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Influence of social interaction on nociceptive-induced changes in locomotor activity in a mouse model of acute inflammatory pain: use of novel thermal assays

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Abstract

Most acute and chronic animal models of pain rely heavily on reflexive assays for evaluating levels of nociception, which involves removing the animal from its normal social environment. Here, we examine and characterize the influence of social interactions on inflammatory pain-evoked changes in movement in two different mouse strains. To produce inflammatory nociception, we injected CFA bilaterally into the hind paws of Balb/c and C3H mice and then recorded exploratory locomotor activity using an automated detector system to first evaluate the effects of social behavior on nociception. Secondly, we determined if carprofen administration altered the effects of social behavior on nociceptive-evoked movement. This methodology was expanded to create a novel thermal activity assay to objectively measure the effect of heat and cold on CFA-evoked animal movement in paired animals. Paired Balb/c and C3H mice exhibited significant hyper-locomotion that lasted for 3 hours post-injection in Balb/c, but only 1 hour post-injection in C3H. Single Balb/c mice only showed increased activity for 1 hour post-injection, while single C3H mice showed no increase. This CFA-induced increase in activity in paired animals was highly inversely correlated with mechanical allodynia as measured using standard Von Frey filaments. Carprofen administration completely blocked this CFA-induced hyperlocomotor activity. Both heat and cold induced a significant increase in locomotor activity in paired mice injected with CFA, while having no effect on activity in control mice injected with saline. The results presented here indicate that social interactions greatly influence inflammatory

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pain-induced changes in locomotor activity and indicate that the use of movement-based assays to evaluate nociception in paired mice may provide an alternative and more sensitive method to quantify nociception and characterize novel analgesic effects over time in the context of social interactions in rodent models of pain.

1. Introduction

While preclinical pain studies have identified a myriad of promising targets and candidate analgesics, there has been little translation to novel pain therapeutics [1]. The pain phenotype in humans involves complex behavioral alterations that are typically never measured in animal models of pain. Thus, the majority of preclinical pain-testing assays evaluate reflex-based responses of animals evoked by noxious or non-noxious stimuli [2-4]. While highly useful, these reflexive assays require restriction of animal movement, which is associated with the development of stress. Moreover, the use of spontaneous behaviors in these tests can be ambiguous and inconsistent between observers, accounting for a plethora of results obtained that do not directly translate in human patient pain settings [5]. Finally, the majority of nociceptive testing is performed on individual animals of certain strains negating the importance of social interactions within strains on pain behavior. Thus, there is an ongoing search for adequate behavioral outcomes that objectively evaluate the pain experienced by rodents. Some recent studies have begun to employ the use of voluntary movements as a way to quantify non-reflexive pain behavior in an attempt to mitigate issues surrounding current reflexive pain testing approaches [6-8]. Thus, nociceptive-induced changes in animal activity can be assessed objectively in an unconfined space over time as a measure of acute or chronic nociception. However, to date, all such studies have been performed using one animal at a time similar to reflexive tests. In this regard, it has been recently shown that individual testing may alter animal behavior and responses to painful stimuli [9,10]. Increasing evidence suggests that social interactions between animals can affect pain behaviors in response to acute stressors [11-13] [14] and in chronic pain states [15, 16]. Furthermore, these behavioral changes may be modulated by the neuroendocrine system as they are in humans [17, 18]. Modulation of pain in different social contexts is an underserved area of study and further investigation is needed to uncover the complex interactions between pain and social behavior as is known to occur in humans [26-28]. A bilateral hind paw inflammation model using Balb/c and C3H mice was selected for this study based on a previous study in rats, which showed that bilateral hind paw inflammation produces significantly greater changes in movement than unilateral inflammation [8].

Our hypothesis for the present study was that social interaction among mice of specific strains influences their pain-induced changes in behavior. In this study we pursued the following objectives: 1) Determine the effect of acute CFA-induced pain on the parameters of locomotor activity in two strains of mice using an activity box; 2) Determine whether nociceptive-induced changes in activity were influenced by social interactions when animals were evaluated alone or together with another mouse in the same activity chamber; 3) Determine if administration of carprofen could alter the observed CFA-induced changes in activity in paired mice; and 4) Determine if thermal hyperalgesia can elicit changes in locomotor activity.

