Beyond Pigmentation: The Role of POMC-Derived Opioid Signaling in Radiation Fatigue, Addiction, and Pain

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Articles closely related to the subject of the thesis

I. **Hermann AL**, Fell GL, Kemény LV, Fung CY, Held KD, Biggs PJ, Rivera PD, Bilbo SD, Igras V, Willers H, Kung J, Gheorghiu L, Hideghéty K, Mao J, Woolf CJ, Fisher DE. β-Endorphin mediates radiation therapy fatigue. Sci Adv. 2022. **IF: 13**

II. Kemény LV, Robinson KC, **Hermann AL**, Walker DM, Regan S, Yew YW, Lai YC, Theodosakis N, Rivera PD, Ding W, Yang L, Beyer T, Loh YE, Lo JA, van der Sande AAJ, Sarnie W, Kotler D, Hsiao JJ, Su MY, Kato S, Kotler J, Bilbo SD, Chopra V, Salomon MP, Shen S, Hoon DSB, Asgari MM, Wakeman SE, Nestler EJ, Fisher DE. Vitamin D deficiency exacerbates UV/endorphin and opioid addiction. Sci Adv. 2021. **IF: 13.934**

III. Robinson KC, Kemény LV, Fell GL, **Hermann AL**, Allouche J, Ding W, Yekkirala A, Hsiao JJ, Su MY, Theodosakis N, Kozak G, Takeuchi Y, Shen S, Berenyi A, Mao J, Woolf CJ, Fisher DE. Reduced MC4R signaling alters nociceptive thresholds associated with red hair. Sci Adv. 2021. **IF: 13.934**

Articles closely not related to the subject of the thesis

I. Mujahid N, Liang Y, Murakami R, Choi HG, Dobry AS, Wang J, Suita Y, Weng QY, Allouche J, Kemeny LV, **Hermann AL**, Roider EM, Gray NS, Fisher DE. A UV-Independent Topical Small-Molecule Approach for Melanin Production in Human Skin. Cell Rep. 2017. **IF: 8.03**

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ABSTRACT

Pro-opiomelanocortin (POMC) is a precursor protein cleaved into multiple peptides, including melanocyte-stimulating hormones (MSH) that regulate pigmentation and β-endorphin, an endogenous opioid involved in pain modulation and reward. While POMC's role in pigmentation is well understood, its broader physiological functions remain unclear.

Fatigue is a common side effect of radiation therapy, yet its mechanisms are poorly understood. Using rodent models, we found that skin-derived β-endorphin increases systemically following radiation, paralleling opioid-like phenotypes such as elevated pain thresholds, Straub tail response, and fatigue-like behavior. These effects were reversed by opioid antagonism and were absent in β-endorphin knockout mice and those lacking keratinocyte p53 expression. These findings suggest that radiation-induced β-endorphin release contributes to fatigue and that opioid antagonists may offer therapeutic potential.

Recent evidence suggested, endogenous opioid signaling influences addiction-related behaviors. Here, we identified a feedback loop where vitamin D deficiency increases UV/β-endorphin-seeking behavior to maximize vitamin D synthesis. However, exogenous opioid use lacks this regulatory feedback, leading to maladaptive reinforcement of opioid dependence. This suggests vitamin D levels may modulate opioid sensitivity and addiction risk.

Finally, we investigated pain sensitivity in red-haired individuals, who exhibit higher nociceptive thresholds and enhanced opioid sensitivity due to melanocortin 1 receptor (MC1R) loss. Our findings indicate that opioid and melanocortin signals antagonize each other in the periaqueductal gray, modulating pain perception.

These studies reveal the broad physiological role **of** POMC-derived opioid signaling in fatigue, addiction, and pain. While POMC is crucial to pigmentation, its systemic effects extend beyond skin color, with implications for cancer therapy, addiction treatment, and pain management.

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List of abbreviations

7-DHC – 7-Dehydrocholesterol

ACTH – Adrenocorticotropic Hormone

ANOVA – Analysis of Variance

BBB – Blood–Brain Barrier

cAMP – Cyclic Adenosine Monophosphate

c-Fos – FBJ Osteosarcoma Oncogene (a marker of neuronal activation)

CREB – cAMP Response Element-Binding Protein

CPP – Conditioned Place Preference

CNS – Central Nervous System

DMSO – Dimethyl Sulfoxide

EDTA – Ethylenediaminetetraacetic Acid

GAPDH – Glyceraldehyde 3-Phosphate Dehydrogenase

HPA – Hypothalamic–Pituitary–Adrenal (axis)

MC1R – Melanocortin 1 Receptor

MC4R – Melanocortin 4 Receptor

MSH – Melanocyte-Stimulating Hormone

OPRM1 – μ-Opioid Receptor 1

p53 – Tumor Protein p53

POMC – Proopiomelanocortin

PBS – Phosphate-Buffered Saline

PAG – Periaqueductal Gray

SEM – Standard Error of the Mean

T-test – Student's t-test

TRP1 – Tyrosinase-Related Protein 1

TRP2 – Tyrosinase-Related Protein 2

Tyr – Tyrosinase

UV – Ultraviolet

UVB – Ultraviolet B

UVR – Ultraviolet Radiation

VDR – Vitamin D Receptor

Vdr−/− – Vitamin D Receptor Knockout (genotype)

