stress reactivity to analgesic effects of morphine, or indeed other drug candidates in pain assays such as those used here is undoubtedly complex.

Linking the temporal pharmacodynamic actions of a drug candidate, here exemplified by the mu opioid receptor morphine, to relevant concentrations within its target tissue(s) is a highly desirable facet of demonstrating appropriate target engagement in drug discovery. In this regard, a potential limitation of our study was the use of a single 60 min time point post-morphine for assessment of efficacy. Whereas the analgesic effects of morphine in rodent assays of acute nociception are mediated centrally, both peripheral and central sites contribute to its anti-hyperalgesic efficacy in inflammatory models (Schafer et al., 1995; Zhang et al., 2003). Accordingly, Tmax for s.c. morphine is reached more rapidly in plasma (0.25 hr = 15min) than in brain where it ranges from 45 to 95 min (Stain et al., 1995; Van Crugten, Somogyi, & Nation, 2000), which aligns well with the efficacy time point used here. When combined with other reports for LEW and F344 rats describing antinociceptive effects of morphine peaking at 30–60 min (Cook et al., 2000), and similar Cmax in blood at this time (Davis et al., 2007), we think it unlikely that pharmacokinetic differences are responsible for the clear strain differences in morphine antinociception observed in our hot plate experiments; observations further supported by the comparable strain effects of morphine in both CFA- and locomotor-studies. These findings do not however consider accumulation of morphine or its metabolites within the CNS, where enhanced analgesic effects of morphine in Spontaneously Hypertensive Rats compared with WKY rats (their normotensive controls) have been linked to higher tissue concentrations within spinal cord and various brain areas, despite similar plasma pharmacokinetic parameters (Bhargava et al., 1992; Gulati & Bhargava, 1990).

Can other mechanisms account for the differences in analgesic efficacy of morphine between strains? Although comparative insights typically need to be gained from studies which have been performed in isolation, a number of possibilities may exist. Despite possessing a similar density of mu-opioid receptors in brain and spinal cord (Herradon, Morales, & Alguacil, 2003; Herradon, Morales, Perez-Garcia

et al., 2003), the intrinsic responsiveness of the receptor and its downstream signalling cascades to agonist stimulation differs between F344 and LEW rats even in the absence of injury (Guitart et al., 1993; Herradon, Morales, & Alguacil, 2003; Herradon, Morales, Perez-Garcia et al., 2003; Selley et al., 2003). Although inflammatory injury of itself fails to change receptor affinity to opioid agonists, it does increase the density of mu-opioid receptor binding sites and G-protein coupling within dorsal root ganglion of Wistar rats (Zollner et al., 2003); this likely explains why inflammation potentiates the overall efficacy of morphine in the current study, especially in inflammatory-prone LEW rats (Juif et al., 2012; O'Malley, Julio-Pieper, O'Mahony, Dinan, & Cryan, 2014; Sternberg et al., 1989). Strain differences in nociceptive processing are not restricted to models of acute and tonic pain. Strain-dependent nociceptive sensitivity has also been reported in various rodent models of neuropathic pain (del Rey et al., 2011; Le Coz, Fiatte, Anton, & Hanesch, 2014; Rode et al., 2007; Yoon, Lee, Lee, Chung, & Chung, 1999; Zeng et al., 2008), wherein a primary pathophysiological mechanism involves a change in descending modulatory control from brainstem structures (e.g., PAG and RVM) to the spinal dorsal horn to facilitate nociceptive transmission (De Felice et al., 2011). Intriguingly, a higher incidence of neuropathic sensitivity in SD rats versus closely-related outbred Holtzman rats (85% vs. 50%, respectively) has been attributed to the protective recruitment of descending inhibition in the latter strain (De Felice et al., 2011). Notably, despite a myriad of side-effects opioids are efficacious in neuropathic pain acting in part by engaging descending monoaminergic pathways (Finnerup, Sindrup, & Jensen, 2010). Whether differential recruitment of such a mechanism accounts in part for the current findings is speculative, although not unreasonable based on the acknowledged role of descending modulation to inflammatory hyperalgesia (Ren & Dubner, 1996).