2. Methods and Materials

2.1 Experimental animals

Young adult, male (20-25 g) Balb/c and C3H (NCI, Bethesda, MD) were used for all experiments. All animals were naïve and healthy at the start of each experiment. The number of animals used for each experiment were based on sample size calculations to generate statistical power for the detection of significant differences (using the formula: $n = \text{sample size} = [\sigma/\delta]^2 \times [Z \text{ alpha} + Z \text{ beta}]^2$ where σ = standard deviation and δ = difference in SD units; where the p-value < 0.05 and Z alpha = 1.65 for all of the experiments). Balb/c and C3H mice were selected because these strains are commonly used in research studies, show average line cross frequencies in an open arm maze, rearing frequencies and grooming behavior in comparison to numerous other mouse strains [19], yet Balb/c mice show large differences in acute inflammatory-induced thermal hypersensitivity in comparison to several other mouse strains including C3H, have a significantly lower withdrawal threshold to von Frey stimulation compared to C3H mice and show significant mechanical hyperalgesia in several models of pain [20]. Animals were maintained on a 12 h light/dark cycle (lights on from 6:30a – 6:30p) and housed four to a cage in their home cages prior to experimentation with food and water *ad libitum* in a research animal resources approved vivarium. All experiments were performed at approximately the same time each day according to the light cycle and animals were habituated for 60 minutes before testing. Separate cohorts of animals were utilized for each series of experiments.

2.2 Ethics Statement

All procedures were performed in accordance with the guidelines recommended by the International Association for the Study of Pain and approved by the Animal Care and Use Committee at the University of Minnesota (protocol #1301A30220).

2.3 Complete Freund's Adjuvant (CFA)-induced inflammatory pain

In this study, we hypothesized that social interaction would affect inflammatory pain-induced changes in movement. To test this hypothesis, we used a CFA-inflammation (Complete Freund's Adjuvant) hind paw model, which we found produces significant changes in normal mouse movement over time. Bilateral inflammation was induced by intraplantar injection of 20 μL of CFA (Sigma, St. Louis, MO) into each hind paw. Injections were administered into the subcutaneous plantar tissue of lightly anesthetized mice. Bilateral injections rather than unilateral injection were performed based on previous research showing that single limb inflammation does not significantly alter home cage locomotion, and that bilateral inflammation is needed to induce significant deficits in this behavior [21]. Following bilateral injection, mice were allowed to recover in cages on a heating pad until ambulatory (~2-5 minutes). None of the animals used in the study showed any signs of dysfunction (e.g. problems with ambulation, lethargy or excessive bleeding) and thus, none of the experimental or control animals were removed during the course of the study. Control mice received an injection of 20 μL of physiological saline, while naïve mice received no injections. Animals were randomly assigned to experimental and control groups.

2.4 Mechanical allodynia

Mechanical sensitivity in Balb/c and C3H animals were tested using a calibrated von Frey filament (#2.83 and #3.61 respectively, Stoelting, Wood Dale, IL) as previously described [22-24]. These von Frey filaments were selected because they produce 0-1 responses out of 10 applications in naïve Balb/c and C3H mice. Briefly, each filament was gently applied for 2–3 seconds to the glabrous skin of the hind paw of a mouse standing on a metal mesh. Each filament was applied 10 times with at least 5 s between applications. The frequency of withdrawal responses was scored at each force and expressed as a percentage of the response out of 10 filament applications (% response $\pm$ SEM). Baseline von Frey measurements were obtained prior to CFA or saline injection and subsequent measurements were taken at 1, 2, 3 and 4 h post-injection. Animals injected with physiological saline as well as naïve animals served as controls for CFA-induced inflammation and needle injection. Behavioral assessments were conducted during the light cycle at approximately the same time each day. All behavioral analyses were performed blindly.