VitD – Vitamin D

1. INTRODUCTION

Pro-opiomelanocortin (POMC) is a multifunctional precursor protein integral to various physiological processes in endocrinology but also in skin physiology. Synthesized primarily in the anterior pituitary gland, POMC undergoes post-translational cleavage to produce several active peptides, notably adrenocorticotropic hormone (ACTH) and melanocyte-stimulating hormones (MSH)[1,2]. These derivatives exert diverse effects, ranging from stress response modulation to immune regulation and energy homeostasis. However, in the skin, a well-established function of POMC is its role in pigmentation through the action of its derivative, α-MSH. α-MSH binds to the melanocortin 1 receptor (MC1R) on melanocytes, stimulating the production of eumelanin, the pigment responsible for brown and black coloration in skin and hair[3]. Mutations or polymorphisms in the MC1R gene can lead to altered pigmentation, as observed in individuals with red hair and fair skin, where a shift towards pheomelanin production occurs[3].

POMC in Skin Biology

Beyond its systemic endocrine functions, POMC plays a significant role within the skin itself. Both keratinocytes and melanocytes in the epidermis can produce POMC and its derivatives, including α-MSH and ACTH[4]. These locally produced peptides contribute to melanogenesis and have been implicated in modulating inflammatory responses and promoting DNA repair mechanisms following ultraviolet (UV) radiation exposure[5]. This suggests a protective, autocrine/paracrine role for POMC-derived peptides in maintaining skin homeostasis.

Endocrine and Systemic Roles of POMC

In the endocrine system, POMC-derived ACTH is crucial for regulating the hypothalamic-pituitary-adrenal (HPA) axis, influencing cortisol production and thus modulating stress responses[1,6]. Additionally, POMC neurons in the hypothalamus are involved in energy balance and appetite control, with α-MSH acting on central melanocortin receptors to suppress food intake[7]. Disruptions in POMC processing or receptor signaling pathways have been associated with metabolic disorders, including obesity and adrenal insufficiency[8].

Collectively, these insights underscore the multifaceted roles of POMC-derived peptides across various physiological domains[6]. However, given that POMC-derived peptides may be responsible for multiple physiologic effects, sometimes even antagonistic effects to each other, the role of skin-derived POMC production still poses an important area of research, especially in complex behaviors, in which skin has been proposed to play significant roles, like fatigue, addiction and nociception.

1.1. Radiation induced fatigue and the skin

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[9]. As shown in some of the early longitudinal studies on radiation-related fatigue, patients undergoing radiation typically begin to experience fatigue 3-4 weeks into a 6-8-week regimen[10–12]. 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[11–13]. While fatigue is a subjective phenomenon measured by patients' self-reporting, it's 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[14].

Although a well-described phenomenon, the mechanism(s) causing radiation fatigue remain uncertain. Notably, fatigue is experienced as often in patients receiving tangential field radiation that penetrates only skin and subcutaneous tissue, as in those receiving radiation to deeper structures[15]. 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[16]. 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[12,13,17–19], 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 upregulate p53, which stimulates expression of pro-opiomelanocortin (POMC) that is post-translationally cleaved into bioactive peptides including the pigmentation-inducing hormone α-MSH and the endogenous opioid β-endorphin[20]. Systemic increases in β-endorphin after UV exposure produce opiate-like behaviors and phenotypes[21], indicating systemic β-endorphin effects following local UV

exposure. Opiate drugs can cause sedation, a common symptom associated with fatigue[22,23], 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.

1.2. Vitamin D and its role in skin-endorphin signaling

Opioid use disorder (OUD) is a major medical challenge that is continuing to increase in the United States. On the basis of the National Survey on Drug Use and Health, in 2018, approximately 10.3 million people aged 12 or older had misused opioids in the past year, and 2 million had an OUD[24]. Abatement of the crisis will require more than a singular focus on opioid prescriptions and must include rapid expansion of effective treatments, including pharmacologic therapies for OUD, harm reduction interventions, and alleviation of social and economic determinants such as physical and psychological trauma and diminishing employment opportunities and life satisfaction[25]. Therefore, causative but preventable environmental factors that contribute to opioid addiction are of great interest[26].

Human studies have suggested that ultraviolet (UV) tanning may be addictive[27,28], exhibiting characteristics highly reminiscent of opioid addiction[29]. Recent preclinical data have identified an endogenous opioid-mediated addiction-like pathway triggered by UV-induced cutaneous synthesis of β-endorphin[21]. Maintenance of UV-dependent vitamin D (VitD) synthesis has been suggested as a driver for the evolution of light skin pigmentation[30]. Therefore, we hypothesized that endorphin mediated UV-seeking behavior might be driven by VitD deficiency to maximize VitD synthesis. A possible consequence would be that VitD deficiency might also sensitize individuals to exogenous (UV-independent) opioids, contributing to opioid addiction. Therefore, a negative feedback loop might exist whereby UV/opioid-seeking behaviors are repressed when VitD levels are restored. This feedback might carry the evolutionary advantage of maximizing VitD synthesis. However, unlike UV exposure, exogenous opioid use is not followed by VitD synthesis (and its opioid suppressive effects), contributing to a maladaptive addictive behavior cycle.