6 | CONCLUSIONS

Using morphine as a candidate analgesic, our data reflect that reliance on a single strain of rat, behavioural assay, even vendor, for purposes of assessing efficacy is not straightforward. A key question that arises is whether a specific strain possess superior translational utility in pain research over others? We are not aware of any overriding evidence supporting the use of commonly used outbred rat strains such as SD or Wistar in this regard, albeit as noted above they may develop a greater degree of neuropathic hypersensitivity compared with other strains (De Felice et al., 2011). Researcher compliance may also be influenced by an anecdotal assumption that the heterogenic diversity of such outbred strains better reflects that of a typical pain patient cohort. Rather, pragmatic issues such as reproductive fecundity and availability, ease of

animal handling, unit costs and adherence to previously published methodologies appear to guide researcher compliance (Festing, 2014). Ultimately, we believe that comparative studies such as ours are crucial for facilitating future discussion and mitigation of data discrepancy within analgesic drug research and development.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. Two of the authors are employed by the pharmaceutical company, H. Lundbeck A/S, but as the company has no commercial interests in the compounds tested in the current study, this affiliation does not result in conflicting interests.

AUTHOR CONTRIBUTIONS

The study was conceptualized by SH, KA, TBP and GM. Experiments were designed by SH and GM and discussed thereafter with KA and TBP. SH performed all experiments and statistical analysis. SH and GM wrote the manuscript and all authors discussed the results and finalized the manuscript.

REFERENCES

Andrews, N. A., Latremoliere, A., Basbaum, A. I., Mogil, J. S., Porreca, F., Rice, A. S., … Whiteside, G. (2016). Ensuring transparency and minimization of methodologic bias in preclinical pain research: PPRECISE considerations. *Pain*, *157*(4), 901–909. https://doi.org/10.1097/j.pain.0000000000000458

Bannon, A. W., & Malmberg, A. B. (2007). Models of nociception: Hotplate, tail-flick, and formalin tests in rodents. *Current Protocols in Neuroscience*, *41*(1), 8.9.1–8.9.16 Chapter 8:Unit 8.9.

Berge, O.-G. (2011). Predictive validity of behavioural animal models for chronic pain. *British Journal of Pharmacology.*, *164*(4), 1195–1206. https://doi.org/10.1111/j.1476-5381.2011.01300.x

Bhargava, H. N., Villar, V. M., Rahmani, N. H., & Larsen, A. K. (1992). Time course of the distribution of morphine in brain regions and spinal cord after intravenous injection to spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Journal of Pharmacology and Experimental Therapeutics*, *261*(3), 1008–1014.

Bodnar, R. J., & Kest, B. (2010). Sex differences in opioid analgesia, hyperalgesia, tolerance and withdrawal: Central mechanisms of action and roles of gonadal hormones. *Hormones and Behavior*, *58*(1), 72–81. https://doi.org/10.1016/j.yhbeh.2009.09.012

Bulka, A., Kouya, P. F., Böttiger, Y., Svensson, J. O., Xu, X. J., & Wiesenfeld-Hallin, Z. (2004). Comparison of the antinociceptive effect of morphine, methadone, buprenorphine and codeine in two substrains of Sprague–Dawley rats. *European Journal of Pharmacology*, *492*(1), 27–34. https://doi.org/10.1016/j.ejphar.2004.03.041

Burke, N. N., Hayes, E., Calpin, P., Kerr, D. M., Moriarty, O., Finn, D. P., & Roche, M. (2010). Enhanced nociceptive responding in two rat models of depression is associated with alterations in monoamine levels in discrete brain regions. *Neuroscience*, *171*(4), 1300–1313. https://doi.org/10.1016/j.neuroscience.2010.10.030

Butler, R. K., & Finn, D. P. (2009). Stress-induced analgesia. *Progress in Neurobiology*, *88*(3), 184–202. https://doi.org/10.1016/j.pneurobio.2009.04.003

Chesler, E. J., Ritchie, J., Kokayeff, A., Lariviere, W. R., Wilson, S. G., & Mogil, J. S. (2003). Genotype-dependence of gabapentin and pregabalin sensitivity: The pharmacogenetic mediation of analgesia is specific to the type of pain being inhibited. *Pain*, *106*(3), 325–335. https://doi.org/10.1016/S0304-3959(03)00330-0

Chesler, E. J., Wilson, S. G., Lariviere, W. R., Rodriguez-Zas, S. L., & Mogil, J. S. (2002). Influences of laboratory environment on behavior. *Nature Neuroscience*, *5*(11), 1101–1102. https://doi.org/10.1038/nn1102-1101

Cicero, T. J., Nock, B., & Meyer, E. R. (1997). Sex-related differences in morphine's antinociceptive activity: Relationship to serum and brain morphine concentrations. *Journal of Pharmacology and Experimental Therapeutics*, *282*(2), 939–944.