2.5 Measurement of locomotor activity (LMA)

Single or paired freely moving mice were placed into 43×43×21 cm Plexiglas activity boxes and horizontal (exploratory activity counts) locomotor activity was measured using an automated movement detector system [25] following full recovery from the injection procedure (see section 2.3 for procedure). This system utilizes multiple activity detectors linked to an OPTO-M3 hub box and subsequently to a computer via a serial bus connection. These movement-recording detectors relay real-time activity measurements through the bus system and then can be recorded and analyzed using the company's Oxymax v5.03 software (Columbus Instruments, Columbus, OH). Bedding, food, and water were placed in locations that did not interfere with the ability of the sensors to record movements of the mice. N values are derived from the number of animals per chamber, i.e. single 1 per box: n = 1 animal using 1 chamber; paired 2 per box: n = 2 animals using 1 chamber. When 2 animals were placed in an activity chamber, these animals were all matched pairs from the same home cage (cagemates). Animals in all groups were acclimated to the activity box for a period of 60 minutes following injection procedure recovery (~2-5 min) prior to any experimental manipulations to avoid the confounds of exposure to a novel environment, which is typically not done in most activity box studies. Locomotor activity (LMA) recording was initiated following this adaptation period in naïve animals and in experimental and control animals following recovery from CFA or vehicle injection, respectively as highlighted in section 2.3 of the methods (~62-65 minutes post-injection prior to sampling activity). LMA was either averaged in 1h measurement blocks or over a 4h time span as indicated in the results and figure legends.

2.6 Drug treatment

Carprofen (5 mg/kg) was administered subcutaneously (s.c.) in 0.1 mL physiological saline (control: 0.1 mL physiological saline only) shortly after bilateral intraplantar injection of either CFA or physiological saline prior to activity chamber measurements. Carprofen was selected for this study because it is typically one of the NSAIDs recommended to treat pain in mice and because it has lower levels of toxicity in mice than other NSAIDs. Carprofen

(Rimadyl) was purchased at the Veterinary clinical pharmacy at the University of Minnesota and came in a ready-made injectable formula. No suspension was prepared prior to injections.

2.7 Thermal locomotor assay (TLA)

It is very challenging to assess highly variable and subjective sensations such as pain and aversion to innocuous temperature in mice, since mice exhibit more erratic behavioral responses than rats and also tend to be less conditional. In an attempt to reduce the conscious and unconscious bias that occurs with most of the current thermal assays, we developed a thermal assay that measures animal locomotion on a thermally controlled platform in an enclosed activity box as described below.

Single or paired mice were placed on the thermal plate in an enclosed activity box (20×16×25 cm³) surrounded by movement recording detectors as described above capable of producing a wide range of temperatures accurately. Single and paired exploratory locomotor activity was measured at 53°C over a 1-min period or at 4°C for a 2-min period. While on the thermal plate, animal movement was tracked using the activity box open field test providing an objective measurement of movement. CFA injected animals were tested prior to CFA injection to determine baseline movement patterns in response to continuous thermal stimuli and again at 2, 4 and 6 h post-injection. Similarly, control animals injected with physiological saline were tested in the same manner alongside their CFA counterparts. Since the mice were not restrained, they could raise their forepaws and hindpaws off of the platform at any time during the brief testing period to avoid thermal damage to the skin. Hind paws were examined at various time points after each exposure for signs of redness and edema formation as one measure of evaluating potential thermal damage to the skin.

2.8 Traditional cold/hot plate hyperalgesia testing

Briefly, the cold/hot plate consisted of an aluminum test surface (20×16 cm³) enclosed in a clear Plexiglas container (25 cm high) maintained at either 4 or 53 ± 0.01 °C respectively by a thermostatically controlled unit with water circulating capabilities (model #LHP-1200CPV; TECA, Chicago, IL). Cold plate: After placing the mouse on the cold plate, the experimenter counted the frequency of withdrawal responses over a 2-min time period. A withdrawal response included behaviors such as licking, shaking, jumping or elevation of the paw above the plate (one response per second was recorded). Hot Plate: After placing the mouse on the hot plate, the experimenter recorded the time in seconds until the first withdrawal response utilizing the same criteria as the cold plate testing. A cut off time of 1 minute was established as to ensure no thermal damage was induced to the paw skin of the mouse.