1.3. Red hair and nociception

Humans and mice with red hair exhibit altered pain thresholds, increased nonopioid analgesic requirements, and enhanced responses to opioid analgesics[31–36]. Red hair in both species is caused by loss-of-function variant alleles of the melanocortin 1 receptor (MC1R), a $G\alpha_s$-coupled receptor expressed on melanocytes, the pigment-producing cells of the skin[37]. MC1R mutant red-haired mice are less sensitive to noxious thermal, mechanical, and chemical stimuli[34]. However, the mechanism of this altered nociception has not been determined, prompting us to examine the mechanistic connection between MC1R and the modulation of nociception.

MC1R function is suppressed in the *Mc1r*$^{e/e}$ mouse strain because of a frameshift mutation resulting in a premature stop codon. This strain (referred to herein as red-haired mice) has yellow/red hair and recapitulates features of red-haired humans including synthesis of red/blond pheomelanin pigment, inability to tan following UV exposure, and increased ultraviolet (UV)–associated skin cancer risk[38,39].

Given suggested earlier evidence regarding altered nociception in red-haired genetic background, we hypothesized that endogenous opioid and melanocortin signaling may be altered in *Mc1r*$^{e/e}$ mouse, resulting in higher nociceptive thresholds.

2. METHODS

2.1. Methods for irradiation-induced fatigue study

Animals

All mice used were 8-week-old males in a C57BL/6 background (Jackson Laboratories, Bar Harbor, ME). Additionally, we used mice lacking the C-terminal end of the POMC polyprotein due to a mutation in both copies of the POMC gene, resulting in lack of β-endorphin[40], to test for changes in pain thresholds with tail radiation. To ablate keratinocyte specific p53 expression, mice with floxed alleles of p53[41] were crossed with a strain expressing Cre recombinase driven by the Keratin 14 promoter[42]. 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 littermates 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 (Jackson Laboratories, Bar Harbor, ME) were used. All animals were maintained on a 12-hour light/dark cycle and were acclimated to the vivarium for at least 1 week prior to 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. 3). All experiments were performed with approval from the Massachusetts General Hospital Institutional Animal Care and Use Committee.

Irradiators

Mouse tail irradiation was performed using a 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 an HVL 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 mmCu 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 (lead shield minimal wall thickness was 1.9 cm). Animals underwent 5 Gy/day ionizing radiation to the tail 5 days per week (Monday – 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 2 Gy/day tail X-irradiation, 5 days per week (Monday - Friday) for 6 weeks. Individual irradiation did not last for more than 2 minutes 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 uL blood was collected from the tail vein into EDTA-containing microvette tubes with 0.6 TIU aprotinin. For certain experiments blood was taken submandibularly. Samples were immediately placed on ice after collection. Samples were centrifuged at 3500 RPM for 20 minutes at 4^{o}C and plasma was collected into separate tubes and stored at –80^{o}C until measurement of β-endorphin. Blood was collected once per week on Fridays in the morning prior to 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 beta-endorphin knockout mice.

Straub Tail Measurement

Straub Tail was measured as previously described[43]. The scoring system was a scale of 0-2 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^{o} ; 1.5 = tail rigid and elevated 11^{o} to 45^{o}; and 2 = tail rigid and elevated 46^{o}-90^{o}). For mice, individual Straub tail values were obtained by averaging 6 Straub score measurements taken in 60 seconds. Straub tail score was calculated similarly for rats, with the slight modification of obtaining individual values by averaging 3 measurements in 30 seconds.

Analgesic Threshold Testing

Mice underwent mechanical threshold testing in the von Frey test[44] and the hot plate test as previously described. In the von Frey test, animals were placed on an elevated wire mesh grid in individual enclosures and acclimated for 30 minutes. 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 out 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^{o}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 i.p. with either 10 mg/kg or 1 mg/kg naloxone hydrochloride (Sigma, St. Louis, MO) or with normal saline prior to 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 8am - 7pm. 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” x 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 experiments displayed in Figure 2, 3 Ethovision XT 9 was used to analyze the open field measurements. For experiment shown in Figure 2 animals were injected with either 5

mg/kg methynaltrexone or with saline, then were kept in their home cages for 30 minutes. Then animals were tested for 30 minutes in the chamber.

Statistics

Prism 8.0 was used for statistical analyses. Repeated measures two-way ANOVA with the Holm-Šídák multiple comparisons test was used to analyze the experiments, as well as a two-tailed, unpaired T test (Fig. 1a).

2.2. Methods used in the vitamin D project

Mice and diets

All mice used in this study were on a C57BL/6 background. *Vdr*$^{-/-}$ mice were provided by M. Damay[46]. All mice were habituated to the holding room for at least a week before starting experiments. On the day of the behavior experiments, mice were moved and habituated to the procedure rooms for at least 30 min before starting experiments.

Experiments and analyses were carried out blindly where it was possible. All animals were used in the study, except for animals that suffered injuries that precluded them from providing data points thus were not included in any measurements. All studies and procedures involving animal subjects were performed in accordance with policies and protocols approved by the Institutional Animal Care and Use Committee at Massachusetts General Hospital.