Clark, J. D. (2016). Preclinical pain research: Can we do better? *Anesthesiology*, *125*(5), 846–849. https://doi.org/10.1097/ALN.0000000000001340

Cook, C. D., Barrett, A. C., Roach, E. L., Bowman, J. R., & Picker, M. J. (2000). Sex-related differences in the antinociceptive effects of opioids: importance of rat genotype, nociceptive stimulus intensity, and efficacy at the mu opioid receptor. *Psychopharmacology (Berl).*, *150*(4), 430–442. https://doi.org/10.1007/s002130000453

Davis, C. M., Roma, P. G., Dominguez, J. M., & Riley, A. L. (2007). Morphine-induced place conditioning in Fischer and Lewis rats: Acquisition and dose-response in a fully biased procedure. *Pharmacology Biochemistry and Behavior*, *86*(3), 516–523. https://doi.org/10.1016/j.pbb.2007.01.013

De Felice, M., Sanoja, R., Wang, R., Vera-Portocarrero, L., Oyarzo, J., King, T., … Porreca, F. (2011). Engagement of descending inhibition from the rostral ventromedial medulla protects against chronic neuropathic pain. *Pain*, *152*(12), 2701–2709. https://doi.org/10.1016/j.pain.2011.06.008

del Rey, A., Yau, H. J., Randolf, A., Centeno, M. V., Wildmann, J., Martina, M., … Apkarian, A. V. (2011). Chronic neuropathic pain-like behavior correlates with IL-1beta expression and disrupts cytokine interactions in the hippocampus. *Pain*, *152*(12), 2827–2835.

Dhabhar, F. S. (2002). Stress-induced augmentation of immune function—The role of stress hormones, leukocyte trafficking, and cytokines. *Brain, Behavior, and Immunity.*, *16*(6), 785–798. https://doi.org/10.1016/S0889-1591(02)00036-3

Dhabhar, F. S., McEwen, B. S., & Spencer, R. L. (1993). Stress response, adrenal steroid receptor levels and corticosteroid-binding globulin levels–a comparison between Sprague-Dawley, Fischer 344 and Lewis rats. *Brain Research*, *616*(1–2), 89–98. https://doi.org/10.1016/0006-8993(93)90196-T

Fecho, K., Manning, E. L., Maixner, W., & Schmitt, C. P. (2007). Effects of carrageenan and morphine on acute inflammation and pain in Lewis and Fischer rats. *Brain, Behavior, & Immunity.*, *21*(1), 68–78. https://doi.org/10.1016/j.bbi.2006.02.003

Fecho, K., & Valtschanoff, J. G. (2006). Acute inflammatory and neuropathic pain in Lewis and Fischer rats.

Journal of Neuroendocrinology, *18*(7), 504–513. https://doi.org/10.1111/j.1365-2826.2006.01442.x

Festing, M. F. (2014). Evidence should trump intuition by preferring inbred strains to outbred stocks in preclinical research. *ILAR Journal*, *55*(3), 399–404. https://doi.org/10.1093/ilar/ilu036

Festing, M. F., & Altman, D. G. (2002). Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR Journal*, *43*(4), 244–258. https://doi.org/10.1093/ilar.43.4.244

Finnerup, N. B., Sindrup, S. H., & Jensen, T. S. (2010). The evidence for pharmacological treatment of neuropathic pain. *Pain*, *150*(3), 573–581. https://doi.org/10.1016/j.pain.2010.06.019

Freet, C. S., Wheeler, R. A., Leuenberger, E., Mosblech, N. A., & Grigson, P. S. (2013). Fischer rats are more sensitive than Lewis rats to the suppressive effects of morphine and the aversive kappa-opioid agonist spiradoline. *Behavioral Neuroscience.*, *127*(5), 763–770. https://doi.org/10.1037/a0033943

Gomez, F., Lahmame, A., de Kloet, E. R., & Armario, A. (1996). Hypothalamic-pituitary-adrenal response to chronic stress in five inbred rat strains: Differential responses are mainly located at the adrenocortical level. *Neuroendocrinology*, *63*(4), 327–337. https://doi.org/10.1159/000126973

