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Anti-nerve growth factor therapy increases spontaneous day/night activity in mice with orthopedic surgery induced pain

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Abstract

Total knee arthroplasty (TKA) and total hip arthroplasty (THA) are two of the most common and successful surgical interventions to relieve osteoarthritis pain. Control of postoperative pain is critical for patients to fully participate in the required physical therapy which is the most influential factor in effective postoperative knee rehabilitation. Currently opiates are a mainstay for managing postoperative orthopedic surgery pain including TKA/THA pain. Recently, issues including efficacy, dependence, overdose and death from opiates have made clinicians and researchers more critical of use of opioids for treating non-malignant skeletal pain. In the present report, a non-opiate therapy using a monoclonal antibody raised against nerve growth factor (anti-NGF) was assessed for its ability to increase the spontaneous activity of the operated knee joint in a mouse model of orthopedic surgery pain induced by drilling and coring the trochlear groove of the mouse femur. Horizontal activity & velocity and vertical rearing were continually assessed over a 20 hour day/night period using automated activity boxes in an effort to reduce observer bias and capture night activity when the mice are most active. At days 1 and 3 post-orthopedic surgery there was a marked reduction in spontaneous activity and vertical rearing; anti-NGF significantly attenuated this decline. The present data suggests that anti-NGF improves limb use in a rodent model of joint/orthopedic surgery and as such anti-NGF may be useful in controlling pain following orthopedic surgeries such as TKA/THA.

Keywords

knee; hip; replacement; analgesia; bone; joint; physical rehabilitation

INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease that is characterized by articular cartilage degeneration, subchondral bone sclerosis, and osteophyte formation. Clinical symptoms include chronic pain, joint instability, stiffness, and radiographic joint space narrowing [8; 31; 42; 79]. OA is the most common cause of disability in adults and greater than seventy

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percent of people aged older than 65 years demonstrate radiographic evidence of the disease [34; 46]. Early management of OA includes physical therapy, weight loss, and nonsteroidal anti-inflammatory drugs (NSAIDs) [17; 53]. With disease progression, pain management frequently becomes more of a challenge and opioid analgesics are commonly used [25; 57; 66; 83]. When opiates no longer control OA pain, orthopedic surgery and replacement of the degenerative joint is a rapidly increasing option used to reduce the pain and restore the functional status of the patient [44].

Postoperative pain following orthopedic surgeries including TKA is a major concern as this pain is moderate to severe in 90% of patients and, if not adequately controlled, interferes with early intense physical therapy that is the most influential factor in effective postoperative rehabilitation [6; 63; 69; 71]. Effective pain management following orthopedic surgeries such as TKA also helps patients to use and maintain bone and muscle mass, as elderly patients show a significantly higher incidence of hip fractures following TKA due to falls as a result of loss of bone and muscle mass [35].

Currently, the two most commonly used pharmacological agents to control post-orthopedic surgery pain are NSAIDs and opiates, both of which have significant unwanted side effects. NSAIDs have been shown to directly or indirectly inhibit bone healing in animal models and, if used over an extended period of time, have significant and sometimes lethal renal, hepatic, and gastrointestinal side effects [19; 24; 48; 68]. While opiates are commonly used to control moderate-to-severe post-orthopedic surgery pain, recent data has suggested that long-term opiate use is associated with loss of functional status, inability to return to work, dependence, overdose and overdose-induced death [32; 75; 77; 83]. Nevertheless, there is a continual increase in the overall use of opioids to manage acute and chronic skeletal pain [5; 56].

While there are several reasons for the lack of new therapies to treat skeletal pain, three major impediments are; lack of rodent models that closely mirror painful human skeletal disorders, lack of widely accepted preclinical behavioral endpoints for measuring skeletal pain that can be easily translated into human clinical trials, and a lack of new, mechanism based therapies that are as effective as NSAIDs and opiates.

In the present report we demonstrate that, following orthopedic surgery to the mouse knee, there was a marked reduction in horizontal activity and velocity of travel, as well as vertical rearing and anti-NGF significantly attenuated this decline. These data suggest anti-NGF attenuates acute orthopedic surgery pain and that monitoring of day/night activity and vertical rearing may offer translational insight into novel analgesics that can relieve orthopedic surgery induced pain such as TKA or THA.

