HEALTH AND MEDICINE

β-Endorphin mediates radiation therapy fatigue

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Fatigue is a common adverse effect of external beam radiation therapy in cancer patients. Mechanisms causing radiation fatigue remain unclear, although linkage to skin irradiation has been suggested. β-Endorphin, an endogenous opioid, is synthesized in skin following genotoxic ultraviolet irradiation and acts systemically, producing addiction. Exogenous opiates with the same receptor activity as β-endorphin can cause fatigue. Using rodent models of radiation therapy, exposing tails and sparing vital organs, we tested whether skin-derived β-endorphin contributes to radiation-induced fatigue. Over a 6-week radiation regimen, plasma β-endorphin increased in rats, paralleled by opiate phenotypes (elevated pain thresholds, Straub tail) and fatigue-like behavior, which was reversed in animals treated by the opiate antagonist naloxone. Mechanistically, all these phenotypes were blocked by opiate antagonist treatment and were undetected in either β-endorphin knockout mice or mice lacking keratinocyte p53 expression. These findings implicate skin-derived β-endorphin in systemic effects of radiation therapy. Opioid antagonism may warrant testing in humans as treatment or prevention of radiation-induced fatigue.

INTRODUCTION

Most cancer patients receiving radiation therapy are treated with fractionated external beam radiation in which a daily dose of ionizing radiation from an external source targets a solid tumor located at a specific anatomic site. Radiation commonly causes fatigue, thus exacerbating one of the most common and distressing symptoms in patients with cancer (*1*). As shown in some of the early longitudinal studies on radiation-related fatigue, patients undergoing radiation typically begin to experience fatigue 3 to 4 weeks into a 6- to 8-week regimen (*2*–*4*). The fatigue may last for approximately 3 weeks after the end of therapy, although in some instances it can persist for longer before recovery to pretreatment levels (*2*–*5*). While fatigue is a subjective phenomenon measured by patients' self-reporting, its clinical significance has been recognized as early as 2000 by the National Comprehensive Cancer Network as an entity deserving attention in the management of cancer patients (*6*).

Although a well-described phenomenon, the mechanism(s) causing radiation fatigue remains uncertain. Notably, fatigue is experienced as often in patients receiving tangential field radiation that penetrates only the skin and subcutaneous tissue, as in those receiving radiation to deeper structures (*7*). Among breast cancer patients, those who receive whole breast irradiation have higher levels of treatment-related fatigue compared to patients who receive partial breast irradiation, which involves radiation to a smaller surface area (*8*). Other studies have also demonstrated that fatigue incidence and severity are more highly correlated with dose and surface area of the radiation field than depth penetrated (*4*, *5*, *9*–*11*), leading us to hypothesize that factors in the skin may play a causative role in radiation-induced fatigue.

Recent studies have identified a cutaneous pathway activated following exposure to ultraviolet (UV) light, in which epidermal keratinocytes up-regulate p53, which stimulates expression of pro-opiomelanocortin (POMC) that is posttranslationally cleaved into bioactive peptides including the pigmentation-inducing hormone α-MSH (melanocyte-stimulating hormone) and the endogenous opioid β-endorphin (*12*). Systemic increases in β-endorphin after UV exposure produce opiate-like behaviors and phenotypes (14), indicating systemic β-endorphin effects following local UV exposure. Opiate drugs can cause sedation, a common symptom associated with fatigue (*15*, *16*), prompting us to ask whether radiation-induced increases in β-endorphin might contribute to the fatigue associated with radiation therapy. To test this, we used rodent tail irradiation to model minimally penetrating radiation therapy.

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RESULTS

Tail irradiation induces fatigue-like behavior in rats together with increased plasma β-endorphin and opioid phenotypes

We first asked whether β-endorphin levels increase systemically in response to tail irradiation treatments. In our initial model, rats received ionizing radiation (2 Gy/day) to the tail, with all other parts of the body protected in lead enclosures. Each regimen consisted of 5 days of daily radiation per week for 6 weeks, after which the radiation stopped. Another group of animals (mock treatments) were kept in lead enclosures for a time equal to radiation administration (approximately 1 min), but no radiation was administered. Plasma β-endorphin increased significantly 4 weeks after the start of daily tail irradiation and returned to baseline 1 week after the termination of irradiation (Fig. 1A). Plasma β-endorphin did not significantly increase during mock irradiation, suggesting that the technical procedures required to administer irradiation did not induce endorphin elevation by triggering a stress response.

We next tested whether this radiation treatment protocol may produce fatigue-like symptoms, as a model for the human condition. We observed that, following irradiation, rats became progressively sedate, as quantified by movement measurements calculated using open-field actimetry (Fig. 1B). While other tests of fatigue and sedation in rodents exist, we chose open-field actimetry (*17*) as a surrogate of fatigue-like behavior because, unlike other comparable tests (*18*, *19*), it produces virtually no added stress and does not require single housing of animals; therefore, it has a low probability of independently affecting endogenous opioid levels while permitting the monitoring of multiple end points over the required time period.

To test the opioid dependency of this fatigue-like phenotype, we pretreated rats with intraperitoneal administration of naloxone (10 mg/kg) before actimetry testing. Despite maintained radiation-induced increases in plasma β-endorphin (Fig. 1, A and B), naloxone was seen to prevent the radiation-induced decreases in actimetry measurements. Mock-irradiated rats showed no change in activity or plasma β-endorphin levels (Fig. 1, A and B). Together,