2.9 Statistical analyses

Data are presented as the mean ± SEM. Statistical analyses was performed with unpaired student's t-tests (two-tailed) and two-way ANOVA with Bonferroni's correction where indicated. Data was graphed using Prism 5.0 (GraphPad Software, La Jolla, CA). The level of significance was set at $p = 0.05$.

3. Results

3.1 Social interactions influence locomotor activity differently by strain in novel environments

Single or paired naïve cagemates of the Balb/c or C3H strain type were placed into novel activity chambers and following a 60-min acclimation period their activity count (exploratory activity count) was averaged over a 4 h time course (Fig. 1). Paired Balb/c animals moved significantly less on average than strain-matched single animals. Interestingly, single C3H animals moved significantly less than Balb/c singles and the significant decrease in activity that was observed with paired Balb/c mice was not observed in C3H animals using the same measurement parameters (Fig. 1).

3.2 Within strain social interactions significantly influence nociceptive-induced changes in locomotor activity

To evaluate the effects of bilateral CFA injection on locomotor activity, all animals were first exposed to the activity chambers for a 60-min adaptation period and then single or paired Balb/c and C3H animals were injected with CFA or saline and placed back into the activity chambers and the numbers of locomotor movements (horizontal activity counts) were assayed over a 4-hour time period (Fig. 2). When activity was quantified and averaged over a 4-hour time period, both CFA-injected Balb/c single and paired mice showed a significant increase in locomotor activity compared to their saline injected counterparts and, but the effect was more significant in paired mice when compared to their counterparts and to CFA-injected single animals (Fig. 2A). When the same experiment was performed in C3H mice, no alterations in activity were observed in single or paired cagemates (Fig. 2B). On-the-other-hand when temporal locomotor activity patterns were analyzed by strain social grouping in 1- hour bins over the 4-hour testing period (data from Fig. 2), singly chambered Balb/c mouse activity was only significantly increased for the first hour post-injection (Fig. 3A), while paired mice activity was significantly increased over the first 3 h of testing (Fig. 3C). When single and paired activity counts were analyzed in C3H animals, only CFA-injected paired animal activity was increased for the first hour post-injection (Fig. 3B v. 3D). Thus, CFA did increase activity in C3H mice, but only during the first hour post-injection, so this effect was not observed when averaged over four hours (Fig. 2B).

3.3 Locomotor activity and mechanical allodynia are inversely correlated

To evaluate the acute effects of CFA-induced mechanical allodynia on locomotor activity between strains, animals were injected bilaterally with either CFA or physiological saline into both the right and left hind paws and their response to von Frey filament application was measured (Fig. 4A&B). Bilateral injection of CFA into the hind paws produced an acute and persistent mechanical allodynia steadily increasing in severity over 4 hours in Balb/c (Fig. 4A). Similarly, mechanical allodynia was produced in C3H, but severity plateaued beginning at the 2hour time point and persisted until 4 hours (Fig. 4B). Next, both single and paired animal exploratory activity were compared in each mouse strain to the mechanical allodynia von Frey scores attained at those same time points (Fig. 4C-F). Steadily increasing time-dependent increases in CFA-induced mechanical allodynia was observed in Balb/c animals and these increases were most strongly inversely correlated to changes in locomotor activity

in paired-chambered animals only (Fig. 4C v. 4E). Interestingly, while C3H animals did develop mechanical allodynia, the degree of allodynia was less than Balb/c mice and it plateaued beginning 2 hours post-injection (Fig. 4A v. 4B). When similar correlational analysis was applied to C3H animals, again only paired animal activity significantly correlated with mechanical allodynia scores. (Fig. 4F). These results indicate a strong inverse correlation between von Frey allodynia scores and nociceptive-induced increases in activity in paired mice only. Since, paired chambering better mimics common social laboratory housing, and since the correlation between movement and von Frey scores were more robust in Balb/c mice, only paired groupings of Balb/c mice were selected for the next set of experiments examining the effect of administration of the non-steroidal anti-inflammatory drug carprofen on CFA- induced changes in activity.