UV irradiation

Mice were dorsally shaved 1 day before the start of radiation exposure. Mice were reshaved if there were patches of fur regrowth, once every 2 weeks. To assess UV-induced analgesia, baselines were acquired before UV exposure. Then, mice were exposed daily to 50 mJ^2 of UVB (using G15T8E UVB bulbs with peak emission of 305 to 310 nm) in the afternoon hours, and analgesia was assayed as described below. For UV-CPP, mice were exposed to 50 mJ/cm^2 per day of UVB (an empirically determined sub-erythematic dose) for 5 days, followed by 25 mJ/cm^2 per day of UVB 5 days/week (Monday to Friday) for 2 weeks. UVB was then applied every day (Monday to Sunday) in incrementally increasing doses (+5 mJ/cm^2 every 4 days) until reaching 50 mJ/cm^2, followed by 50 mJ/cm^2 every day for 1 week.

UV CPP testing

Procedures were followed as described[21]. Briefly, the apparatus used for CPP assays consisted of a chamber with black interior and dim lighting and a chamber with white interior and bright lighting, connected by a smaller gray "neutral" chamber. Before baseline preference assessments, mice were pretreated with UVB as indicated above. Baseline place preferences before conditioning were assessed by placing the mice in the neutral chamber and recording the times spent in the white chamber over the next 10 min. On the subsequent 8 days, conditioning took place in which mice were exposed to either UVB (50 mJ/cm^2) or mock UV 15 min before placing them in the white chamber, and all animals were exposed to mock UV 15 min before placement in the black chamber. Conditioning time in each chamber was 30 min. For each mouse, there were at least 4 hours between the two conditioning sets. A day after the last conditioning, place preferences were tested again for 10 min.

Quantitative polymerase chain reaction and western blot of brain samples

To obtain nucleus accumbens and VTA samples, mice were intraperitoneally injected with morphine (5 mg/kg) or saline and were cervically dislocated at different time points (30, 60, 90, or 180 min) after injections. Mice receiving saline injections were euthanized 60 min after injection and were used as control groups for all morphine-treated groups. Brains were then immediately flash frozen in isopentane and stored in −80°C.

Punches were taken from coronal sections of the mouse brain for both NACC and VTA. For NACC, the *aci* and *fmi* white matter structures were used as landmarks to determine Bregma 1.94. Bilateral punches with a diameter of 1.5 mm were then taken 2 mm in depth with *aci* at the center of the punch, to ensure that both the accumbens core and shell were within the punch. To reach the VTA, coronal sections were taken until white matter structures of the cingulum (*cg*) were rounded and the dentate gyrus of the hippocampus began to form ventrally and caudally, as observed at Bregma of −2.92 mm. Bilateral punches with a diameter of 1.5 mm were then taken at ~1.0 mm in depth at a location of 0.5 mm lateral and from the midsagittal plane and 0.5 mm from the most ventral portion of the brain. Location of punches is displayed in Fig. 8. RNA and proteins were then isolated as recently published[47].

2.3. Methods to study nociception in the red haired genetic background

Mice

All experiments were performed with male mice at least 8 weeks of age. Mice were bred on site. All experiments were performed with approval from the Massachusetts General Hospital Institutional Animal Care and Use Committee. All mice used were on the C57BL/6J background or had been backcrossed to the C57BL/6J background at least 10 generations. *Mc1r*$^{e/e}$ mice were obtained from The Jackson Laboratory[48]. Mice were used for single experiments, except the same groups of mice were used for measuring thermal and mechanical nociception and for the effects of different pharmacological compounds.

Drugs

Naltrexone hydrochloride (N3136), and melanotan II acetate salt (M8693) were purchased from Sigma-Aldrich; SHU 9119 (NC9447656), naloxonazine hydrochloride (059110), and DAMGO (11711) were from Thermo Fisher Scientific; and methylnaltrexone bromide (787933) was from McKesson. All drug administrations to mice were by intraperitoneal injection or intrathecally, when specified..

Nociceptive testing

The hot plate assay was performed as originally described[49] on a 52°C hot plate with a maximum cutoff time of 20 s.

PAG cannulation

For cannulation, mice underwent isoflurane anesthesia. Cannulas were positioned to target the ventral lateral PAG (coordinates used bregma −4.5 mm and lateral 0.5 mm) with a custom-made infusion system (P1 Technologies, VA, USA). Custom-made guide cannulas were cut at 2.2-mm length. For intra-PAG injections, an internal cannula of 2.5-mm length was used to administer phosphate-buffered saline (PBS), naloxone, or melanotan under isoflurane anesthesia in 2-µl volume. Nociceptive thresholds were then measured using the hot plate assay. The same animals were used to test all pharmacologic agents for this experiment.

cAMP measurements

RPHNs (Lonza, R-HTH-507) were seeded in poly-D-lysine– and laminin-coated wells at a density of 50,000 cells per well. RPHNs were cultured in primary neuron growth medium

(Lonza, CC-3256) containing growth supplements (Lonza, CC-4462). Medium was changed every 48 to 96 hours. On day 11 after seeding cells, serum starvation was performed for 30 min, and then cells were treated first with either 1 μM DAMGO or PBS for 7.5 min and then with either 1 μM [Nle4,D-Phe7]-α-MSH (M8764, Sigma-Aldrich) or water (vehicle control) for 10 min. Cells were incubated at 37°C during treatments. Ten minutes after [Nle4,D-Phe7]-α-MSH or water treatment, cells were lysed and processed for cAMP measurements with cAMP-GLO Assay (V1501, Promega, WI, USA) according to the manufacturer's instructions.