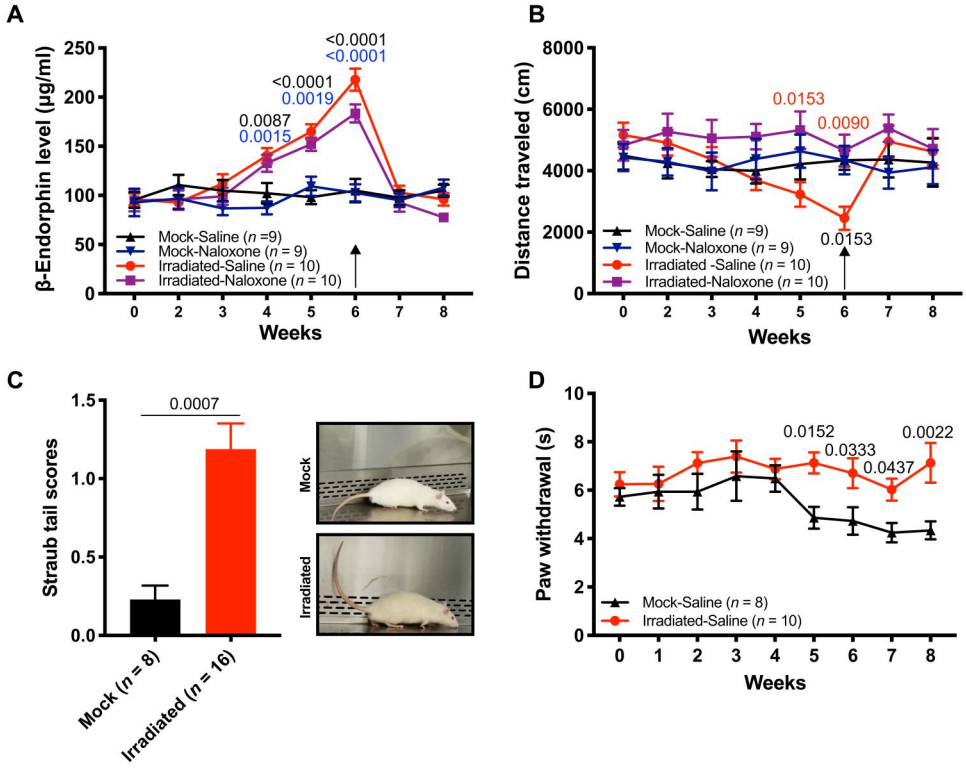

Fig. 1. Tail radiation increases β-endorphin levels, depresses motor activity, and induces other opiate phenotypes in rats. Sprague-Dawley rats underwent tail radiation or mock treatment (2 Gy/day) 5 days/week for 6 weeks. Error bars in the figure indicate ±SEM. Numbers in brackets indicate numbers of animals per group. Arrows in (A) and (B) indicate the last day of radiation exposure or mock treatment (week 6, day 42). In (A) and (B), 6 weeks of tail radiation (5 days/week) or mock exposure were done on all groups followed by two additional weeks after tail treatment. (**A**) Plasma β-endorphin levels for each of the groups. Two-way analysis of variance (ANOVA) with the Holm-Šídák multiple comparisons test revealed significant differences between groups that are indicated above the graphs. Black numbers indicate differences between Mock-Saline and Irradiated-Saline; blue numbers indicate differences between Mock-Naloxone and Irradiated-Naloxone groups. (**B**) Distance traveled in 30 min by open-field actimetry was measured once weekly as described in (A). Rats were administered intraperitoneal saline injection or naloxone injection (10 mg/kg) before actimetry testing. Two-way ANOVA with the Holm-Šídák multiple comparisons test revealed significant differences between groups that are indicated above the graphs. Red numbers indicate difference between Irradiated-Naloxone and Irradiated-Saline. Black number indicates difference between Irradiated-Saline and Mock-Saline. All other groups showed no significant change from baseline. (**C**) Straub tail reaction is observed after 5 weeks of tail radiation (bottom), but not in mock-irradiated animals (top). Value of significance was determined by two-tailed unpaired *t* test. (**D**) Thermal nociceptive threshold increased in irradiated animals. Two-way ANOVA with the Holm-Šídák multiple comparisons test revealed significant differences between the groups that are indicated above the graphs. Black numbers indicate differences between Mock and Irradiated rats.

these data suggest that radiation-induced fatigue is associated with systemic elevations in β-endorphin and may respond to administration of an opiate receptor antagonist.

As both systemic elevations of β-endorphin (*13*) and systemic opiates have been associated with multiple behavioral effects, we asked whether the observed increases in plasma β-endorphin after ionizing radiation may also be associated with other phenotypes associated with opioid signaling. With administration of exogenous opiates, rodents demonstrate μ-opioid receptor–dependent nociceptive threshold elevations (*20*) and Straub tail (*21*), which is a central μ-opioid receptor–dependent contraction of the sacrococcygeus dorsalis muscle at the base of the tail that results in rigidity and elevation of the tail. Following tail irradiation, we observed Straub tail (Fig. 1C) as well as an elevation in nociceptive threshold (Fig. 1D). Although stress has been shown previously to trigger opioid-dependent Straub tail phenomenon in rats (*22*), we have observed a difference between mock-treated and mock-irradiated rats, suggesting the direct role of irradiation in Straub tails.

Given the requirement for peripheral β-endorphin synthesis in these opiate-like phenotypes (*13*), we investigated the role of the peripheral nervous system in fatigue-like behavior. We observed that the blood-brain barrier–permeable antagonist naloxone can prevent fatigue-like behavior in rats (Fig. 1B), so we tested the blood-brain barrier–impermeable compound methylnaltrexone, which when injected peripherally only blocks peripheral opioid receptors. However, peripheral methylnaltrexone did not prevent the development of any fatigue-like behavior end points (velocity, time spent not moving, and distance traveled) (fig. S1, A to C), suggesting that central opioid signaling is critical for mediating fatigue-like behavior. Collectively, these findings suggest that minimally penetrating chronic irradiation can increase a systemic elevation of β-endorphin and induce fatigue-like behavior along with measurable alterations in several other opiate phenotypes.

Plasma β-endorphin elevations in tail-irradiated mice

To elucidate the underlying mechanism behind radiation-induced elevations in blood β-endorphin, we used several genetic mouse models. Mice exhibit paradoxical hyperlocomotion responses to opiates (fig. S2), in line with previous observations (*23*–*29*), in contrast to rats (*30*) and humans; therefore, they do not represent an ideal model system to study the involvement of skin-derived endogenous opioids in a tail irradiation–induced fatigue model. Furthermore, previous observations showed that β-endorphin does not change locomotor behavior in mice (*31*). However, mice represent a rich source of genetic models, which enables the dissection of the pathway through which radiation induces plasma β-endorphin levels. Using a crossover design, we treated mice with daily tail irradiation (with body shielding similar to the rat studies above) and switched the two groups reciprocally between mock irradiation and ionizing radiation after 6 weeks. Plasma β-endorphin increased significantly 2 weeks after the start of daily tail irradiation and returned to baseline 1 week after the transition from tail irradiation to mock irradiation (Fig. 2A). Plasma β-endorphin did not significantly increase during mock irradiation, but upon initiation of tail irradiation in the previously mock-treated group, β-endorphin significantly increased after 1 week of tail irradiation (Fig. 2A). Tail irradiation–mediated increases in β-endorphin were greater in these mouse experiments than in the rat studies above. This may be due to the lower daily radiation doses administered to rats, which more closely model radiation dose fractions administered to cancer patients. As expected from DNA damage–induced up-regulation of POMC and POMC-derived peptides [as in response to UV exposure (*12*, *13*)], we observed increased local pigmentation in irradiated skin areas, but not in nonirradiated skin areas or in mock-irradiated animals (Fig. 2B). We did not observe hyperpigmentation of rat tails upon radiation (fig. S3), because Sprague-Dawley rats carry a mutation in the tyrosinase gene that makes them albino (*32*). This missense mutation (R299H) is conserved across all albino rat strains (*29*, *33*) and has been described in patients with oculocutaneous albinism type I (*34*). Because of the lack of tyrosinase activity, it is expected that the melanocytes of these rats are incapable of producing pigment, despite upstream activation by POMC-derived MSH. This inability to produce pigment in albino rats is similar in albino mice and albino humans. Thus, this difference is not a species-dependent difference in DNA damage response upon irradiation in keratinocytes because irradiation induces POMC-derived endorphin elevation and multiple opioid phenotypes in both species. Rather, this observation is due to the inability of albino melanocytes to produce pigment upon MSH stimulation, independent of the upstream DNA damage response. Collectively, our results are consistent with previous observations that ionizing radiation induces DNA damage responses that, like UV radiation, can trigger p53-mediated downstream effects (*35*, *36*).

Radiation induces opioid-mediated behaviors in parallel with plasma β-endorphin increases that are inhibited by pharmacologic opioid antagonism

We next asked whether the observed increases in plasma β-endorphin are functionally significant in mice. Similar to our findings in rats, we observed that tail-irradiated mice exhibit the Straub tail sign (Fig. 2C). The sign was noticeable within 4 weeks of initiating radiation, and tails returned to normal within 1 week of stopping radiation, while controls that were initially mock-irradiated demonstrated no evidence of Straub tail, but did develop Straub tail after initiation of tail irradiation at the beginning of week 7 (crossover design) (Fig. 2C). In irradiated animals, Straub tail was reversed by administration of naloxone, but not by saline (Fig. 2, D and E), suggesting involvement of an endogenous opioid pathway. While these studies used male animals, we separately compared tail irradiation effects on female mice and observed identical effects (fig. S4).

Mechanical (von Frey assay) and thermal (hot plate assay) pain sensitivity were also measured during the tail radiation regimen. Both mechanical nociceptive thresholds and thermal nociceptive response latencies increased significantly with tail irradiation and returned to baseline within 1 week of stopping tail irradiation (Fig. 3, A and B). These changes paralleled increases in plasma β-endorphin levels (Fig. 3C). Tail-irradiated mice treated with the opiate antagonist naloxone before pain threshold testing session showed no increases in pain thresholds (Fig. 3, A and B) despite increases in plasma β-endorphin levels (Fig. 3C).