METHODS

Animals

Experiments were performed on 50 adult male C3H/HeJ mice (numbers represent three independent experiments) (Jackson Laboratories, Bar Harbor, ME, USA), approximately 8-9 weeks old, weighing 22-30g at the time of surgery. Thirty mice were used to establish the

timeline and effect of orthopedic surgery on activity, rearing and velocity of movement and 20 mice were used to test the efficacy of anti-NGF (10 anti-NGF, 10 vehicle) in attenuating these changes. Animals were individually housed (AAALAC approved SPF facility, Lab Products IVC 750 cages, 6.75"×12.25"×5", with ¼" corn cob bedding and nestlet) for at least 2 weeks prior to baseline recordings and continued throughout the duration of the experiment. After one week of acclimation to the housing facility, all mice were numbered via eartag. For each experiment, odd numbered mice were assigned to a group and even numbered mice to another group prior to any testing or surgical procedures. All mice underwent orthopedic surgery after naïve baselines were obtained. Mice were housed in accordance with National Institutes of Health Guidelines and kept in a vivarium maintained at 22°C with a 12h alternating light–dark cycle (7am–7pm) and provided food and water *ad libitum*. All procedures adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Institutional Animal Care and Use Committee at the University of Arizona (Tucson, AZ, USA).

Orthopedic Surgery

An arthrotomy was performed as described previously with some alterations [49]. In short, following induction of general anesthesia with ketamine/xylazine (100 mg/kg ketamine and 10 mg/kg xylazine; s.c.), a 1-centimeter incision was made in the skin overlying the knee on the lateral aspect, parallel to the femur. The skin over the knee was reflected and the joint was exposed by transposing the patella medially following blunt dissection through the lateral parapatella tissues with the knee in flexion. A 0.5mm in diameter hole was drilled in the center of the trochlear groove of the femur using a pneumatic dental high-speed hand piece, avoiding the cruciate ligaments. A pin was inserted into the intramedullary canal to core the marrow space. The drill site was sealed with a dental amalgam plug (Dentsply, Milford, DE). The knee was extended and the patella returned to its normal position in the trochlear groove. To minimize medial patella luxation, the fascia of the vastus muscles and the parapatella tissues near the knee were secured back in position using a horizontal mattress suture prior to the closure of the skin. Wound closure was achieved with two 7-mm auto wound clips (Becton Dickinson, Sparks, MD). Animals recovered from anesthesia on heating pads, and received injections of antibiotic (Baytril, 85mg/kg, s.c.) and sterile saline (1mL, s.c.). After recovering from surgery, animals remained individually housed to avoid animals fighting which increases the likelihood of displacement of the patella.

Radiography

While under general anesthesia, high-resolution x-ray images of the mediolateral plane of the ipsilateral femur were obtained during and after surgery using a Faxitron MX-20 digital cabinet x-ray system (Faxitron/Bioptics, Wheeling, IL). Animals also had x-ray images taken (under light anesthesia-50 mg/kg ketamine and 5 mg/kg xylazine; s.c.) on day 7 post-orthopedic surgery as X-ray images easily show if patellar displacement has occurred. X-ray images were not taken on days 1 and 3 because previous experience in our laboratory has shown that a significant increase in patellar displacement occurs at these time points due to the animal's struggles during immobilization for injection and the effects of anesthesia, which can over-stress the weakened and healing tissues. It is important to note the possibility

exists that the animals could have experienced intermittent luxation during movement which we could not detect with x-ray images.

Exclusion Criteria

Animals were excluded from the experiment under three conditions; surgical complications, a loss of more than 20% of their pre-surgery weight or if patella displacement had occurred, as identified through radiography. The final number of mice used for the two timeline experiments was 28 (one mouse was lost due to surgical complications and one mouse was removed due to patellar displacement). The final number of mice used for the anti-NGF experiment was 17 (one mouse in the anti-NGF group and two mice in the vehicle group were lost due to patellar displacement).

Assessment of Locomotor Activity

For the timeline experiments, mice were assessed at day -7 (baseline) and on days 1, 3, 7 and 12 post-orthopedic surgery for locomotor activity (horizontal activity and velocity) and vertical rearing episodes. For the anti-NGF experiment, mice were assessed at day -7 (baseline) and on days 1 and 3 post-orthopedic surgery for locomotor activity (horizontal activity and velocity) and vertical rearing episodes. Animals were placed individually in plexiglass boxes (16×16×11.75 inches) containing a thin layer of bedding and a 1-inch square of Napa Nectar (Systems Engineering, Napa CA, USA). Locomotor activity and rearing episodes were assessed via open field monitoring by arrays of photo-beam sensors that use beam breaks to determine the location of each animal at all times (Omnitech Electronics, Columbus OH, USA). Locomotor activity and rearing episodes were monitored for 20 hours beginning at 1200 hours (noon) in a light (12 hour light/dark, 7am-7pm) and temperature controlled (22°-26° C) testing room that remained closed to any other activity. Fusion software (Omnitech Electronics) was used to analyze and store the above parameters.