Statistics

For pairwise comparisons in the nociceptive assays and serum peptide measurements, two-tailed *t* tests were used. For the cAMP experiment and MSH time course experiment, one-way analysis of variance (ANOVA) was used with Dunnett's multiple comparison test. For all other experiments, two-way ANOVA with Sidak's multiple comparison test was used. For all statistical tests, Prism version 7.0a for Mac OS X (GraphPad Software, La Jolla, CA, USA) was used.

3. RESULTS

In order to introduce all projects in a meaningful manner in this Results section, I am citing a few earlier results that were also published in my co-first-authored manuscript, but I did not incorporate the Figures of those results in this Thesis, as they were done by my colleague and co-first Author, Dr. Gillian Fell. However, referring to those earlier results are important in order to fully evaluate all experiments and figures in this Thesis that were done by me.

3.1. Skin β-endorphin can trigger radiation-induced fatigue

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

Earlier, we initially asked whether β-endorphin levels increase systemically in response to tail irradiation treatments[50]. In this initial model, rats received 2 Gy/day ionizing radiation 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 after the start of daily tail irradiation and returned to baseline one week after the termination of irradiation. Plasma β-endorphin did not significantly increase during mock irradiation, suggesting that the technical procedures required to administer irradiation did not induce endorphin elevation through triggering a stress response.

Also earlier, we tested whether this radiation treatment protocol may produce fatigue-like symptoms, as a model for the human condition[50]. We observed that, following irradiation, rats became progressively sedate, as quantified by movement measurements calculated using open field actimetry. While other tests of fatigue and sedation in rodents exist, we chose open field actimetry[51] as a surrogate of fatigue-like behavior because, unlike other comparable tests[52,53], 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 endpoints over the required time period.

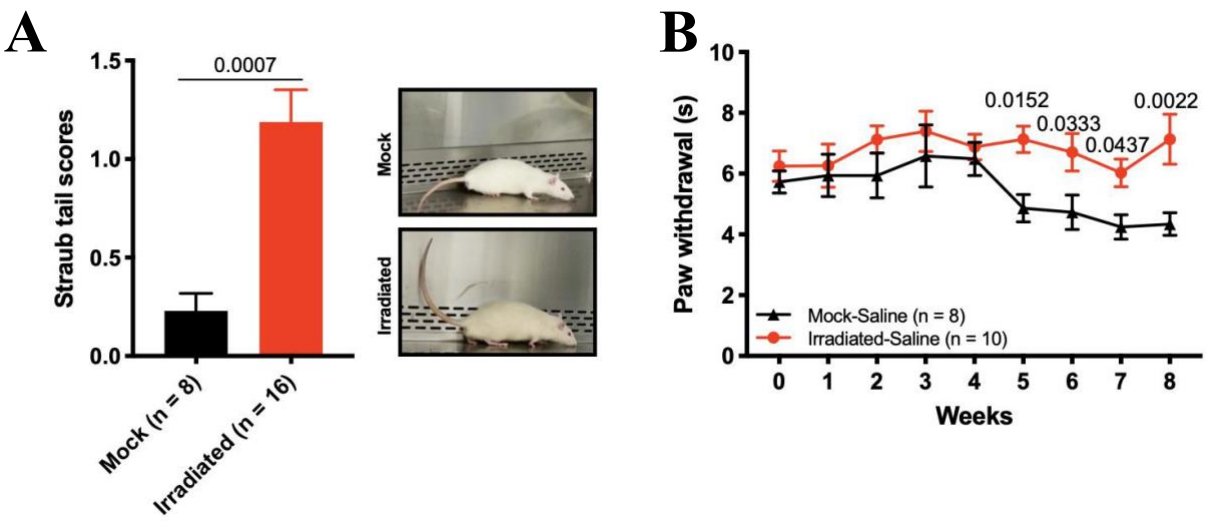


Figure 1. Tail radiation increases induces opiate phenotypes in rats . *Sprague-Dawley rats underwent 2 Gy/day tail radiation or mock treatment 5 days/week for 6 weeks. Error bars in the figure indicate +/−standard error of the mean (SEM) numbers in brackets indicate numbers of animals per group.* ***a)*** *Straub tail reaction is observed after 5 weeks of tail radiation (bottom picture), but not in mock-irradiated animals (top picture). Value of significance was determined by two-tailed unpaired t-test.* ***b)*** *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.*

To test the opioid dependency of this fatigue-like phenotype, earlier we pre-treated rats with intraperitoneal administration of 10mg/kg naloxone prior to actimetry testing[50]. Despite maintained radiation-induced increases in plasma β-endorphin, 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. Together these data suggested 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[21] 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[54] and Straub tail[43], 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. 1a) as well as an elevation in nociceptive threshold (Fig. 1b). Although stress has been shown previously to trigger opioid dependent Straub-tail phenomenon in rats [55], we have observed a difference between mock treated and irradiated rats, suggesting the direct role of irradiation in Straub tails.

Given the requirement for peripheral β-endorphin synthesis in these opiate-like phenotypes[21], we investigated the role of the peripheral nervous system in fatigue-like behavior. As earlier we observed that the blood-brain barrier-permeable antagonist naloxone can prevent fatigue-like behavior in rats[50], 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 endpoints (velocity, time spent not moving and distance traveled) (Fig. 2a, b, c), suggesting that central opioid signaling is critical for mediating fatigue-like behavior.