Despite reversal of radiation-induced behavioral effects by naloxone administration, tail-irradiated mice administered naloxone before nociceptive sensitivity testing demonstrated significantly greater increases in systemic β-endorphin levels compared with tail-irradiated mice administered saline (Fig. 3C). Mock-irradiated controls with or without naloxone pretreatment showed no significant change in mechanical or thermal pain tolerance, or in plasma

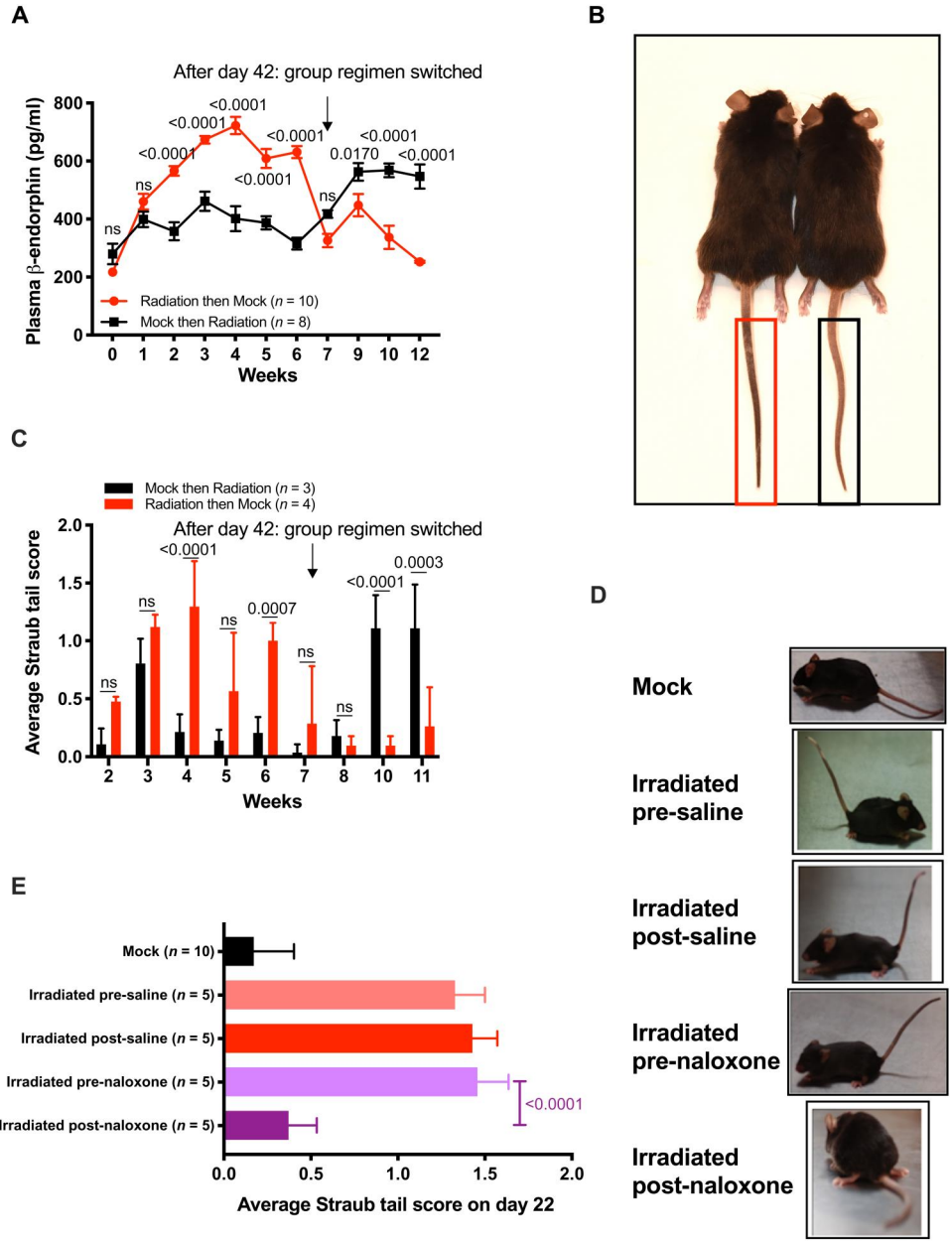

Fig. 2. Tail ionizing radiation exposure in mice increases plasma β-endorphin levels and induces opioid-dependent Straub tail. (**A**) Weekly plasma β-endorphin levels in wild-type mice in a crossover experiment in which, initially, one group of mice underwent mock irradiation, while a second group of mice underwent tail radiation (5 Gy/day) 5 days/week for 6 weeks. After week 6 (day 42), the groups switched treatment regimens, and the treatments continued for six more weeks. Error bars indicate ±SEM. Two-way ANOVA with the Holm-Šídák multiple comparisons test revealed significant differences between the groups that are indicated above the graph. Black numbers indicate differences between Radiation then Mock and Mock then Radiation. (**B**) Increased local pigmentation of the tail (radiation-exposed area) in a tail-irradiated mouse after 6 weeks of ionizing radiation (left), while no pigmentation is observed on the tail of a mock-treated mouse (right). (**C**) Straub tail scores in mock-irradiated mice and mice administered tail radiation (5 Gy/day) 5 days/week for 6 weeks. After 6 weeks (day 42), the groups switched regimens [the same regimens as in (A) but with separate groups of mice]. Two-way ANOVA with the Holm-Šídák multiple comparisons test revealed significant differences between the groups that are indicated above the graph. (**D**) Naloxone reverses Straub tail induced by tail irradiation. Representative photos of mice after tail irradiation or mock treatment and 20 min after saline or naloxone injection described in (E). (**E**) On week 4 of tail irradiation, mice were administered intraperitoneal saline or naloxone (10 mg/kg), and Straub tail scores were measured 20 min after administration. One-way ANOVA with the Holm-Šídák multiple comparisons test revealed significant differences between groups that are indicated above the graph. Error bars in this figure indicate ±SEM. ns, not significant.