Guitart, X., Kogan, J. H., Berhow, M., Terwilliger, R. Z., Aghajanian, G. K., & Nestler, E. J. (1993). Lewis and Fischer rat strains display differences in biochemical, electrophysiological and behavioral parameters: Studies in the nucleus accumbens and locus coeruleus of drug naive and morphine-treated animals. *Brain Research*, *611*(1), 7–17. https://doi.org/10.1016/0006-8993(93)91770-S

Gulati, A., & Bhargava, H. N. (1990). Characteristics of central binding sites for [3H] DAMGO in spontaneously hypertensive rats. *Life Sciences.*, *47*(2), 159–166. https://doi.org/10.1016/0024-3205(90)90229-K

Herradon, G., Morales, L., & Alguacil, F. L. (2003). Differences of [mu]-opioid receptors between Lewis and F344 rats. *Life Sciences*, *73*(12), 1537–1542. https://doi.org/10.1016/S0024-3205(03)00479-X

Herradon, G., Morales, L., Perez-Garcia, C., & Alguacil, L. F. (2003). The contribution of alpha2-adrenoceptor and opioid receptor mechanisms to antinociception differs in Lewis and Fischer 344 rats. *European Journal of Pharmacology*, *465*(3), 251–256. https://doi.org/10.1016/S0014-2999(03)01532-2

Hestehave, S., Munro, G., Pedersen, T. B., & Abelson, K. S. (2017). Antinociceptive effects of voluntarily ingested buprenorphine in the hot-plate test in laboratory rats. *Laboratory Animals.*, *51*(3), 264–272. https://doi.org/10.1177/0023677216668553

Hoffmann, O., Plesan, A., & Wiesenfeld-Hallin, Z. (1998). Genetic differences in morphine sensitivity, tolerance and withdrawal in rats. *Brain Research*, *806*(2), 232–237. https://doi.org/10.1016/S0006-8993(98)00768-9

Juif, P. E., Anton, F., & Hanesch, U. (2012). Pain behavior and spinal cell activation due to carrageenan-induced inflammation in two inbred rat strains with differential hypothalamic-pituitary-adrenal axis reactivity. *Physiology & Behavior*, *105*(4), 901–908. https://doi.org/10.1016/j.physbeh.2011.11.008

Julius, D., & Basbaum, A. I. (2001). Molecular mechanisms of nociception. *Nature*, *413*(6852), 203–210. https://doi.org/10.1038/35093019

Kosten, T. A., & Ambrosio, E. (2002). HPA axis function and drug addictive behaviors: Insights from studies with Lewis and Fischer 344 inbred rats. *Psychoneuroendocrinology*, *27*(1–2), 35–69. https://doi.org/10.1016/S0306-4530(01)00035-X

Kristensen, P. J., Heegaard, A. M., Hestehave, S., Jeggo, R. D., Bjerrum, O. J., & Munro, G. (2017). Vendor-derived differences

in injury-induced pain phenotype and pharmacology of Sprague-Dawley rats: Does it matter? *European Journal of Pain*, *21*(4), 692–704. https://doi.org/10.1002/ejp.973

Lacroix-Fralish, M. L., & Mogil, J. S. (2009). Progress in genetic studies of pain and analgesia. *Annual Review of Pharmacology and Toxicology*, *49*, 97–121. https://doi.org/10.1146/annurev-pharmtox-061008-103222

Lariviere, W. R., & Mogil, J. S. (2010). The genetics of pain and analgesia in laboratory animals. *Methods in Molecular Biology*, *617*, 261–278. https://doi.org/10.1007/978-1-60327-323-7

Lariviere, W. R., Sattar, M. A., & Melzack, R. (2006). Inflammation-susceptible Lewis rats show less sensitivity than resistant Fischer rats in the formalin inflammatory pain test and with repeated thermal testing. *Journal of Neurophysiology*, *95*(5), 2889–2897. https://doi.org/10.1152/jn.00608.2005

Le Coz, G. M., Fiatte, C., Anton, F., & Hanesch, U. (2014). Differential neuropathic pain sensitivity and expression of spinal mediators in Lewis and Fischer 344 rats. *BMC Neuroscience*, *15*, 35. https://doi.org/10.1186/1471-2202-15-35

Lewejohann, L., Reinhard, C., Schrewe, A., Brandewiede, J., Haemisch, A., Gortz, N., … Sachser, N. (2006). Environmental bias? Effects of housing conditions, laboratory environment and experimenter on behavioral tests. *Genes, Brain, & Behavior.*, *5*(1), 64–72.