Drug Treatment

NGF sequestering antibody (mAb911, a kind gift from Dr. David Shelton and Dr. Kris Poulsen, Rinat/Pfizer, San Francisco CA, USA) was given at a dose of 10mg/kg (i.p.) 7 and 2 days before orthopedic surgery. This dose was chosen as it significantly reduced evoked pain behaviors in preclinical models of skeletal pain [49; 54]. Mice were assigned to a group via even or odd numbered eartags. Anti-NGF and vehicle solutions were prepared in equal volumes and labeled A and B by an individual not associated with testing the animals. The individual giving the injections was blinded as to the identity of each solution.

Statistical Analysis

All values are expressed as means $\pm$ SEM. All data were found to be normally distributed; consequently, parametric methods were used for statistical analyses. The time-effect data among the different observation days were analyzed using 2-factor ANOVA, followed by Tukey's multiple comparisons test with adjusted P values. One-way ANOVA followed by Tukey's multiple comparisons test was used when comparing the areas under the time-effect curves among the different observational days. Student's t-test was used when comparing 2

means. Significance level was set at $p = 0.05$. All statistical analyses were performed using Prism (GraphPad, La Jolla, CA).

RESULTS

Orthopedic surgery transiently reduces horizontal locomotor activity (distance traveled)

Orthopedic pain was generated in animals by drilling into the trochlear groove of the right femur, avoiding the cruciate ligaments (Figure 1). Horizontal activity during the different observation days were significantly different ($F_{(4,91)}=20.97$; $P<0.0001$; 2-factor ANOVA, followed by Tukey's multiple comparisons test) (Figure 2A-D). Animals exhibited profound and significant reductions in distance traveled on days 1 and 3 post-surgery at the first hour of exploratory behavior (hour 1 Baseline = 3767 ± 267 cm; hour 1 Day 1 = 2575 ± 254 cm ($p=0.0023$); hour 1 Day 3 = 2372 ± 269 cm ($p=0.0006$); 1-factor ANOVA, followed by Tukey's multiple comparisons test) and most hours during the dark phase (Figure 2A, B, and E respectively). On day 7 post-orthopedic surgery, there was no significant reduction in distance traveled at the first hour of exploratory behavior (Figure 2E). However, at various hours during the dark phase there remained significant reductions in distanced traveled (Figure 2C). By day 12 post-orthopedic surgery, all activity time-points have returned to baseline values (Figure 2D and E). There were no significant differences ($p>0.05$, 1-factor ANOVA, followed by Tukey's multiple comparisons test) in locomotor activity during the light phase after the initial exploratory hour when compared among the different observational periods (Figure 2F).

The mice showed significant ($p<0.001$; 2-factor ANOVA, followed by Tukey's multiple comparisons test) increases in locomotor activity during the dark phase of the light/dark cycle, which is consistent with the nocturnal nature of mice. In order to better visualize the differences in peak nocturnal activity among the baseline and post-injury observation periods, the areas under the time-effect curves were measured between 1900 and 2200 hours, which we found to be a time of peak locomotor activity. Nighttime horizontal activity was significantly ($F_{(4,91)}=13.82$; $p<0.0001$; 1-factor ANOVA, followed by Tukey's multiple comparisons test) reduced from baseline on Days 1 and 3, was reduced by not significantly on day 7, and returned to baseline levels on Day 12 (Figure 2G; AUC-Baseline = 5842 ± 464 cm, AUC-Day 1 = 2387 ± 291 cm, AUC-Day 3 = 2943 ± 258 , AUC-Day 7 = 4436 ± 421 , AUC-Day 12 = 5835 ± 775).

Representative tracings of horizontal locomotor activity from a 30-minute period during the dark phase (2000-2030 hours) are shown in figure 3. Visually significant reductions in horizontal activity are apparent on days 1 and 3 in the open field apparatus when compared to naïve baseline and to day 12.