3.4 Administration of carprofen blocks the development of hyperlocomotor activity caused by acute noxious inflammation in paired animals

To determine whether a nonsteroidal anti-inflammatory drug (NSAID) used to treat inflammation could prevent hyperlocomotor activity observed during acute inflammation in Balb/c animals (Figs. 2A & 3C), carprofen (Rimadyl) was administered to paired mice following bilateral CFA or saline injection (Figs. 5A & 5B). Treatment with carprofen significantly reduced the CFA-induced hyperlocomotor activity during a 4 hour time bin following injection (Fig. 5A; all groups v. CFA) with its effects lasting for 3 h (Fig. 5B). No drug effects on exploratory locomotor activity were seen in any of the control animals (Fig. 5A & 5B; $p > 0.05$; v. saline + CAR and/or saline alone).

3.5 The thermal locomotor assay (TLA) provides better sensitivity than traditional reflexive testing in detecting thermal hyperalgesia

Traditional thermal nociceptive testing such as the hot plate paw withdrawal latency (PWL) or spontaneous behavior counting on the cold plate typically involves measurement of reflexive behaviors to a thermal stimulus in individual animals. Here, PWL (Fig. 6A) and the number of spontaneous behaviors (Fig. 6D) were recorded at baseline (BL) prior to injection of CFA or saline into the hind paws and then again at 2, 4 and 6 h following injections in single Balb/c animals. A reduction in PWL was observed in animals bilaterally injected with CFA at all time points tested following a 1 min exposure to a plate at 53°C (Fig. 6A). No significant changes in the number of spontaneous behaviors were observed in CFA or saline mice when exposed to a cold plate for 2 min at 4°C (Fig. 6D).

In a new thermal assay, we inserted a thermal plate device in an activity box, which allowed us to measure the effect of thermal stimulation on locomotor activity at the same temperatures used for the thermal reflexive testing above (Figs. 6B & 6C for hot and Figs. 6E & 6F for cold respectively). These thermal effects on locomotor activity were evaluated at 2, 4 and 6 h following bilateral saline or CFA injection into the hind paws of single and paired animals and compared to baseline values determined prior to injections. Compared to the activity of saline-injected control mice, only CFA-injected paired animals exhibited significantly increased movement beginning 2 h after injection and lasting for 4 h at both temperatures (53°C and 4°C) tested (Figs. 6C & 6F respectively). No significant increases in locomotor activity were evident in saline-injected control animals at either temperature

throughout the course of this experiment or in singly tested animals. As evident here, the use of the thermal locomotor assay (TLA) can provide similar data to traditional testing (Figs. 6A & 6D) while simultaneously providing superior sensitivity for cold hyperalgesia and accounting for the effects of social interactions in paired mice (Fig. 6D v. Fig. 6F). Examination of the hind paws at the end of the experiment did not indicate any redness or edema induced by the thermal testing.

4. Discussion and conclusion

Recently, Martin and colleagues chronicled the effects of pain and social behavior in humans and rodents [9]. There is increasing data to suggest that modulation of acute pain can be altered in different social contexts in both humans and rodents, which underscores the importance of understanding the effects of social behavior on painful outcomes both experimentally and clinically from a biopsychosocial perspective [26-28]. In this regard, Cobos et al. recently reviewed the experimental evidence showing that rodents subjected to sustained pain change their behavior markedly and that these behavioral consequences of pain can be detected by behavioral tests such as locomotor activity that are not limited exclusively to reflexive hypersensitivity [1]. Recent analysis of locomotor activity has been utilized as a means to study inflammation in an acute pain models involving carrageenan, CFA or lipopolysaccharide (LPS) in individual animals [6-8, 29, 30], yet to our knowledge, this study is the first to document these assays in a murine CFA-induced inflammatory pain locomotor activity model, to incorporate a novel thermal locomotion assay and more importantly to characterize the effects of social behavior on its outcome in two different mouse strains. Data from Brown et al. and Krahe and colleagues suggests acute pain outcomes in human patients can be optimized through social buffering techniques [31, 32]. These positive effects are also observed in rodents where behavioral responses are modified through social buffering [33]. Non-painful environmental responses are also affected by social grouping. Lower levels of stress-related behaviors and plasma hormones were apparent when rats were paired in novel environments [34]. Similarly, recent work from Pitzer and colleagues suggests that isolated versus group housing of animals has significant implications on mechanical/cold hyperalgesia as well as body weight fluctuations in a spared nerve injury (SNI) model [35]. Likewise, in our study, decreased locomotor activity was observed in naïve paired Balb/c animals compared to their isolated counterparts.