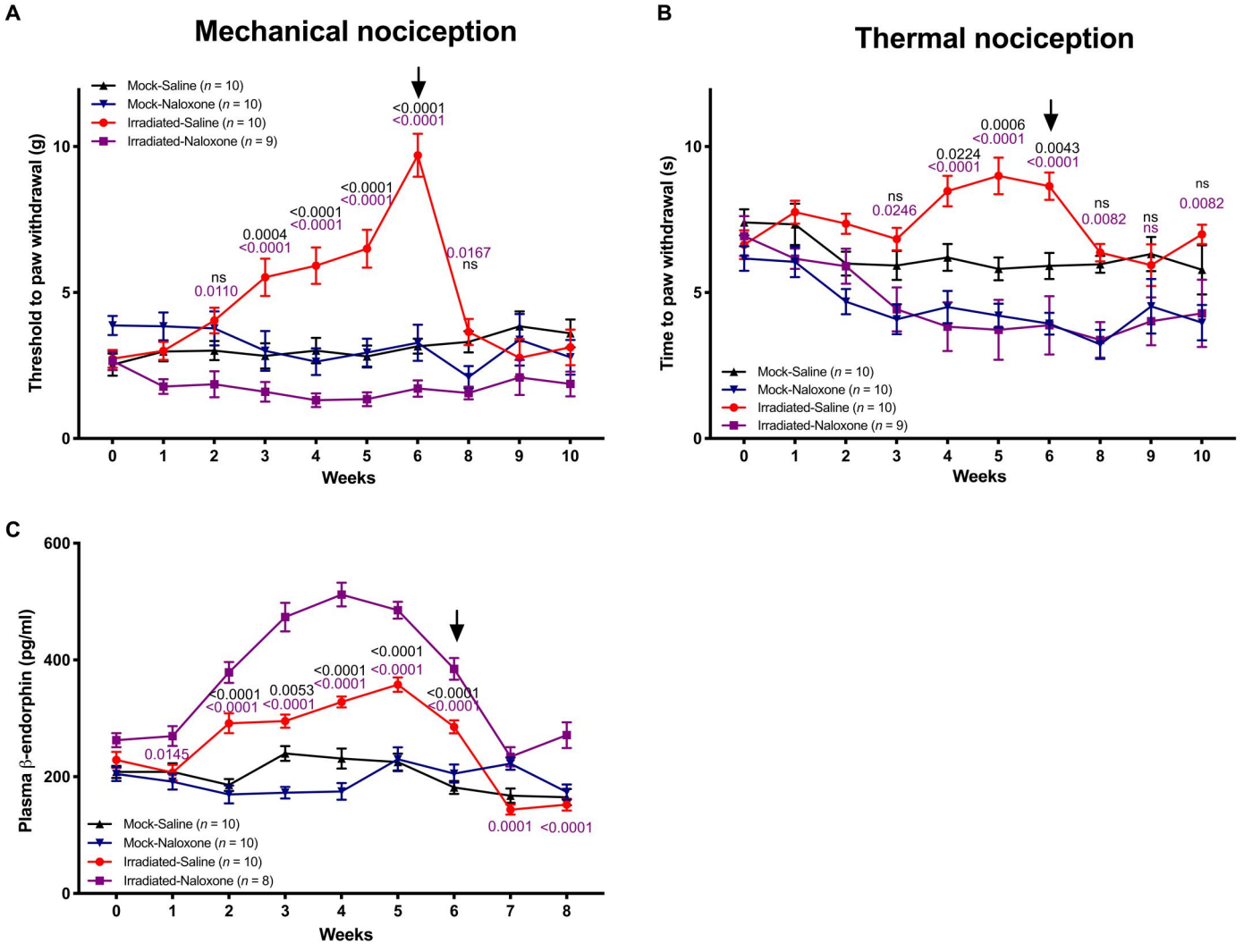

Fig. 3. Increased analgesic thresholds induced by tail irradiation are reversed by opioid antagonists. After week 6 of tail irradiation or mock exposure, mice were assayed for two additional weeks after tail treatment. Arrows indicate the final day of radiation or mock exposure for each group (Mock or Radiation Stop). Two-way ANOVA with the Holm-Šídák multiple comparisons test revealed significant differences between groups that are indicated above the graphs. Black numbers indicate difference between Irradiated-Saline and Mock-Saline groups. Purple numbers indicate differences between Irradiated-Saline and Irradiated-Naloxone groups. Error bars in the figure indicate ±SEM. (**A**) Mechanical (von Frey assay) (top) and (**B**) thermal (hot plate assay) (middle) analgesic thresholds in mice that were tail-irradiated (5 Gy/day ionizing radiation) or mock-treated 5 days/week for 6 weeks and administered intraperitoneal injection of either saline or naloxone (10 mg/kg) before analgesic testing. (**C**) Plasma β-endorphin levels of mice described in (A) and (B).

β-endorphin levels (Fig. 3, A to C), suggesting that the brief restraint without radiation does not trigger a stress response modifying β-endorphin levels.

Radiation-induced opioid-mediated behaviors are dependent upon β-endorphin and keratinocyte-specific p53 expression

To test whether these radiation-induced changes in sensory nociceptive threshold are β-endorphin dependent, β-endorphin–null mice (*37*) underwent the tail radiation regimen. As shown in Fig. 4 (A and B), β-endorphin–null mice demonstrated no significant change in mechanical or thermal pain threshold with radiation. Similarly, β-endorphin–null mice did not display Straub tail after irradiation (Fig. 4C). These studies suggest that radiation-induced increases in β-endorphin produce changes in opioid receptor–dependent phenotypes.

Because up-regulation of POMC and production of cutaneous β-endorphin in response to UV exposure has been shown to be p53 dependent (*12*, *13*), we tested whether mice with keratinocyte-specific deletion of p53 fail to elevate plasma β-endorphin in response to tail irradiation. We observed that these mice showed no significant increase in plasma β-endorphin upon chronic low-dose tail irradiation (Fig. 4D), consistent with a keratinocyte-specific p53-dependent process. These data suggest that the keratinocyte p53–POMC–β-endorphin pathway is required to elevate plasma β-endorphin levels after tail irradiation. In line with the lack of plasma β-endorphin elevations, mice with keratinocyte-specific p53 knockout did not have elevated nociceptive thresholds

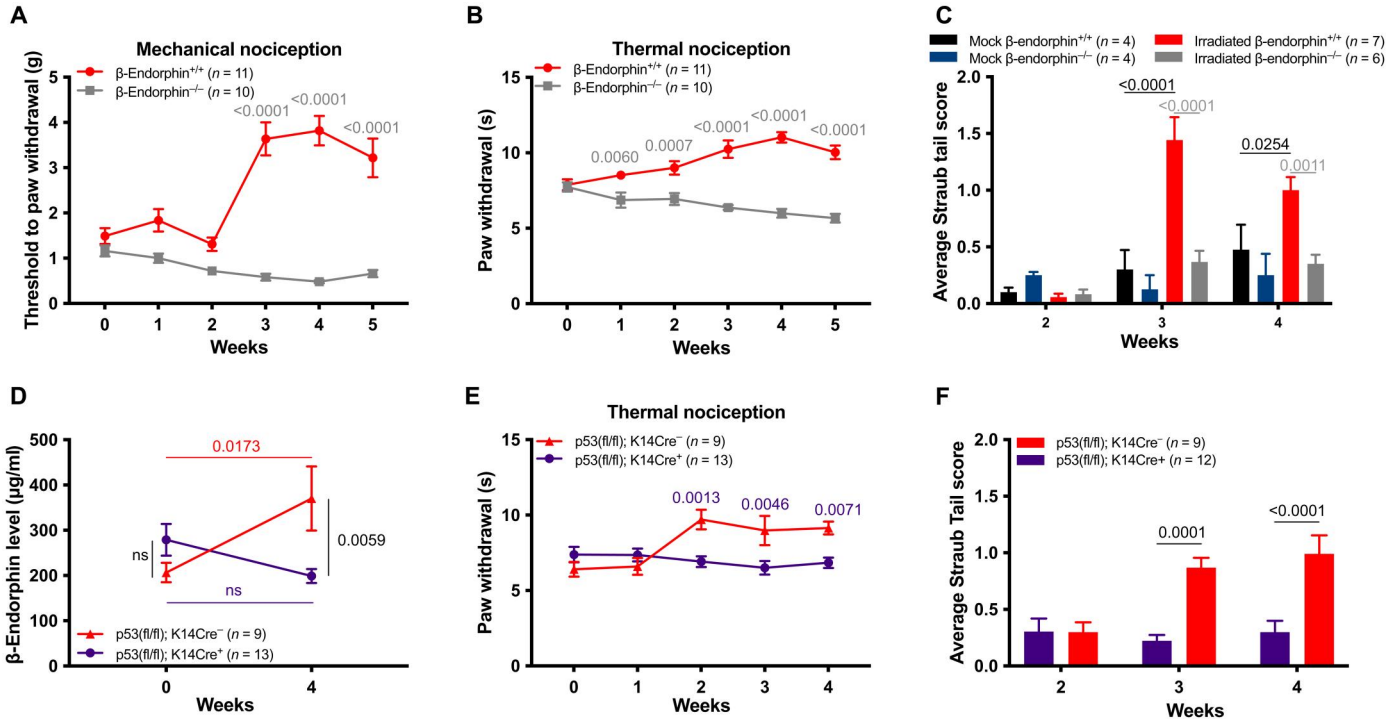

Fig. 4. Radiation-induced Straub tail and elevated nociceptive thresholds depend on β-endorphin and on keratinocyte-specific p53 expression. (**A**) Mechanical (von Frey assay) and (**B**) thermal (hot plate assay) analgesic thresholds in β-endorphin wild-type and β-endorphin–null mice over 5 weeks of tail radiation (5 Gy/day) 5 days/week. (**C**) Straub tail was observed starting 3 weeks after irradiation in β-endorphin wild-type mice, but not in mock-treated or β-endorphin–null mice. (**D**) β-Endorphin and (**E**) thermal analgesic threshold (hot plate assay) elevations are absent in p53fl/fl mice expressing Cre under the keratinocyte-specific promoter K14 but are present in p53fl/fl mice with no Cre. Mice were treated weekly with tail radiation (5 Gy/day) 5 days/week. (**F**) Straub tail was absent in K14Cre;p53fl/fl mice, but was present in p53fl/fl mice after 3 and 4 weeks of the tail radiation regimen described in (D) and (E). Error bars in the figure indicate ±SEM. Two-way ANOVA with the Holm-Šídák multiple comparisons tests revealed significant differences between the groups that are indicated above the graphs.

(Fig. 4E) and did not display Straub tail (Fig. 4F). These results collectively support our model that radiation-induced DNA damage in keratinocytes increases plasma β-endorphin levels that are required for the opioid behaviors.

DISCUSSION

This study provides mechanistic insight into a debilitating side effect of cancer radiation therapy. Previous mechanistic analyses of radiation-induced fatigue have suggested roles for systemic inflammation, in which an association was observed between cytokine levels and fatigue in some studies (*38*, *39*), but not others (*40*). Additional research has suggested that radiation-induced fatigue may arise from the emotional and psychological toll of having cancer (*41*); however, a correlation between the presence of clinical anxiety or depression and fatigue has not been consistently observed (*40*). While the current study suggests a role for radiation-induced production of β-endorphin as a contributor to fatigue, it is plausible that the clinical syndrome involves combinatorial influences of these and other mechanisms.

The fact that mice do not display fatigue in a tail irradiation model is expected and does not contradict previous observations (*42*–*45*), as this model was used not to explain a unifying mechanism behind radiation-induced fatigue but to demonstrate a previously unidentified skin-specific mechanism contributing to fatigue, which can be reliably measured in rats. Certainly, there are multiple mechanisms contributing to fatigue in both humans and animal models. Previous studies investigating peripheral irradiation in mice investigate a likely different mechanism that is supported by (i) different irradiation protocols and (ii) the different kinetics of development of fatigue-like behavior.