The effect of orthopedic surgery on the number of vertical rearing episodes

The numbers of vertical rearing episodes among the different observation days were significantly different ($F_{(4,91)}=20.79$; $P<0.0001$; 2-factor ANOVA, followed by Tukey's multiple comparisons test) (Figure 4A-D). There were significant reductions in rearing episodes on days 1 and 3 post-orthopedic surgery at the first hour of exploratory behavior

(hour 1 Baseline = 205.3 ± 14.9 ; hour 1 Day 1 = 130.4 ± 13.4 ($p=0.0005$); hour 1 Day 3 = 129.0 ± 14.0 ($p=0.0005$; 1-factor ANOVA, followed by Tukey's multiple comparisons) and most hours during the dark phase (Figure 4A, B and E respectively). On day 7 post-orthopedic surgery, there was no significant reduction in the number of rears at the first hour of exploratory behavior (Figure 4E). However, as with distance traveled, at various hours during the dark phase there remained a significant reduction in rears (Figure 4C). By day 12 post-orthopedic surgery, all activity time-points have returned to baseline values (Figure 4D and E). There were no significant differences ($p>0.05$; 1-factor ANOVA, followed by Tukey's multiple comparisons) in locomotor activity during the light phase after the initial exploratory hour when compared among the different observational periods (Figure 4F).

The mice showed significant ($p<0.001$) increases in mean numbers of rearing episodes during the dark phase of the light/dark cycle, which is consistent with what was seen with locomotor activity. In order to better visualize the differences in peak nocturnal activity among the baseline and post-injury observation periods, the areas under the time-effect curves were measured 1900 and 2200 hours. Peak nighttime number of rearing episodes was significantly ($F_{(4,91)}=18.02$; $p<0.0001$; 1-factor ANOVA, followed by Tukey's multiple comparisons test) reduced from baseline on Days 1 and 3, was not reduced significantly on day 7 and returned to baseline levels on Day 12 (Figure 4G; AUC-Baseline = 521 ± 40.1 , AUC-Day 1 = 147 ± 18.9 , AUC-Day 3 = 246 ± 29.1 , AUC-Day 7 = 401 ± 43.1 , AUC-Day 12 = 557 ± 88.3).

The effect of orthopedic surgery on horizontal average velocity

The overall horizontal average velocity of locomotor activity among the different observation days were significantly different ($F_{(4,91)}=10.77$; $P<0.0001$; 2-factor ANOVA, followed by Tukey's multiple comparisons test) (Figure 5A-D). There was no significant reduction in horizontal average velocity on days 1 and 3 post-orthopedic surgery at the first hour of exploratory behavior (hour 1 Baseline = 7.2 ± 0.2 cm/s; hour 1 Day 1 = 7.3 ± 0.3 cm/s ($p=0.775$); hour 1 Day 3 = 7.1 ± 0.3 cm/s ($p=0.831$); 1-factor ANOVA, followed by Tukey's multiple comparisons test) (Figure 5E), but there was a significant increase in horizontal velocity on day 7 post-orthopedic surgery at the first hour of exploratory behavior (hour 1 Day 7 = 8.3 ± 0.2 cm/s ($p=0.01$; 1-factor ANOVA, followed by Tukey's multiple comparisons test; Figure 5E). By day 12 post-orthopedic surgery, all activity time-points have returned to baseline values (Figure 5D and E). There were no significant differences ($p>0.05$; 1-factor ANOVA, followed by Tukey's comparisons test) in horizontal average velocity during the light phase after the initial exploratory hour when compared among the different observational periods (Figure 5F).

In order to better visualize the differences in peak nocturnal activity among the baseline and post-injury observation periods, the areas under the time-effect curves were measured between 1900 and 2200 hours. Nocturnal horizontal average velocity was significantly ($F_{(4,91)}=9.528$; $p<0.0001$; 1-factor ANOVA, followed by Tukey's multiple comparisons test) reduced from baseline on days 1 and 3, but not on days 7 and 12 (Figure 5G; AUC-Baseline = 21 ± 0.38 , AUC-Day 1 = 17 ± 0.72 , AUC-Day 3 = 19 ± 0.57 , AUC-Day 7 = 20 ± 0.58 , AUC-Day 12 = 22 ± 0.52 cm/s).