Liang, D. Y., Guo, T., Liao, G., Kingery, W. S., Peltz, G., & Clark, J. D. (2006). Chronic pain and genetic background interact and influence opioid analgesia, tolerance, and physical dependence. *Pain*, *121*(3), 232–240. https://doi.org/10.1016/j.pain.2005.12.026

Marchand, F., Perretti, M., & McMahon, S. B. (2005). Role of the immune system in chronic pain. *Nature Reviews Neuroscience*, *6*(7), 521–532. https://doi.org/10.1038/nrn1700

Mellon, R. D., & Bayer, B. M. (1998). Evidence for central opioid receptors in the immunomodulatory effects of morphine: Review of potential mechanism(s) of action. *Journal of Neuroimmunology*, *83*(1–2), 19–28. https://doi.org/10.1016/S0165-5728(97)00217-8

Mogil, J. S., Chesler, E. J., Wilson, S. G., Juraska, J. M., & Sternberg, W. F. (2000). Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. *Neuroscience & Biobehavioral Reviews.*, *24*(3), 375–389. https://doi.org/10.1016/S0149-7634(00)00015-4

Mogil, J. S., Lichtensteiger, C. A., & Wilson, S. G. (1998). The effect of genotype on sensitivity to inflammatory nociception: Characterization of resistant (A/J) and sensitive (C57BL/6J) inbred mouse strains. *Pain*, *76*(1–2), 115–125. https://doi.org/10.1016/S0304-3959(98)00032-3

Morgan, D., Cook, C. D., & Picker, M. J. (1999). Sensitivity to the discriminative stimulus and antinociceptive effects of mu opioids: Role of strain of rat, stimulus intensity, and intrinsic efficacy at the mu opioid receptor. *Journal of Pharmacology and Experimental Therapeutics*, *289*(2), 965–975.

Munro, G. (2009). Pharmacological assessment of the rat formalin test utilizing the clinically used analgesic drugs gabapentin, lamotrigine, morphine, duloxetine, tramadol and ibuprofen: Influence of low and high formalin concentrations. *European Journal of Pharmacology*, *605*(1), 95–102. https://doi.org/10.1016/j.ejphar.2009.01.004

Munro, G., Erichsen, H. K., Rae, M. G., & Mirza, N. R. (2011). A question of balance – Positive versus negative allosteric modulation of GABAA receptor subtypes as a driver of analgesic efficacy in rat models of inflammatory and neuropathic pain. *Neuropharmacology*, *61*(1–2), 121–132. https://doi.org/10.1016/j.neuropharm.2011.03.017

Negus, S. S., Vanderah, T. W., Brandt, M. R., Bilsky, E. J., Becerra, L., & Borsook, D. (2006). Preclinical assessment of candidate analgesic drugs: Recent advances and future challenges. *Journal of Pharmacology and Experimental Therapeutics*, *319*(2), 507–514. https://doi.org/10.1124/jpet.106.106377

O'Malley, D., Julio-Pieper, M., O'Mahony, S. M., Dinan, T. G., & Cryan, J. F. (2014). Differential visceral pain sensitivity and colonic morphology in four common laboratory rat strains. *Experimental Physiology*, *99*(2), 359–367. https://doi.org/10.1113/expphysiol.2013.076109

Pardon, M. C., Gould, G. G., Garcia, A., Phillips, L., Cook, M. C., Miller, S. A., … Morilak, D. A. (2002). Stress reactivity of the brain noradrenergic system in three rat strains differing in their neuroendocrine and behavioral responses to stress: Implications for susceptibility to stress-related neuropsychiatric disorders. *Neuroscience*, *115*(1), 229–242. https://doi.org/10.1016/S0306-4522(02)00364-0

Pare, W. P. (1994). Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats. *Physiology & Behavior*, *55*(3), 433–439. https://doi.org/10.1016/0031-9384(94)90097-3

Pechnick, R. N. (1993). Effects of opioids on the hypothalamo-pituitary-adrenal axis. *Annual Review of Pharmacology and Toxicology*, *33*, 353–382. https://doi.org/10.1146/annurev.pa.33.040193.002033

Ren, K., & Dubner, R. (1996). Enhanced descending modulation of nociception in rats with persistent hindpaw inflammation. *Journal of Neurophysiology*, *76*(5), 3025–3037. https://doi.org/10.1152/jn.1996.76.5.3025