Most papers published on peripheral irradiation target the lower abdominal or pelvic area of mice, not focusing on the tail, which is external to the body cavities and internal organs. This is an important distinction as the other studies involve proportionally much less skin (and therefore proportionally less activation of this pathway) and much more soft tissue and abdominal/pelvic organ involvement (and proportionally more of their tissue damage). The differential tissue involvement suggests potentially different mechanisms contributing to fatigue. Previously, peripherally secreted cytokines have been suspected to mediate the central fatigue-like effects after irradiation (*46*–*48*), which we believe explains why these different models of irradiating significant non-skin tissue might cause fatigue-like behavior in mice, independently of skin-endorphin synthesis. Our method focused on a skin-specific mechanism that may play a more minimal role in the pelvic/abdomen/brain irradiation models.

It is possible that lower doses could also trigger endorphin elevation and fatigue development, most likely necessitating longer monitoring. There are likely to be interesting considerations that influence the threshold for clinical fatigue. We believe that these questions warrant further human studies. However, some studies done

in human previously suggested a skin surface area–dependent nature of fatigue-like behavior (*8*), which is in line with our observations that skin-derived signal mediates fatigue-like behavior.

The kinetics of fatigue-like behavior in other models also differ from our model as they use more limited numbers of radiation doses within a short time frame (days), and the fatigue-like behavior is observed quite rapidly within days of the last radiation (*42*, *44*), unlike in our model and in most human contexts. Our rat model phenocopies human fatigue, as the symptoms do not start right away, but after a delay of a few weeks, which is consistent with the kinetics of endorphin level buildup in the serum that we observed. The different kinetics of developing fatigue-like behavior further argues that different mechanisms are responsible for fatigue-like behavior in the various irradiation models, and therefore, the previous observations regarding fatigue-like behavior in mice do not contradict our findings. Our results complement previous studies with an additional skin-specific mechanism that previously was not appreciated—only suspected based on correlation of irradiation surface area and severity of fatigue symptoms in humans (*8*). While human studies are still needed to further validate the mechanism identified here, many key aspects of the pathway have previously been demonstrated in both human and rodent skin including UV induction of p53, POMC, and β-endorphin (*12*, *13*). Additional variables regulating plasma endorphin levels (circadian rhythm, stressors from blood draw, and UV exposure) might make it difficult to measure radiation-induced endorphin elevations in the plasma in man, similar to UV radiation (*13*). However, the behavior effects of opioid blockade might reverse the effects of chronic opioid signaling, similar to the observed effects of naloxone in frequent tanners where, despite the lack of demonstrated endorphin elevation upon tanning (*49*, *50*), naloxone has been shown to elicit behavior effects (*51*, *52*).

Fatigue-like symptoms have been measured by running wheel (*18*), treadmill (*19*), and open-field (*17*, *53*–*58*) experimental methods. While all three assays are potential indicators of fatigue, we used open-field actimetry as a measure of fatigue-like behavior because it does not require individual housing of the animals and permits numerous measurements over multiple weeks (the time frame over which plasma elevations of β-endorphin occur).

The studies reported here have used a variety of genetic models to elucidate the underlying mechanism connecting ionizing radiation to up-regulation of plasma β-endorphin. We observed that the cutaneous response to ionizing radiation requires p53 function within keratinocytes, similar to what was previously observed after UV radiation (*12*, *13*). This pathway appears to be evolutionarily conserved and is present in rats and humans as well. It is also responsible for the UV-tanning response (*12*) and the addiction-like effects of UV radiation (*13*). Here, we extend previous studies and demonstrate that ionizing radiation triggers keratinocyte-specific, p53-regulated β-endorphin synthesis, which promotes multiple opiate behaviors that are preventable by opiate antagonism. In conclusion, mice, rats, and humans have identical cutaneous DNA damage responses that lead to very similar opioid phenotypes after UV and ionizing radiation. The only established known difference among these species lies in the central opioid response; this species-specific difference in response between mice and rats has been previously explored and mechanistically explained (*23*).

Previously, the role of cancer cells and cell death–induced changes in fatigue-like behavior has been investigated (*59*). Some studies have implicated the role of cytokines released by cancer cells in promoting fatigue in rodents and in humans (*38*, *39*), but in some, there was no evidence for the role of systemic inflammation (*40*). Our data do not contradict these studies; rather, they highlight the importance of skin-derived endorphin synthesis that is independent of cancer cell death. We hypothesize that, when patients with cancer undergo radiation therapy, multiple mechanisms can operate simultaneously, with each contributing to fatigue-like behavior. Tumor cells may also express POMC (*60*, *61*); however, their role in regulating behavior warrants further investigation. Here, we attempted to decouple cancer-derived signals from skin-derived signals, because of previous observations that the exposed skin area is correlated with the development of fatigue. Our study has identified skin-derived endorphin to play a role in mediating fatigue-like behavior, independent of cancer cell–derived signals.

The observation that acute peripheral opioid receptor blockade was not able to prevent fatigue-like behavior suggests that peripheral p53 and peripherally synthetized β-endorphin ultimately act through the central nervous system (CNS). The chronic peripheral irradiation might gradually increase peripheral endorphin levels that might directly increase central opioid signaling despite their low blood-brain barrier permeability. Alternatively, peripheral endorphin synthesis could possibly activate the CNS indirectly through dorsal root ganglia neurons. In that case, the chronic β-endorphin signaling would involve peripheral opioid receptors, whose acute blockade is not sufficient to reverse the chronic activation of CNS opioid signaling. However, it is also possible that there are alternative targets of p53 in keratinocytes that contribute to central opioid signaling.

The precise mechanism through which β-endorphin may induce sedation is uncertain, but it is notable that UV radiation also elevates circulating β-endorphin (*13*) and has been associated with fatigue (albeit anecdotally, e.g., a "day at the beach"). While sedation associated with exogenous opiates may be accompanied by other symptoms not observed in the radiation fatigue syndrome, it is plausible that endogenous β-endorphin may not phenocopy these agents. Opioid-mediated inhibition of the hypothalamic-pituitary-adrenal axis has been implicated in the pathophysiology of chronic fatigue syndrome (*62*), although this point remains debated (*63*) and inconclusive given small sample sizes in reporting studies.

We would also like to highlight the importance of validation of the work with human studies. Although technically challenging, it would be advantageous to confirm that irradiation exposure raises β-endorphin in humans to a level similar to UV irradiation and also that radiation-induced DNA damage may lead to fatigue. These studies could further the evidence that a single pathway triggered by different stimuli (UV and irradiation) may lead to the same behavioral phenotype. Note that in humans UV exposure has been associated with greater levels of outdoor activity, extended life expectancy, and decreases in multiple causes of mortality independently of vitamin D (*64*), so the mechanistic extrapolation of these preclinical results to the human scenario will be important.

This study highlights a potential therapeutic strategy: the use of naloxone or other opioid antagonists for pharmacologic treatment or prevention of radiation-induced fatigue in certain cancer patients. Although we used naloxone in the study to investigate the role of opioid signaling in multiple end points shortly after injections, naltrexone might be a better choice clinically because of its

longer duration of action and oral administration. Patients who require opiates for pain management would not be recommended for such treatment, but the use of opioid antagonist agents such as naltrexone is anticipated to be a relatively benign pharmacologic intervention in patients without such specific contraindications. For otherwise functional cancer patients limited by radiation therapy–induced fatigue, opioid antagonism might potentially offer a safe and beneficial means to improve quality of life and activity levels during radiation therapy. Future studies will be required to evaluate potential safety and efficacy of such an approach in the clinic.

METHODS

Animals

All mice used were 8-week-old males in a C57BL/6 background, with the exception that 8-week-old female C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were used in the Straub tail test described in Fig. 2 (D and E) and fig. S4. In addition, we used mice lacking the C-terminal end of the POMC polyprotein because of a mutation in both copies of the POMC gene, resulting in lack of β-endorphin (*37*), to test for changes in pain thresholds with tail radiation. To ablate keratinocyte-specific p53 expression, mice with floxed alleles of p53 (*65*) were crossed with a strain expressing Cre recombinase driven by the keratin 14 promoter (*66*). Throughout the study, we compared −/− animals to +/+ animals, as on certain backgrounds the +/− phenotype does not fully recapitulate the +/+ phenotype. Generating litters containing both −/− and +/+ genotypes in sufficiently high numbers was unfortunately not feasible throughout the study; therefore, littermate controls were not used in this study. Experiments were blinded when possible.