The orthopedic surgery-induced reduction in horizontal activity, initial exploratory vertical rearing and nighttime horizontal velocity are attenuated by administration of anti-NGF

Animals were preemptively treated with an antibody against NGF administered 7 and 2 days before orthopedic surgery. Animals treated with anti-NGF exhibited significantly greater horizontal activity on days 1 ($t_{(14)}=2.799$; $p=0.0142$) and 3 ($t_{(14)}=3.162$; $p=0.0069$) post-orthopedic surgery during the initial exploratory hour when compared to vehicle treated animals (Figure 6A; Vehicle Day 1 = 2110 ± 564 cm, anti-NGF Day 1 = 4235 ± 506 cm; Vehicle Day 3 = 1979 ± 568 cm, anti-NGF Day 3 = 3824 ± 269 cm). The number of vertical rearing episodes after anti-NGF treatment on days 1 and 3 post-orthopedic surgery during the initial exploratory hour was also significantly increased when compared to vehicle (Figure 6C; Vehicle Day 1 = 114 ± 27.0 , anti-NGF Day 1 = 189 ± 22.4 ; $t_{(14)}=2.165$, $p=0.0481$; Vehicle Day 3 = 110 ± 24.3 , anti-NGF Day 3 = 171 ± 15.8 ; $t_{(14)}=2.199$, $p=0.0452$). Animals treated with anti-NGF exhibited an increase in horizontal velocity on days 1 and 3 post-orthopedic surgery during the initial exploratory hour compared to vehicle treated animals, but the effect was not significant (Figure 6E; Vehicle Day 1 = 6.33 ± 0.386 cm/s, anti-NGF Day 1 = 6.66 ± 0.327 cm/s; $t_{(14)}=0.6576$, $p=0.5214$; Vehicle Day 3 = 6.43 ± 0.571 cm/s, anti-NGF Day 3 = 7.17 ± 0.348 cm/s; $t_{(14)}=1.15$, $p=0.2693$).

Animals treated with anti-NGF exhibited significantly higher horizontal activity on day 1 post-orthopedic surgery during peak nocturnal activity (1900-2200 hours) compared to vehicle treated animals (Figure 6B; Vehicle Day 1 = 2611 ± 495 cm, anti-NGF Day 1 = 6964 ± 1626 cm; $t_{(14)}=2.281$, $p=0.0387$). The number of vertical rearing episodes after anti-NGF treatment during peak nocturnal activity increased when compared to vehicle, but the effect was not significant (Figure 6D; Vehicle Day 1 = 196.3 ± 45.6 , anti-NGF Day 1 = 397.3 ± 94.9 ; $t_{(14)}=1.741$, $p=0.1036$). However, as with horizontal activity, animals treated with anti-NGF exhibited a significant increase in horizontal velocity on day 1 post-orthopedic surgery during peak nocturnal activity compared to vehicle treated animals (Figure 6F; Vehicle Day 1 = $16.0\pm.9$ cm/s, anti-NGF Day 1 = $20.5.8\pm1.0$ cm/s; $t_{(14)}=3.225$, $p=0.005$). On day 3 post-orthopedic surgery during peak nocturnal activity (1900-2200 hours), animals treated with anti-NGF exhibited increased horizontal activity, vertical rearing and horizontal velocity compared to vehicle treated animals, but the increases were not significant.

The importance of monitoring spontaneous activity and rearing when animals are normally the most active

Figure 7 shows representations of three different time periods for measuring activity, depicted here by the 20-hour Day 1 horizontal activity graph (Figure 7A). The first 30 minutes in a testing apparatus is the time when rodents explore a new environment, depicted here by the tracings recorded at 1200-1230 hours (Figure 7B). This initial exploration is followed by an extended period of virtual inactivity that lasts until the dark phase, depicted here by the tracings recorded at 1400-1430 hours (Figure 7C). The greatest amount of spontaneous locomotor activity occurs during the first 3 hours of dark, depicted here by the tracings recorded at 2000-2030 hours (Figure 7D).

DISCUSSION

Translating results from preclinical models of skeletal pain into human clinical trials

Acute and chronic human skeletal pain remains a difficult pain to fully control using currently available analgesics [16; 52; 81]. In light of this significant unmet clinical need, several preclinical rodent models have been developed [3; 4; 7; 49; 54; 59; 65; 72; 82]. While many of these preclinical rodent models of skeletal pain mirror the bone or joint pathology that drives human skeletal pain, there are several major challenges in behaviorally assessing pain and analgesia in these models.

The first major problem in measuring pain in rodent preclinical models is that in most cases the behaviors are performed during daylight hours (7am–7pm) which is the time that rodents are normally least active or sleeping [23; 30; 37; 39; 41; 81]. As human clinical trials examining the efficacy of an analgesic on relieving skeletal pain usually focus on when the patient is normally awake, and not when they are sleeping, it is not clear how one can readily translate results obtained from rodents during the time when the animal is least active vs. assessments conducted when humans are normally most active.