Emotional states clearly play a role in social interactions and influence behaviors in both humans and animals. Rats have been shown to relay stress to one another via vocalizations and chemical odors [36-38] causing changes in pain behavior. Moreover, recent data from Langford et al. [12] shows that mice can transmit pain between cagemates (contagious pain), resulting in elevated pain hypersensitivity in paired animals. Similarly, our data mirrors this behavioral phenomenon with respect to nociceptive-induced changes in locomotor activity, which is clearly more robust among paired mice. Increased locomotor activity following bilateral injection of CFA was observed in both isolated and paired Balb/c animals, but this effect was greatly magnified when animals were paired lasting for 3-hours post-injection. Moreover, C3H paired mice, but not single mice, showed increased locomotor activity following CFA injection. If movement over the 4-hour post-injection time point is averaged, there is a significant increase in movement in single CFA injected Balb/c mice that is

amplified in paired CFA injected mice as illustrated in figure 2. On-the-other-hand, when movement is analyzed in 1 hour bins as illustrated in figure 3, there is a clear decrease in CFA-induced movement over time, which is why animal movement is inversely correlated with von Frey mechanical allodynia scores as illustrated in figure 4 when movement is analyzed in 1 hour increments as opposed to movement averaged over the 4-hour time period of analysis. Conversely, if movement is averaged over 4 hours rather than examining 1 hour bins, there is a positive correlation between von Frey scores and movement. This underscores the need to carefully consider how movement data is analyzed and to standardize measurements over time.

While the advantages to using objective open field locomotor activity testing over traditional reflexive assays are well documented [6, 30], one major drawback is that isolated animals are typically tested in both reflexive and locomotor assays, which can dramatically affect pain-related behaviors [39, 40]. Furthermore, few if any studies have considered the benefits of comparison between traditional assays and open field-testing, offering a potentially stronger conclusion regarding the pain state of the animal. Lastly, in the majority of studies using locomotor assays to evaluate pain, animals are typically not habituated to the novel environment, which potentially confounds the data by not taking into account the effect of novelty and stress on activity. In this study, we demonstrated that not only is locomotor activity well correlated to traditional mechanical hyperalgesia scores, but also that social grouping has significant effects on exploratory behavior and acute pain sensitivity. The multiple comparisons between these two tests suggests that not only is locomotor activity sensitive to expressions of mechanical pain in mice, but that these pain-evoked movements may have patterns unique and deeply rooted in social behavior and mouse strain dynamics.

Locomotor movement has been used to characterize nociceptive effects in response to administration of common NSAIDs and opioids, but many investigators mention sedative opioid effects or they utilize joint models that may restrict movement outright [41, 42]. It is well established that NSAIDs reduce inflammation and nociception in acute animal models [43] and this effect can be objectively quantified using locomotor activity. In this regard, Zhu and colleagues showed that NSAIDs can reverse the CFA-induced decrease in activity in individually tested rats [8]. In this study, carprofen reversed the effect of acute CFA-induced inflammatory pain on movement in paired Balb/c mice. Thus, a single dose of carprofen successfully reduced total CFA-evoked hyperlocomotion back to the activity levels of saline-injected controls for a period of 3 h post administration, while having no effect on normal control locomotor behavior. This indicates that antinociceptive drug effects can be evaluated using paired mice in this locomotive assay.