For open-field actimetry experiments using rats, 8-week-old male Sprague-Dawley rats (The Jackson Laboratory, Bar Harbor, ME) were used. All animals were maintained on a 12-hour light/12-hour dark cycle and were acclimated to the vivarium for at least 1 week before starting experiments. All animal experiments were performed in accordance with institutional policies and Institutional Animal Care and Use Committee–approved protocols.

Animals that suffered injuries that precluded them from providing data points were not included in any measurements. This comprised 8 animals out of nearly 200 total. Separately, one mouse was noticed to exhibit a freezing behavior following morphine injection, which appeared to represent a potentially misplaced injection. The values for this mouse in open-field testing suggested it to be an outlier by the ROUT test; thus, it was excluded (fig. S2). All experiments were performed with approval from the Massachusetts General Hospital Institutional Animal Care and Use Committee.

Irradiators

Mouse tail irradiation was performed using the Gammacell 40 Exactor with a Cs-137 radiation source (MDS Nordion). Rat tail irradiation was performed using a Siemens Stabilipan 2 irradiator operating at 250 kVp with a Half-value layer of 0.4-mm Cu and a dose rate of 1.89 Gy/min, or a Precision x-ray 225-kVp unit with 0.5-mm Cu and a dose rate of 2.07 Gy/min. Tube output was regularly monitored, and x-ray dosimetry is traceable to standards by the National Institute of Standards and Technology.

Tail irradiation and blood draws

Mice were placed in a lead restrainer custom made to protect the heads and bodies of the mice from radiation, with tails protruding from a designated hole for the tails (the lead shield minimal wall thickness was 1.9 cm). Animals underwent ionizing radiation (5 Gy/day) to the tail 5 days/week (Monday to Friday) for 6 weeks. For rat tail irradiation, separate custom-made lead restrainers were made (wall thickness was 0.635 cm), each with a rear hole for tail protrusion, and animals underwent tail x-irradiation (2 Gy/day), 5 days/week (Monday to Friday) for 6 weeks. Individual irradiation did not last for more than 2 min to minimize restraint and to prevent immobilization stress, which was not observed, as evidenced by the lack of endorphin alterations in the mock-treated groups.

Mock-irradiated animals were restrained by the same restrainers as the irradiated animals and placed in the irradiator for the same amount of time as when the radiation would be administered, but without the irradiator running. Then, they were removed and placed back in their cages. This way, we helped to ensure that all animals experienced the same environment (and potential stress) during handling and the radiation procedure.

For blood draws, animals were placed in a species-specific standard restrainer and 100 μl of blood was collected from the tail vein into EDTA-containing microvette tubes with 0.6 Trypsin Inhibitory Unit (TIU) of aprotinin. For certain experiments, blood was taken submandibularly. Samples were immediately placed on ice after collection. Samples were centrifuged at 3500 rpm for 20 min at 4°C, and plasma was collected into separate tubes and stored at −80°C until measurement of β-endorphin. Blood was collected once per week on Fridays in the morning before tail irradiation for the day. β-Endorphin was measured by radioimmunoassay (Phoenix Pharmaceuticals). We have validated that the kit does not show any signal in samples obtained from β-endorphin knockout mice.

Straub tail measurement

Straub tail was measured as previously described (*21*). The scoring system was a scale of 0 to 2 based on the rigidity and angle of tail elevation from horizontal (0, relaxed tail and no elevation; 1, tail rigid and elevated up to 10°; 1.5, tail rigid and elevated 11° to 45°; and 2, tail rigid and elevated 46° to 90°). For mice, individual Straub tail values were obtained by averaging six Straub score measurements taken in 60 s. Straub tail score was calculated similarly for rats, with the slight modification of obtaining individual values by averaging three measurements in 30 s.

Analgesic threshold testing

Mice underwent mechanical threshold testing in the von Frey test (*67*) and the hot plate test (*68*) as previously described. In the von Frey test, animals were placed on an elevated wire mesh grid in individual enclosures and acclimated for 30 min. The plantar surface of each left hind paw was poked 10 times with fibers calibrated to deliver specific pressures. Increasing pressures were delivered until 2 of 10 responses of paw flinching, fluttering, or licking in response to a poke. In the hot plate test, animals were placed on a 52°C plate surrounded by Plexiglas walls, and time to a response was measured. Responses included paw licking or fluttering, jumping, or attempt to escape the enclosure.

Animals underwent this testing twice weekly in the morning. In select experiments, mice were injected intraperitoneally with

naloxone hydrochloride (10 or 1 mg/kg) (Sigma-Aldrich, St. Louis, MO) or with normal saline before the acclimation period.

Open-field actimetry

Eight-week-old Sprague-Dawley rats underwent open-field actimetry testing. Testing was carried out during the light cycle between 8 a.m. and 7 p.m. Groups were randomized to avoid any batch effect, i.e., all groups were equally likely to be tested in morning/afternoon hours. The apparatus consists of a 17″ × 17″ chamber with Plexiglas walls and an open top, equipped with three 16-beam arrays that detect motion and a computer that calculates distance traveled based on breaks in the beams (Med Associates, St. Albans, VT). Animals underwent actimetry testing once weekly. For experiment displayed in Fig. 1, animals were injected intraperitoneally with either naloxone hydrochloride (10 mg/kg) or normal saline and then acclimated to the chamber for 30 min before testing. Data collected were distance traveled (centimeters) in 30 min.

For experiments displayed in fig. S1, 2 Ethovision XT 9 was used to analyze the open-field measurements. For experiment shown in fig. S1, animals were injected with either methylnaltrexone (5 mg/kg) or saline and then were kept in their home cages for 30 min. Then, animals were tested for 30 min in the chamber.

Statistics

Prism 8.0 was used for statistical analyses. Repeated-measures two-way analysis of variance (ANOVA) with the Holm-Šídák multiple comparisons test was used to analyze the experiments, as well as a two-tailed, unpaired *t* test (Fig. 1C) and ordinary one-way ANOVA (Fig. 2E).

Supplementary Materials

This PDF file includes:

Figs. S1 to S4

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. R. Stasi, L. Abriani, P. Beccaglia, E. Terzoli, S. Amadori, Cancer-related fatigue. *Cancer* **98**, 1786–1801 (2003).
2. D. B. Greenberg, J. Sawicka, S. Eisenthal, D. Ross, Fatigue syndrome due to localized radiation. *J. Pain Symptom Manage.* **7**, 38–45 (1992).
3. P. B. Jacobsen, C. L. Thors, Fatigue in the radiation therapy patient: Current management and investigations. *Semin. Radiat. Oncol.* **13**, 372–380 (2003).
4. J. T. Hickok, G. R. Morrow, J. A. Roscoe, K. Mustian, P. Okunieff, Occurrence, severity, and longitudinal course of twelve common symptoms in 1129 consecutive patients during radiotherapy for cancer. *J. Pain Symptom Manage.* **30**, 433–442 (2005).
5. M. Back, V. Ahern, G. Delaney, P. Graham, A. Steigler, C. Wratten; New South Wales Breast Radiation Oncology Group, Absence of adverse early quality of life outcomes of radiation therapy in breast conservation therapy for early breast cancer. *Australas. Radiol.* **49**, 39–43 (2005).
6. V. Mock, A. Atkinson, A. Barsevick, D. Cella, B. Cimprich, C. Cleeland, J. Donnelly, M. A. Eisenberger, C. Escalante, P. Hinds, P. B. Jacobsen, P. Kaldor, S. J. Knight, A. Peterman, B. F. Piper, H. Rugo, P. Sabbatini, C. Stahl; National Comprehensive Cancer Network, NCCN practice guidelines for cancer-related fatigue. *Oncology (Williston Park)* **14**, 151–161 (2000).
7. R. Barbarino, D. Janniello, P. Morelli, M. D. Falco, S. Cicchetti, L. di Murro, G. Tortorelli, P. Bagalà, D. di Cristino, A. Murgia, S. Terenzi, E. Ponti, B. Tolu, R. Santoni, Fatigue in patients undergoing radiation therapy: An observational study. *Minerva Med.* **104**, 185–191 (2013).
8. N. K. Taunk, B. G. Haffty, S. Chen, A. J. Khan, C. Nelson, D. Pierce, S. Goyal, Comparison of radiation-induced fatigue across 3 different radiotherapeutic methods for early stage breast cancer. *Cancer* **117**, 4116–4124 (2011).
9. B. A. Jereczek-Fossa, H. R. Marsiglia, R. Orecchia, Radiotherapy-related fatigue. *Crit. Rev. Oncol. Hematol.* **41**, 317–325 (2002).
10. M. Hofman, J. L. Ryan, C. D. Figueroa-Moseley, P. Jean-Pierre, G. R. Morrow, Cancer-related fatigue: The scale of the problem. *Oncologist* **12** (Suppl. 1), 4–10 (2007).
11. A. L. Schwartz, L. M. Nail, R. N. S. Chen, P. Meek, A. M. Barsevick, M. E. King, L. S. Jones, Fatigue patterns observed in patients receiving chemotherapy and radiotherapy. *Cancer Invest.* **18**, 11–19 (2000).
12. R. Cui, H. R. Widlund, E. Feige, J. Y. Lin, D. L. Wilensky, V. E. Igras, J. D'Orazio, C. Y. Fung, C. F. Schanbacher, S. R. Granter, D. E. Fisher, Central role of p53 in the suntan response and pathologic hyperpigmentation. *Cell* **128**, 853–864 (2007).
13. G. L. Fell, K. C. Robinson, J. Mao, C. J. Woolf, D. E. Fisher, Skin β-endorphin mediates addiction to UV light. *Cell* **157**, 1527–1534 (2014).
14. Lajos V. Kemény, Kathleen C. Robinson, Andrea L. Hermann, Deena M. Walker, Susan Regan, Yik Weng Yew, Yi Chun Lai, Nicholas Theodosakis, Phillip D. Rivera, Weihua Ding, Liuyue Yang, Tobias Beyer, Yong-Hwee E. Loh, Jennifer A. Lo, Anita A. J. van der Sande, William Sarnie, David Kotler, Jennifer J. Hsiao, Mack Y. Su, Shinichiro Kato, Joseph Kotler, Staci D. Bilbo, Vanita Chopra, Matthew P. Salomon, Shiqian Shen, Dave S. B. Hoon, Maryam M. Asgari, Sarah E. Wakeman, Eric J. Nestler, David E. Fisher, Vitamin D deficiency exacerbates UV/endorphin and opioid addiction. *Science Advances* **7**2021). 10.1126/sciadv.abe4577.
15. J. E. Reissig, A. M. Rybarczyk, Pharmacologic treatment of opioid-induced sedation in chronic pain. *Ann. Pharmacother.* **39**, 727–731 (2005).
16. S. O'Mahony, N. Coyle, R. Payne, Current management of opioid-related side effects. *Oncology (Williston Park)* **15**, 61–73 (2001).
17. F. S. Lira, A. M. Esteves, G. D. Pimentel, J. C. Rosa, M. K. Frank, M. O. Mariano, J. Budni, J. Quevedo, R. V. dos Santos, M. T. de Mello, Sleep pattern and locomotor activity are impaired by doxorubicin in non-tumor-bearing rats. *Sleep Sci.* **9**, 232–235 (2016).
18. J. Goh, W. Ladiges, Voluntary wheel running in mice. *Curr. Protoc. Mouse Biol.* **5**, 283–290 (2015).
19. J. P. Dougherty, D. A. Springer, M. C. Gershengorn, The treadmill fatigue test: A simple, high-throughput assay of fatigue-like behavior for the mouse. *J. Vis. Exp.* , 54052 (2016).
20. H. W. Matthes, R. Maldonado, F. Simonin, O. Valverde, S. Slowe, I. Kitchen, K. Befort, A. Dierich, M. L. Meur, P. Dollé, E. Tzavara, J. Hanoune, B. P. Roques, B. L. Kieffer, Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* **383**, 819–823 (1996).
21. D. L. J. Bilbey, H. Salem, M. H. Grossman, The anatomical basis of the straub phenomenon. *Br. J. Pharmacol. Chemother.* **15**, 540–543 (1960).
22. R. J. Katz, J. Gelbart, Endogenous opiates and behavioral responses to environmental novelty. *Behav. Biol.* **24**, 338–348 (1978).
23. K. Kuschinsky, O. Hornykiewicz, Effects of morphine on striatal dopamine metabolism: Possible mechanism of its opposite effect on locomotor activity in rats and mice. *Eur. J. Pharmacol.* **26**, 41–50 (1974).
24. D. A. Brase, H. H. Loh, E. L. Way, Comparison of the effects of morphine on locomotor activity, analgesia and primary and protracted physical dependence in six mouse strains. *J. Pharmacol. Exp. Ther.* **201**, 368–374 (1977).
25. U. Filibeck, C. Castellano, A. Oliverio, Development of morphine-induced changes of activity in the mouse. *Brain Res.* **254**, 602–605 (1981).
26. G. J. Gwynn, E. F. Domino, Genotype-dependent behavioral sensitivity to mu vs. kappa opiate agonists. I. Acute and chronic effects on mouse locomotor activity. *J. Pharmacol. Exp. Ther.* **231**, 306–311 (1984).
27. D. Marona-Lewicka, J. Vetulani, Neurochemical correlates of differences in responses to psychotropic drugs. I. Apomorphine and morphine effects on locomotor activity of C57/BL and Balb/C mice. *Pol. J. Pharmacol. Pharm.* **41**, 431–438 (1989).
28. K. Mizuo, M. Narita, K. Miyagawa, M. Narita, E. Okuno, T. Suzuki, Prenatal and neonatal exposure to bisphenol-A affects the morphine-induced rewarding effect and hyperlocomotion in mice. *Neurosci. Lett.* **356**, 95–98 (2004).
29. N. Kitanaka, J. Kitanaka, M. Takemura, Modification of morphine-induced hyperlocomotion and antinociception in mice by clorgyline, a monoamine oxidase-A inhibitor. *Neurochem. Res.* **31**, 829–837 (2006).
30. G. A. Mickley, K. E. Stevens, J. M. Burrows, G. A. White, G. L. Gibbs, Morphine tolerance offers protection from radiogenic performance deficits. *Radiat. Res.* **93**, 381–387 (1983).
31. R. G. Browne, D. S. Segal, Behavioral activating effects of opiates and opioid peptides. *Biol. Psychiatry* **15**, 77–86 (1980).
32. T. Kuramoto, S. Nakanishi, M. Ochiai, H. Nakagama, B. Voigt, T. Serikawa, Origins of albino and hooded rats: Implications from molecular genetic analysis across modern laboratory rat strains. *PLOS ONE* **7**, e43059 (2012).
33. W. M. Blaszczyk, L. Arning, K. P. Hoffmann, J. T. Epplen, A tyrosinase missense mutation causes albinism in the Wistar rat. *Pigment Cell Res.* **18**, 144–145 (2005).