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.

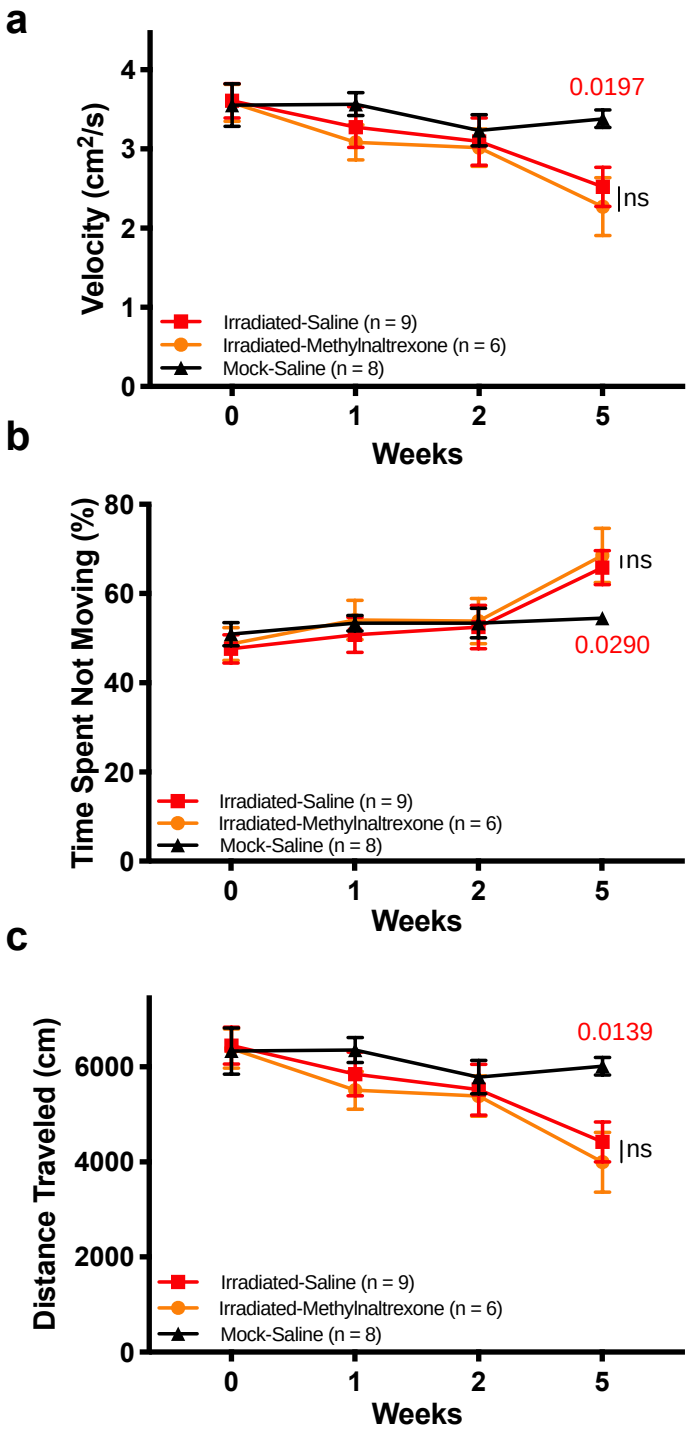


Figure 2. Chronic tail irradiation changes multiple endpoints of rat locomotion. *Using the open field, additional endpoints of fatigue-like behavior were measured in rats.* ***a)*** *velocity decreased,* ***b)*** *time spent not moving increased and* ***c)*** *total distance travelled decreased in irradiated rats injected i.p. with saline or 5 mg/kg methylnaltrexone compared to rats undergoing mock radiation. Two-way ANOVA with the Holm-Šídák multiple comparisons test revealed significant differences between groups that are indicated above the graphs. Error bars indicate +/– SEM. Red numbers indicate differences between Irradiated-Saline and Mock-Saline groups.*

Plasma β-endorphin elevations in tail-irradiated mice.

To elucidate the underlying mechanism behind radiation-induced elevations in blood β-endorphin, we utilized several genetic mouse models. Mice exhibit paradoxical hyperlocomotion responses to opiates (Fig. 3), in line with prior observations[56–62], in contrast to rats[63] 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, prior observations that β-endorphin does not change locomotor behavior in mice[64].