Traditional pain-evoked responses are limited by end points. Clinically, this poses a significant challenge because of the subjective component to patient care and the diverse biopsychosocial interactions that alter patient prognoses [20, 43]. Common animal assays involving radiant heat application such as hot plate (where paw licking and jumping are measured) or Hargreaves' test (where latency to withdrawal is measured) have been and continue to be extensively used in pain models of inflammation. As documented by Le Bars and colleagues [44], when a very weak source of energy (such as a radiant heat source) is used, a weak variation in threshold will translate mathematically into a large variation in

reaction time, which is one limitation of these thermal assays. Moreover, for the Hargreave's test, extensive habituation is required to prevent excessive movement in order to collect data. Ultimately, the limited nature of these tests makes it difficult to assess novel analgesics for the clinic. In our study, we utilized a new combinational approach for assessing thermal hyperalgesia. A traditional hot/cold plate was fitted with activity movement sensors to objectively measure heat-evoked movement on the plate in animals injected with CFA or saline over a 1-min time period for heat and over a 2-min period for cold. Furthermore, our method allows for simultaneous testing of more than one animal at a time. Our results suggest that an increase in locomotor activity and its subsequent maintenance may be influenced by paired animal interactions. In combination with acute models of inflammatory pain [3], thermal-evoked locomotor activity testing may provide additional translational value above and beyond what is already known when pain in the context of social interactions is considered. We are currently examining broader applications of this methodology and are evaluating different temperatures and surfaces and their effects on activity.

Many of the limitations on using locomotor activity in models of pain are amenable or even negated if certain social conditions are taken into account. Detecting differences in normal animals versus animals in pain is difficult as the novelty of the testing environment decreases [43, 45]. Our data suggests that using paired animals and habituating them to the activity box environment can overcome the limitation of novelty. While we have observed some reduction in exploratory activity in naïve, saline and CFA-injected animals through repeated exposure to the testing environment in smaller studies, the effect of a novel environment is most apparent in isolated animals. By habituating single and paired animals, any reductions in activity due to novelty were greatly reduced or eliminated. Our results indicate that social interactions have important effects on acute inflammatory pain-induced changes in activity and demonstrate the critical role of a multifaceted approach to the study of nociception in the laboratory. While we used mice from the same home cage in these studies and did not use stranger dyads as did Martin et al. [46] or Gioiosa et al. [47], preliminary data suggests that the CFA-induced changes in activity of stranger dyads is different than familiar dyads and will be the topic of a subsequent report.

Conclusion

Here we established an objective approach to ambient and thermal hypersensitivity locomotor assessment in a murine model of acute CFA-induced inflammatory pain. We demonstrate that nociception is highly influenced by social grouping, strain and animal interaction. Thus, Balb/c mice are more sensitive to CFA inflammation than C3H mice and this is clearly evident in our analysis of the effect of CFA on locomotor activity, which is significantly increased in paired Balb/c compared to single Balb/c or paired C3H mice. We believe that using a paired approach to study acute pain in animal models has more similarities to human acute pain than studies of isolated animals, which is the traditional approach used in rodent pain studies. Moreover, the nociceptive-induced changes in activity observed in paired animals can be rescued using a common NSAID and has broad implications on pain-evoked responses in the context of social interaction. In future studies, additional testing using other pharmacological drug classes and pain models may prove

highly fruitful in developing a deeper understanding of the effect of social interaction on both acute and chronic pain.

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Highlights

- Normal locomotion is affected by social interactions in mice
- Social interactions alter nociceptive-induced changes in locomotion
- Carprofen blocks the effect of social interaction on nociception
- A novel thermal locomotion assay allows detection of nociception in paired mice

Fig. 1. Assessment of social interactions on normal locomotor behavior in Balb/c and C3H mouse strains

Exploratory activity was averaged over 4 hours in naïve Balb/c and C3H single or paired animals in an activity chamber. Paired Balb/c animals moved significantly less than strain-matched single animals (** $p < 0.01$). Inter-strain comparisons revealed increased activity in single Balb/c animals compared to C3H singles (** $p < 0.01$). Data shown as mean ± SEM; $n = 6/\text{single}$, $n = 12/\text{paired}$; student's unpaired t-test.