Rice, A. S., Cimino-Brown, D., Eisenach, J. C., Kontinen, V. K., Lacroix-Fralish, M. L., Machin, I., … Stohr, T. (2008). Animal models and the prediction of efficacy in clinical trials of analgesic drugs: A critical appraisal and call for uniform reporting standards. *Pain*, *139*(2), 243–247. https://doi.org/10.1016/j.pain.2008.08.017

Rode, F., Thomsen, M., Broløs, T., Jensen, D. G., Blackburn-Munro, G., & Bjerrum, O. J. (2007). The importance of genetic background on pain behaviours and pharmacological sensitivity in the rat spared serve injury model of peripheral neuropathic pain. *European Journal of Pharmacology*, *564*(1–3), 103–111. https://doi.org/10.1016/j.ejphar.2007.02.009

Sams-Dodd, F. (1998). Effects of continuous D-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. *Neuropsychopharmacology*, *19*(1), 18–25. https://doi.org/10.1016/S0893-133X(97)00200-5

Schafer, M., Imai, Y., Uhl, G. R., & Stein, C. (1995). Inflammation enhances peripheral mu-opioid receptor-mediated analgesia, but not mu-opioid receptor transcription in dorsal root ganglia. *European Journal of Pharmacology*, *279*(2–3), 165–169. https://doi.org/10.1016/0014-2999(95)00150-J

Selley, D. E., Herbert, J. T., Morgan, D., Cook, C. D., Picker, M. J., & Sim-Selley, L. J. (2003). Effect of strain and sex on mu opioid receptor-mediated G-protein activation in rat brain. *Brain Research Bulletin*, *60*(3), 201–208. https://doi.org/10.1016/S0361-9230(03)00014-5

Shir, Y., Zeltser, R., Vatine, J. J., Carmi, G., Belfer, I., Zangen, A., … Seltzer, Z. (2001). Correlation of intact sensibility and neuropathic pain-related behaviors in eight inbred and outbred rat strains and selection lines. *Pain*, *90*(1–2), 75–82. https://doi.org/10.1016/S0304-3959(00)00388-2

Soignier, R. D., Taylor, B. K., Baiamonte, B. A., Lee, F. A., Paul, D., & Gould, H. J. 3rd (2011). Measurement of CFA-induced hyperalgesia and morphine-induced analgesia in rats: Dorsal vs plantar mechanical stimulation of the hindpaw. *Pain Medicine*, *12*(3), 451–458. https://doi.org/10.1111/j.1526-4637.2011.01066.x

Sorge, R. E., Martin, L. J., Isbester, K. A., Sotocinal, S. G., Rosen, S., Tuttle, A. H., … Mogil, J. S. (2014). Olfactory exposure to males, including men, causes stress and related analgesia in rodents. *Nature Methods*, *11*(6), 629–632. https://doi.org/10.1038/nmeth.2935

Stain, F., Barjavel, M. J., Sandouk, P., Plotkine, M., Scherrmann, J. M., & Bhargava, H. N. (1995). Analgesic response and plasma and brain extracellular fluid pharmacokinetics of morphine and morphine-6-beta-D-glucuronide in the rat. *Journal of Pharmacology and Experimental Therapeutics*, *274*(2), 852–857.

Sternberg, E. M., Young, W. S. 3rd, Bernardini, R., Calogero, A. E., Chrousos, G. P., Gold, P. W., & Wilder, R. L. (1989). A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptococcal cell wall-induced arthritis in Lewis rats. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(12), 4771–4775. https://doi.org/10.1073/pnas.86.12.4771

Sudakov, S. K., Goldberg, S. R., Borisova, E. V., Surkova, L. A., Turina, I. V., Rusakov, D., & Elmer, G. I. (1993). Differences in morphine reinforcement property in two inbred rat strains: Associations with cortical receptors, behavioral activity, analgesia and the cataleptic effects of morphine. *Psychopharmacology (Berl).*, *112*(2–3), 183–188. https://doi.org/10.1007/BF02244908

Terner, J. M., Barrett, A. C., Cook, C. D., & Picker, M. J. (2003). Sex differences in (-)-pentazocine antinociception: Comparison to morphine and spiradoline in four rat strains using a thermal nociceptive assay. *Behavioural Pharmacology*, *14*(1), 77–85. https://doi.org/10.1097/00008877-200302000-00008