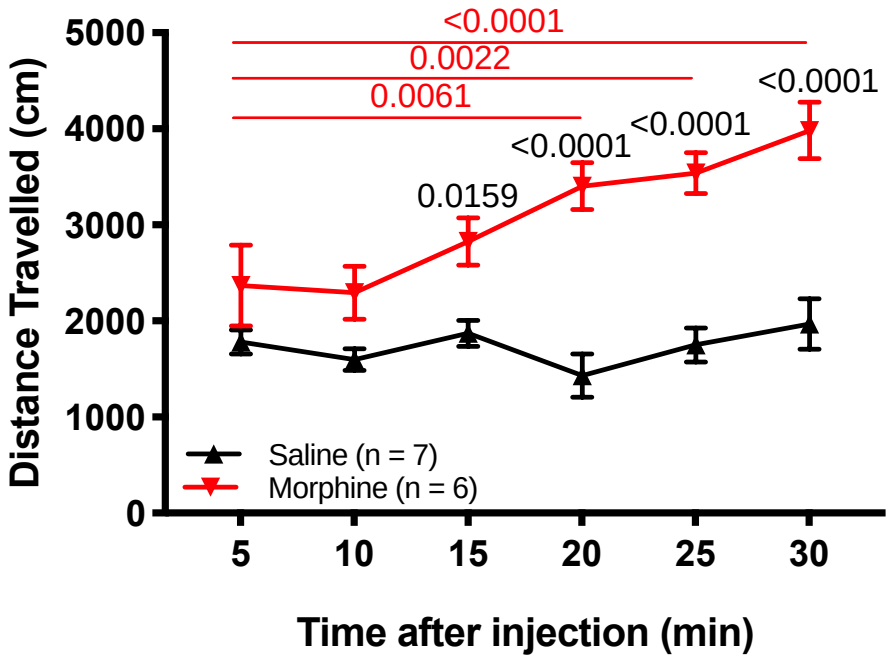


Figure 3. Morphine increases locomotion of C57BL/6 mice. *Mice were injected i.p. with 10 mg/kg morphine or saline and placed into an open field chamber. Distance travelled was monitored in 5-minute bins. One morphine-treated mouse exhibited freezing behavior after injection, likely representing a potentially misplaced injection. The values for this mouse in open field testing suggested it to be an outlier by ROUT-test, and its data points were thus removed from the experiment. Two-way ANOVA with the Holm-Šídák multiple comparisons test revealed significant differences between groups that are indicated above the graphs. Black numbers indicate the p value for differences between the Saline and Morphine groups, while red numbers indicate the p value for comparing the timepoint to the first 5 min bin in morphine treated animals. Error bars indicate +/− SEM.*

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 previously treated mice with daily tail irradiation (with body shielding similar to the rat studies above) and switched the 2 groups reciprocally between mock irradiation and ionizing radiation after 6 weeks[50]. Plasma β-endorphin increased significantly two weeks after the start of daily tail irradiation and returned to baseline one week after the transition from tail irradiation to mock irradiation. 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 one week of tail irradiation. Tail irradiation-mediated increases in β-endorphin were greater in these mouse experiments than in the rat studies[50]. 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 upregulation of POMC and POMC-derived peptides (as in response to UV exposure[20,21]), we observed increased local pigmentation in irradiated skin areas, but not in non-irradiated skin areas or in mock-irradiated animals (Fig. 4).

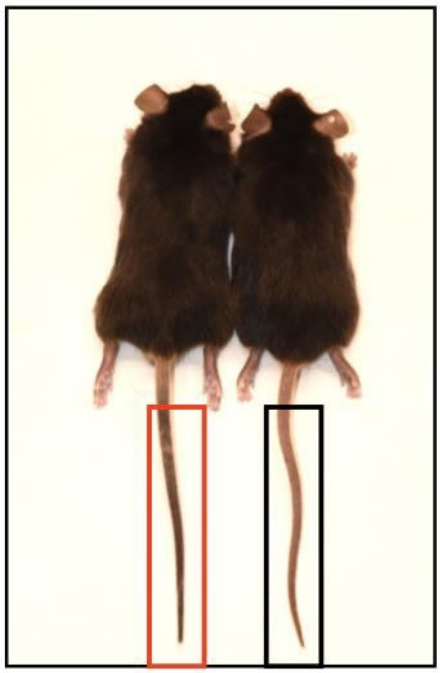


***Figure 4**. **Tail ionizing radiation exposure in mice increases pigmentation in mice.** Increased local pigmentation of the tail (radiation-exposed area) in a tail-irradiated mouse after 6 weeks of ionizing radiation (left), while no pigmentation is observed on the tail of a mock-treated mouse (right).*

Although, we have seen consistent hyperpigmentation in mice tails upon radiation (Fig. 4)., we did not observe hyperpigmentation of rat tails upon radiation (Fig. 5), because Sprague-Dawley rats carry a mutation in the tyrosinase gene that makes them albino[65]. This missense mutation (R299H) is conserved across all albino rat strains[62,66] and has been described in patients with oculocutaneous albinism type I[67]. Due to 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 which, like UV radiation, can trigger p53-mediated downstream effects[68,69].

Figure 5. Sprague-Dawley albino rats do not show hyperpigmentation in response to irradiation. *Rats underwent 8 weeks of 5 days/week tail irradiation (2 Gy/day) or mock irradiation. The Figure uses animals from the experiment shown in Figure 1a, b.*

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

Earlier, we asked whether the observed increases in plasma β-endorphin are functionally significant in mice[50]. Similar to our findings in rats, we had observed that tail-irradiated mice exhibit the Straub tail sign. The sign was noticeable within four 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 seven, (cross-over design). In irradiated animals, Straub tail was reversed by administration of naloxone, but not by saline, suggesting involvement of an endogenous opioid pathway[50]. While these studies utilized male animals, we separately compared tail irradiation effects on female mice and observed identical effects.