A second major problem with current assessments of rodent skeletal pain behaviors is that nearly all rodent pain behaviors are evoked and not spontaneous. For example, currently the most commonly used endpoint in skeletal pain behaviors is skin hypersensitivity as assessed by von Frey (mechanical testing) or the Hargreaves method (thermal) [2; 20; 51; 67]. While it may be peripheral and/or central sensitization [15; 21; 29; 50; 60; 64] that is driving this skeletal injury induced skin hypersensitivity, from the perspective of a patient with skeletal pain due to osteoarthritis or TKA/THA, evoked skin hyperalgesia is rarely the major pain complaint [18; 55; 62]. Rather the major complaint of patients with orthopedic surgery pain is the spontaneous pain that arises upon use of the bone/joint that interferes with their quality of life, functional status, and ability to participate in the needed physical therapy and rehabilitation [10]. Indeed, it has recently been questioned whether analgesics that relieve skin hypersensitivity accurately predicts the extent to which the analgesic will also reduce the underlying skeletal pain [28; 70] and at the very least, it is not clear how one would design, perform a power analysis, or determine what endpoints would be measured in a human clinical trial that is based principally on the ability of an analgesic to relieve skin hypersensitivity.

Third, even when “skeletal pain related behaviors” such as limb guarding, flinching or weight bearing are assessed, these behaviors are evoked in that the animal is removed from its home cage during their normal sleep period and their behaviors assessed during the least active period of the animal's day [23; 30; 37; 39; 41; 81]. Equally important, all observer based assessments of pain are subject to unavoidable observer differences and bias [74; 76]. Thus, what is a guard or flinch to one observer may be scored differently with another observer. Additionally, when the analgesic therapy under examination has either a large therapeutic effect or a noticeable side effect, it becomes even more difficult to fully remove observer bias.

In light of the above challenges, in the present report day/night horizontal activity, horizontal velocity, and episodes of vertical rearing were assessed in mice with orthopedic surgery induced skeletal pain. The animals were continuously monitored for a 20 hour period before orthopedic surgery and then at days 1, 3, 7 and 12 following surgery using a system that continually measures spontaneous locomotion and hindlimb rearing for precise analysis of circadian patterns. At days 1 and 3 post-orthopedic surgery, mice showed a marked decline in horizontal activity and vertical rearing. In all the mice, activity was greatest during the first 30 minutes they were placed in the activity boxes (the exploratory period) and for the first 3 hours following the onset of the dark cycle (peak night activity). Interestingly, it was during these times when the animals were most active that one observes the greatest differences between the naïve animals and animals that had orthopedic surgery. At other time periods during both the day and night, when the animals were less active and there was little spontaneous rearing, very small or no significant differences were noted between naïve animals and animals that had orthopedic surgery.

The present results clearly show that there are significant differences in horizontal activity and vertical rearing episodes in C3H mice at days 1 & 3 post-orthopedic surgery vs. their baseline behaviors. In contrast, by days 7 and 12 post-surgery these differences are largely gone presumably due to healing of the injured skeleton. However, there are several additional controls/experiments that may provide insight in the strengths and limitations of using mouse activity to measure orthopedic surgery induced pain. These include; testing a non-surgical group at the identical time as the animals which had orthopedic surgery, running a sham group which had only a skin incision, and identifying and using a strain of mice that may have significantly higher baseline activity than the C3H mice used in the present experiments [22]. Experiments do suggest that naïve C3H mice do not appreciably change their horizontal activity or vertical rearing over a 14 day observation period (unpublished observations) and skin incision alone does not induce significant changes in skeletal pain behaviors measured by guarding and flinching [49]. However, running these additional controls and strains of mice on the same day, in the same 20 hours activity apparatus used here, has the potential to better define the strengths and weaknesses of using activity boxes to measure skeletal pain and assessing the efficacy of analgesics in relieving skeletal pain.

Spontaneous movement has been validated and successfully used as an outcome measure in cat [26; 27] and dog [11-14; 61; 78; 80] spontaneous models of chronic OA musculoskeletal pain, and it is well established that activity is an important measure of musculoskeletal pain-associated disability in humans [9; 35; 43; 58; 73]. Similarly, with skeletal pain following orthopedic surgery, our data indicate that mice show a marked reduction in levels of spontaneous movement and rearing and thus have significantly reduced “functional status” compared to their non-operated controls. The present data also emphasize the importance that, just as in humans with skeletal pain, focusing on measuring spontaneous activity based behaviors during the period when the animal or human is most active, greatly improves the ability to capture the reduction in functional status that is the hallmark in animals and humans with chronic skeletal pain.