Terner, J. M., Barrett, A. C., Lomas, L. M., Negus, S. S., & Picker, M. J. (2006). Influence of low doses of naltrexone on morphine antinociception and morphine tolerance in male and female rats of four strains. *Pain*, *122*(1–2), 90–101. https://doi.org/10.1016/j.pain.2006.01.019

Terner, J. M., Lomas, L. M., Smith, E. S., Barrett, A. C., & Picker, M. J. (2003). Pharmacogenetic analysis of sex differences in opioid antinociception in rats. *Pain*, *106*(3), 381–391. https://doi.org/10.1016/j.pain.2003.08.008

Tonelli, L., Webster, J. I., Rapp, K. L., & Sternberg, E. (2001). Neuroendocrine responses regulating susceptibility and resistance to autoimmune/inflammatory disease in inbred rat strains. *Immunological Reviews*, *184*, 203–211. https://doi.org/10.1034/j.1600-065x.2001.1840118.x

Trang, T., Al-Hasani, R., Salvemini, D., Salter, M. W., Gutstein, H., & Cahill, C. M. (2015). Pain and poppies: The good, the bad, and the ugly of opioid analgesics. *The Journal of Neuroscience*, *35*(41), 13879–13888. https://doi.org/10.1523/JNEUROSCI.2711-15.2015

Turner, P. V., Pekow, C., Vasbinder, M. A., & Brabb, T. (2011). Administration of substances to laboratory animals: Equipment considerations, vehicle selection, and solute preparation. *Journal of the American Association for Laboratory Animal Science*, *50*(5), 614–627.

Vaccarino, A. L., & Couret, L. C. Jr (1995). Relationship between hypothalamic-pituitary-adrenal activity and blockade of tolerance to morphine analgesia by pain: A strain comparison. *Pain*, *63*(3), 385–389. https://doi.org/10.1016/0304-3959(95)00069-0

Van Crugten, J. T., Somogyi, A. A., & Nation, R. L. (2000). Effect of uranyl nitrate-induced renal failure on morphine disposition and antinociceptive response in rats. *Clinical and Experimental Pharmacology and Physiology*, *27*(1–2), 74–79. https://doi.org/10.1046/j.1440-1681.2000.03206.x

Vit, J. P., Clauw, D. J., Moallem, T., Boudah, A., Ohara, P. T., & Jasmin, L. (2006). Analgesia and hyperalgesia from CRF receptor modulation in the central nervous system of Fischer and Lewis rats. *Pain*, *121*(3), 241–260. https://doi.org/10.1016/j.pain.2005.12.024

Woolfolk, D. R., & Holtzman, S. G. (1995). Rat strain differences in the potentiation of morphine-induced analgesia by stress. *Pharmacology, Biochemistry and Behavior*, *51*(4), 699–703. https://doi.org/10.1016/0091-3057(94)00440-T

Wu, H. H., & Wang, S. (2010). Strain differences in the chronic mild stress animal model of depression. *Behavioural Brain Research.*, *213*(1), 94–102. https://doi.org/10.1016/j.bbr.2010.04.041

Yoon, Y. W., Lee, D. H., Lee, B. H., Chung, K., & Chung, J. M. (1999). Different strains and substrains of rats show different levels of neuropathic pain behaviors. *Experimental Brain Research*, *129*(2), 167–171. https://doi.org/10.1007/s002210050886

Zeng, Q., Wang, S., Lim, G., Yang, L., Mao, J., Sung, B., … Mao, J. (2008). Exacerbated mechanical allodynia in rats with depression-like behavior. *Brain Research*, *1200*, 27–38. https://doi.org/10.1016/j.brainres.2008.01.038

Zhang, R. X., Lao, L., Qiao, J. T., & Ruda, M. A. (2003). Strain differences in pain sensitivity and expression of preprodynorphin mRNA in rats following peripheral inflammation. *Neuroscience Letters*, *353*(3), 213–216. https://doi.org/10.1016/j.neulet.2003.09.043

Zimmermann, M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*, *16*(2), 109–110. https://doi.org/10.1016/0304-3959(83)90201-4

Zollner, C., Shaqura, M. A., Bopaiah, C. P., Mousa, S., Stein, C., & Schafer, M. (2003). Painful inflammation-induced increase in mu-opioid receptor binding and G-protein coupling in primary afferent neurons. *Molecular Pharmacology*, *64*(2), 202–210. https://doi.org/10.1124/mol.64.2.202