34. R. K. Tripathi, K. M. Strunk, L. B. Giebel, R. G. Weleber, R. A. Spritz, Tyrosinase gene mutations in type I (tyrosinase-deficient) oculocutaneous albinism define two clusters of missense substitutions. *Am. J. Med. Genet.* **43**, 865–871 (1992).
35. S. W. Lowe, S. Bodis, A. McClatchey, L. Remington, H. E. Ruley, D. E. Fisher, D. E. Housman, T. Jacks, p53 status and the efficacy of cancer therapy in vivo. *Science* **266**, 807–810 (1994).
36. A. Ziegler, A. S. Jonason, D. J. Leffellt, J. A. Simon, H. W. Sharma, J. Kimmelman, L. Remington, T. Jacks, D. E. Brash, Sunburn and p53 in the onset of skin cancer. *Nature* **372**, 773–776 (1994).
37. M. Rubinstein, J. S. Mogil, M. Japón, E. C. Chan, R. G. Allen, M. J. Low, Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3995–4000 (1996).
38. V. De Sanctis, L. Agolli, V. Visco, F. Monaco, R. Muni, A. Spagnoli, B. Campanella, M. Valeriani, G. Minniti, M. F. Osti, C. Amanti, P. Pellegrini, S. Brunetti, A. Costantini, M. Alfò, M. R. Torrisi, P. Marchetti, R. M. Enrici, Cytokines, fatigue, and cutaneous erythema in early stage breast cancer patients receiving adjuvant radiation therapy. *Biomed. Res. Int.* **2014**, 523568 (2014).
39. D. B. Greenberg, J. L. Gray, C. M. Mannix, S. Eisenthal, M. Carey, Treatment-related fatigue and serum interleukin-1 levels in patients during external beam irradiation for prostate cancer. *J. Pain Symptom Manage.* **8**, 196–200 (1993).
40. H. Geinitz, F. B. Zimmermann, P. Stoll, R. Thamm, W. Kaffenberger, K. Ansorg, M. Keller, R. Busch, D. van Beuningen, M. Molls, Fatigue, serum cytokine levels, and blood cell counts during radiotherapy of patients with breast cancer. *Int. J. Radiat. Oncol. Biol. Phys.* **51**, 691–698 (2001).
41. P. B. Jacobsen, K. A. Donovan, B. J. Small, H. S. Jim, P. N. Munster, M. A. Andrykowski, Fatigue after treatment for early stage breast cancer: A controlled comparison. *Cancer* **110**, 1851–1859 (2007).
42. M. Renner, R. Feng, D. Springer, M. K. Chen, A. Ntamack, A. Espina, L. N. Saligan, A murine model of peripheral irradiation-induced fatigue. *Behav. Brain Res.* **307**, 218–226 (2016).
43. B. S. Wolff, M. A. Renner, D. A. Springer, L. N. Saligan, A mouse model of fatigue induced by peripheral irradiation. *J. Vis. Exp.* , 55145 (2017).
44. B. S. Wolff, S. A. Raheem, L. N. Saligan, Comparing passive measures of fatigue-like behavior in mice. *Sci. Rep.* **8**, 1–12 (2018).
45. B. S. Wolff, S. A. Raheem, S. A. Alshawi, J. M. Regan, L. R. Feng, L. N. Saligan, Induction of fatigue-like behavior by pelvic irradiation of male mice alters cognitive behaviors and BDNF expression. *PLOS ONE* **15**, e0235566 (2020).
46. J. E. Bower, P. A. Ganz, M. L. Tao, W. Hu, T. R. Belin, S. Sepah, S. Cole, N. Aziz, Inflammatory biomarkers and fatigue during radiation therapy for breast and prostate cancer. *Clin. Cancer Res.* **15**, 5534–5540 (2009).
47. J. E. Bower, D. M. Lamkin, Inflammation and cancer-related fatigue: Mechanisms, contributing factors, and treatment implications. *Brain. Behav. Immunity* **30**, S48–S57 (2013).
48. C. Xiao, J. J. Beitler, K. A. Higgins, K. Conneely, B. Dwivedi, J. Felger, E. C. Wommack, D. M. Shin, N. F. Saba, L. Y. Ong, J. Kowalski, D. W. Bruner, A. H. Miller, Fatigue is associated with inflammation in patients with head and neck cancer before and after intensity-modulated radiation therapy. *Brain Behav. Immun.* **52**, 145–152 (2016).
49. T. Gambichler, A. Bader, M. Vojvodic, A. Avermaete, M. Schenk, P. Altmeyer, K. Hoffmann, Plasma levels of opioid peptides after sunbed exposures. *Br. J. Dermatol.* **147**, 1207–1211 (2002).
50. M. Kaur, A. Liguori, A. B. Fleischer Jr., S. R. Feldman, Plasma beta-endorphin levels in frequent and infrequent tanners before and after ultraviolet and non-ultraviolet stimuli. *J. Am. Acad. Dermatol.* **54**, 919–920 (2006).
51. M. Kaur, A. Liguori, A. B. Fleischer Jr., S. R. Feldman, Side effects of naltrexone observed in frequent tanners: Could frequent tanners have ultraviolet-induced high opioid levels? *J. Am. Acad. Dermatol.* **52**, 916 (2005).
52. M. Kaur, A. Liguori, W. Lang, S. R. Rapp, A. B. Fleischer Jr., S. R. Feldman, Induction of withdrawal-like symptoms in a small randomized, controlled trial of opioid blockade in frequent tanners. *J. Am. Acad. Dermatol.* **54**, 709–711 (2006).
53. A. Chi, C. Kang, Y. Zhang, L. Tang, H. Guo, H. Li, K. Zhang, Immunomodulating and antioxidant effects of polysaccharide conjugates from the fruits of Ziziphus jujube on chronic fatigue syndrome rats. *Carbohydr. Polymers.* **122**, 189–196 (2015).
54. A. Chi, Y. Zhang, Y. Kang, Z. Shen, Metabolic mechanism of a polysaccharide from Schisandra chinensis to relieve chronic fatigue syndrome. *Int. J. Biol. Macromol.* **93**, 322–332 (2016).
55. A. Chi, Z. Shen, W. Zhu, Y. Sun, Y. Kang, F. Guo, Characterization of a protein-bound polysaccharide from Herba Epimedii and its metabolic mechanism in chronic fatigue syndrome. *J. Ethnopharmacol.* **203**, 241–251 (2017).
56. C. Shao, Y. Ren, Z. Wang, C. Kang, H. Jiang, A. Chi, Detection of urine metabolites in a rat model of chronic fatigue syndrome before and after exercise. *Biomed. Res. Int.* **2017**, 1–12 (2017).
57. C. Shao, J. Song, S. Zhao, H. Jiang, B. Wang, A. Chi, Therapeutic effect and metabolic mechanism of a selenium-polysaccharide from ziyang green tea on chronic fatigue syndrome. *Polymers (Basel).* **10**, 1269 (2018).
58. T. Yi, L. Qi, J. Li, J. J. le, L. Shao, X. Du, J. C. Dong, Moxibustion upregulates hippocampal progranulin expression. *Neural Regen. Res.* **11**, 610–616 (2016).
59. S. Yang, S. Chu, Y. Gao, Q. Ai, Y. Liu, X. Li, N. Chen, A narrative review of cancer-related fatigue (CRF) and its possible pathogenesis. *Cell* **8**, 738 (2019).
60. A. White, M. F. Stewart, W. E. Farrell, S. R. Crosby, P. M. Lavender, P. R. Twentyman, L. H. Rees, A. J. L. Clark, Pro-opiomelanocortin gene expression and peptide secretion in human small-cell lung cancer cell lines. *J. Mol. Endocrinol.* **3**, 65–70 (1989).
61. D. W. Ray, A. C. Littlewood, A. J. Clark, J. R. Davis, A. White, Human small cell lung cancer cell lines expressing the proopiomelanocortin gene have aberrant glucocorticoid receptor function. *J. Clin. Invest.* **93**, 1625–1630 (1994).
62. L. V. Scott, F. Burnett, S. Medbak, T. G. Dinan, Naloxone-mediated activation of the hypothalamic-pituitary-adrenal axis in chronic fatigue syndrome. *Psychol. Med.* **28**, 285–293 (1998).
63. W. J. Inder, T. C. R. Prickett, R. T. Mulder, Normal opioid tone and hypothalamic-pituitary-adrenal axis function in chronic fatigue syndrome despite marked functional impairment. *Clin. Endocrinol.* **62**, 343–348 (2005).
64. R. B. Weller, Sunlight has cardiovascular benefits independently of vitamin D. *Blood Purif.* **41**, 130–134 (2016).
65. S. Marino, M. Vooijs, H. van der Gulden, J. Jonkers, A. Berns, Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. *Genes Dev.* **14**, 994–1004 (2000).
66. H. R. Dassule, P. Lewis, M. Bei, R. Maas, A. P. McMahon, Sonic hedgehog regulates growth and morphogenesis of the tooth. *Development* **127**, 4775–4785 (2000).
67. K. Y. Kwan, A. J. Allchorne, M. A. Vollrath, A. P. Christensen, D. S. Zhang, C. J. Woolf, D. P. Corey, TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* **50**, 277–289 (2006).
68. J. S. Mogil, S. G. Wilson, K. Bon, S. E. Lee, K. Chung, P. Raber, J. O. Pieper, H. S. Hain, J. K. Belknap, L. Hubert, G. I. Elmer, J. M. Chung, M. Devor, Heritability of nociception II. 'Types' of nociception revealed by genetic correlation analysis. *Pain* **80**, 83–93 (1999).

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