Targeting nerve growth factor/TrkA for the relief of orthopedic surgery pain

In exploring possible alternatives to NSAIDs or opiates to control postoperative orthopedic surgery pain, we focused on anti-NGF [16; 23; 30; 38; 39; 41; 45; 47; 52]. Previous studies in humans with osteoarthritis and low back pain showed that anti-NGF reduced these skeletal pains by 40% and 30% respectively [40; 45], and dramatic improvement in function and activity was seen in cat [27] and dog [47] spontaneous OA models following administration of anti-NGF. In the present study, during the times the animals were most active, anti-NGF increased horizontal activity, velocity and vertical rearing but had no effect in increasing activity or rearing when the mice were normally less active. Thus, anti-NGF appears to be relieving skeletal pain, which results in increased use of the skeleton, rather than simply driving a hyper-locomotion which would boost the activity at all day and nighttime points examined. The present results also suggest that human clinical trials targeting the relief of orthopedic surgery pain including that due to TKA/THA may be a fruitful approach in gaining approval of anti-NGF and TrkA inhibitors for human skeletal pain. Thus, the time needed to assess analgesic efficacy and the surveillance period for unwanted side effects would be weeks/months for TKA/THA rather than months to years required for osteoarthritis and low back pain.

The present results in mice may shed some light on an issue that was raised in human clinical trials of anti-NGF for the relief of OA pain [1]. In these human studies, while it was shown that anti-NGF therapy was efficacious in relieving pain due to OA, a small but significant number of elderly (>65 years old) patients with moderate-to-severe OA pain on the highest dose of anti-NGF and an NSAID needed earlier-than-expected joint replacement [33; 45]. In light of these results, it was not clear whether this earlier-than-expected joint replacement was due to greater use of the diseased joint (i.e. excessive use and loading of the bone/joint) [36] or a direct and delirious effect anti-NGF plus the NSAID were having on the bone or joint [52]. The present data demonstrates that in a mouse model of orthopedic surgery pain, anti-NGF does increase activity and rearing in animals with the injured skeleton suggesting that monitoring and advising patients as to the overuse of their skeleton may minimize the orthopedic surgeries required to repair excessive use-induced injury to the aging/diseased bone and joint.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Summary

Using an automated system that continually monitored rodent activity, sequestration of NGF increased spontaneous day/night activity and rearing in mice with acute post-orthopedic surgery pain.

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Day 0: Orthopedic Surgery

Figure 1. Radiographic images of a mouse femur showing the orthopedic surgery procedure performed in the present study

Orthopedic surgery pain was induced by drilling a 0.5mm hole in the center of the trochlear groove of C3H/HeJ male mice, which were 8-9 weeks old at the time of the surgery, using a pneumatic dental high-speed hand piece. A pin was then inserted into the intramedullary canal to core the marrow space and the drill site was sealed with a dental amalgam plug. This procedure induced injury to the articular cartilage, subchondral bone, trabecular bone and marrow in the knee joint of the distal head of the femur.

Figure 2. Horizontal locomotor activity is transiently reduced following orthopedic surgery

The activity of the mice was continuously recorded (A-D) for 20 hours, from 1200 hours (noon) to 0800 hours the following morning under a 12 hour light/dark cycle (0700-1900 hours light, 1900-0700 hours dark). Horizontal activity during the different observation days following orthopedic surgery were significantly different (Mean $\pm$ SEM, * indicates $p < 0.05$ as determined by 2-factor ANOVA and Tukey's *post-hoc* test for multiple comparisons). Figures E,F and G show the areas under the time-effect curves (AUC) for the initial exploratory hour (E), the 6 hours of light that immediately followed the exploratory hour

(F), and the first 3 hours of night activity (F) corresponding to the most active period. At days 1 and 3 post-orthopedic surgery, horizontal activity, as measured as area under the curve, was significantly reduced during the initial exploratory hour (E), and the first 3 hours of night activity (G) but not during 6 hours of light that immediately followed the exploratory hour (F) (* indicates $p < 0.05$ as determined by one-way ANOVA followed by Tukey's *post-hoc* multiple comparisons test). On day 7 and 12 post-orthopedic surgery, there was no statistically significant difference in horizontal activity as measured by AUC.