Fig. 2. Balb/c, but not C3H social interactions significantly influence nociceptive-induced changes in locomotor activity

To evaluate the social effects of bilateral CFA injection on locomotor activity in Balb/c and C3H animals, single or paired animals from each strain were placed into activity chambers and the numbers of locomotor movements (horizontal activity counts) were assayed over a 4-hour time period. When activity was quantified and averaged over a 4-hour time period, both Balb/c CFA-injected single mice ($**p < 0.01$) and paired mice ($***p < 0.001$) showed a significant increase in locomotor activity compared to their saline injected counterparts, but the effect was more significant in paired mice when compared to their respective counterparts and to CFA-injected single animals ($*p < 0.05$). These single and paired effects were not observed in C3H ($p > 0.05$). Data shown as mean $\pm$ SEM; $n = 6$ /single, $n = 12$ /paired; student's unpaired t-test.

Fig. 3. Temporal patterns of the effect of social interaction on CFA-induced locomotor activity
When temporal locomotor activity patterns were analyzed within strains in the context of single versus paired (socially interactive) animals in 1 hour bins over the 4-hour testing period, the activity of singly chambered Balb/c mice was only significantly increased for the first hour following injection (A), while paired mouse activity was significantly maintained over the first 3 hours of testing (C). Unlike Balb/c animals, single C3H animals showed no differences in movement (B), however; paired animals showed increased movement during the first hour of activity when compared to saline counterparts (D). Data shown as mean $\pm$ SEM; $n = 6/\text{single}$, $n = 12/\text{paired}$; * $p < 0.05$, *** $p < 0.001$; two-way ANOVA.

Fig 4. CFA-induced hyperalgesia inversely correlates with locomotor activity

The developmental time course of acute mechanical allodynia in Balb/c (A) and C3H (B) mice. Single and paired animals were assayed for exploratory locomotor activity at 1, 2, 3 and 4 hours post-CFA injection and movement was correlated to nociceptive mechanical allodynia scores attained within each strain indicated. Von Frey scores were highly and significantly inversely correlated with activity in paired mice indicating a clear relationship between mechanical allodynia and activity. Data shown as mean $\pm$ SEM; r^2 values indicated in graphs, $n = 6$ /single, $n = 12$ /paired, $p < 0.05$; student's unpaired t-test and linear regression analysis.

Fig 5. Administration of carprofen blocks the development of hyperlocomotor activity caused by acute noxious inflammation

Locomotor activity was assayed for 4 hours following either bilateral CFA injection or physiological saline in paired Balb/c animals. Carprofen (5 mg/kg, s.c.) was administered 10 min after injection of CFA or saline subcutaneous injection of carprofen significantly reduced CFA-induced hyperlocomotor activity over a 4 hour time period (A) and for a period of 3 hours post-injection when the data was analyzed in 1 hour bins (B). Data shown as mean $\pm$ SEM, $n = 12/\text{group}$; $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$; student's unpaired t-test (A) and two-way ANOVA (B).

Fig. 6. The thermal locomotor assay (TLA) provides better sensitivity than traditional reflexive testing in detecting thermal hyperalgesia

A thermal plate device was inserted in an activity box allowing the measurement (B & C and E & F) of the effects of thermal stimulation on locomotor activity at the same temperatures used for the thermal reflexive testing (A & D). The effect of CFA-induced thermal hyperalgesia on locomotor activity was evaluated at baseline (BL) and at 2, 4 and 6 hours in single (B & E) and paired mice (C & F) following bilateral saline or CFA injection ($n = 12/\text{group}$). Single animals injected with CFA exposed to the 53°C hot plate displayed a significant decrease in PWL compared to the PWL of saline-injected control mice (A). Conversely CFA-injected animals did not display significant changes in the number of spontaneous behaviors when exposed to a 4°C cold plate over a 2-min testing period (D). In contrast, there was a significant increase in paired locomotor activity only in CFA-injected animals compared to saline-injected control mice at both 2 and 4 hours post-injection at both temperatures (C & F). Thus, locomotor activity provides equivalent or better sensitivity to traditional thermal reflexive tests, accounts for social interactions and is without the subjectivity that is often associated with reflexive thermal testing. Data shown as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, two-way ANOVA