Figure 3. Representative tracings of the horizontal locomotor activity of a single mouse in the activity box, pre- and post-orthopedic surgery, during the 30 minute nighttime period when mice normally display the greatest spontaneous activity

Note that compared to pre-surgery naïve baseline activity, there is a marked reduction in horizontal activity 1 & 3 days post-surgery and this spontaneous activity returns to baseline levels by day 12 post-surgery.

Figure 4. The number of rearing episodes is transiently reduced following orthopedic surgery
 The activity of the mice was continuously recorded (A-D) for 20 hours, from 1200 hours (noon) to 0800 hours the following morning under a 12 hour light/dark cycle (0700-1900 hours light, 1900-0700 hours dark). The number of vertical rearing episodes during the different observation days following orthopedic surgery were significantly different (Mean $\pm$ SEM, * indicates $p < 0.05$ as determined by 2-factor ANOVA and Tukey's *post-hoc* test for multiple comparisons). Figures E, F and G show the areas under the time-effect curves (AUC) for the initial exploratory hour (E), the 6 hours of light that immediately followed the exploratory hour (F), and the first 3 hours of night activity (F) corresponding to the most active period. At days 1 and 3 post-orthopedic surgery, vertical rearing, as measured as area under the curve, was significantly reduced during the initial exploratory hour (E), and the first 3 hours of night activity (G) but not during 6 hours of light that immediately followed the exploratory hour (F) (* indicates $p < 0.05$ as determined by one-way ANOVA followed by Tukey's *post-hoc* multiple comparisons test). On day 7 and 12 post-orthopedic surgery, there was no statistically significant difference in vertical rearing as measured by AUC.

Figure 5. The horizontal velocity of mice shows at best a small and transient reduction following orthopedic surgery

The activity of the mice was continuously recorded (A-D) for 20 hours, from 1200 hours (noon) to 0800 hours the following morning under a 12 hour light/dark cycle (0700-1900 hours light, 1900-0700 hours dark). Horizontal velocity during the different observation days following orthopedic surgery were significantly different (Mean $\pm$ SEM, * indicates $p < 0.05$ as determined by 2-factor ANOVA and Tukey's *post-hoc* test for multiple comparisons).

Figures E, F and G show the areas under the time-effect curves (AUC) for the initial exploratory hour (E), the 6 hours of light that immediately followed the exploratory hour (F), and the first 3 hours of night activity (F) corresponding to the most active period. The only 3 time points which show statistically significant difference in AUC are at day 7 in exploratory behavior and days 1 and 3 during peak night activity (* indicates $p < 0.05$ as determined by oneway ANOVA followed by Tukey's *post-hoc* multiple comparisons test).

Figure 6. Anti-NGF therapy increases some, but not all, measures of horizontal activity, vertical rearing and horizontal velocity following orthopedic surgery

Anti-NGF (10mg/kg, s.c.) or vehicle was given 7 and 2 days prior to orthopedic surgery. The activity of the mice was continuously recorded for 20 hours, from 1200 hours (noon) to 0800 hours the following morning under a 12 hour light/dark cycle (0700-1900 hours light, 1900-0700 hours dark). Figures A-F show the areas under the time-effect curves (AUC) for the initial exploratory hour (A, C & E) and the first 3 hours of night activity (B, D & F) corresponding to the most active period. At days 1 and 3 post-orthopedic surgery, horizontal activity and episodes of rearing were significantly increased during the initial exploratory hour (A & C). Horizontal activity was also significantly increased during the first 3 hours of night activity on day 1 (B), whereas horizontal velocity was only significantly increased during the first 3 hours of night activity on day 1 (F) (Mean±SEM, *= $p < 0.05$ as determined by Student's t-test).

Figure 7. Representative tracings from single mice showing that the efficacy of anti-NGF in increasing horizontal activity is most evident when the mouse is normally the most active
 The tracings are representative 30 minute recordings of horizontal locomotor activity during the initial exploratory hour (B: 1200-1230 hours), the daylight phase (C: 1400-1430 hours) and the dark phase (D: 2000-2030 hours) portions of the recordings for naïve baseline, 1 day post-orthopedic surgery and 1 day post-orthopedic surgery + anti-NGF. The line graph to the left of the tracings (A) illustrates the overall activity of each group of animals. These data suggest that the most informative time points to examine when assessing the ability of an analgesic to increase the horizontal activity is when the mice are normally most active (B and D) in contrast to (C) when the mice are least active.