Mechanical (von Frey assay) and thermal (hot plate assay) pain sensitivity were also measured during the tail radiation regimen in earlier studies[50]. 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. These changes paralleled increases in plasma β-endorphin levels. Tail-irradiated mice treated with the opiate antagonist naloxone prior to pain threshold testing session showed no increases in pain thresholds despite increases in plasma 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, we examined β-endorphin null mice[40] using the tail radiation regimen. As shown in Figures 6a and 6b, 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. 6c). These studies suggest that radiation-induced increases in β-endorphin produce changes in opioid receptor dependent phenotypes.

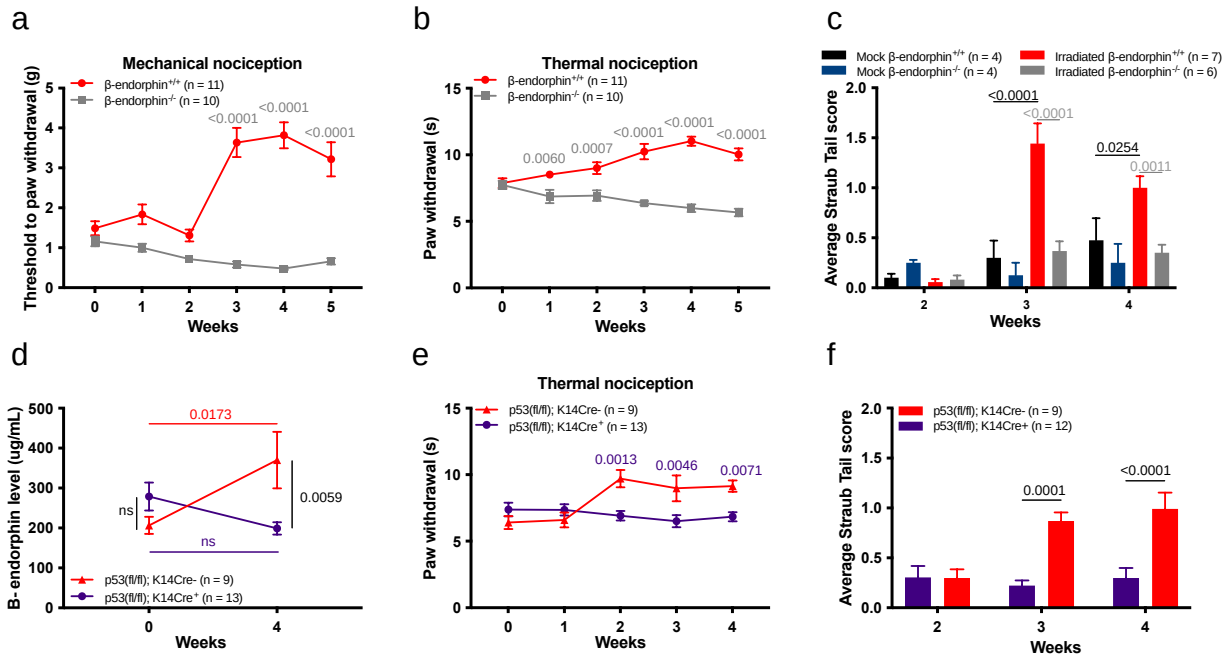


Figure 6. Radiation-induced Straub tail and elevated nociceptive thresholds depend on β-endorphin and on keratinocyte-specific p53 expression. a) *Mechanical (von Frey assay) and* ***b)*** *thermal (hot plate assay) analgesic thresholds in β-endorphin wild type and β-endorphin null mice over 5 weeks of 5 Gy/day tail radiation 5 days per week.* ***c)*** *Straub tail was observed starting 3 weeks after irradiation in β-endorphin wild type, 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 5 Gy/day tail radiation 5 days per 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.*

Since upregulation of POMC and production of cutaneous β-endorphin in response to UV exposure has been shown to be p53-dependent[20,21], 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. 6d), 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. 6e) and did not display Straub tail (Fig. 6f). 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.

3.2. Vitamin D deficiency drives UV seeking behavior

VitD deficiency increases UV radiation–induced reward

We asked whether VitD deficiency might sensitize to physiologic endogenous opioid signaling in response to UV radiation. Because the addiction-like effects of UV were previously shown to be mediated by endorphin/opioid signaling[21], we anticipated that the VDR$^{-/-}$ state would derepress opioid/CPP responses. We used *Vdr*$^{-/-}$ mice to ablate effects of UV-induced de novo cutaneous VitD synthesis. We examined whether VitD deficiency might alter UV-seeking behavior. Mice were conditioned with either UV or mock radiation in a CPP apparatus and tested for place preference (Fig 7A). UV exposure induced a strong CPP in the *Vdr*$^{-/-}$ mice, in contrast to a minimal and insignificant trend toward preference in *Vdr* wild-type mice (Fig. 7B). UV has numerous effects that are independent of VitD; it is notable that the UV responses observed here in *Vdr*$^{-/-}$ were phenocopied by morphine treatments in VitD deficient as well as *Vdr*$^{-/-}$ mice[70], suggesting that the CPP effects were opiate/opioid mediated. These results suggest that the lack of VitD signaling sensitizes individuals to the rewarding effects of UV, in line with an adaptive feedback loop in which deficiencies in VitD signaling increase UV/opioid reward to maximize VitD synthesis, whereas correcting VitD signaling restores normal sensitivity to UV (illustrated in Fig. 7C).

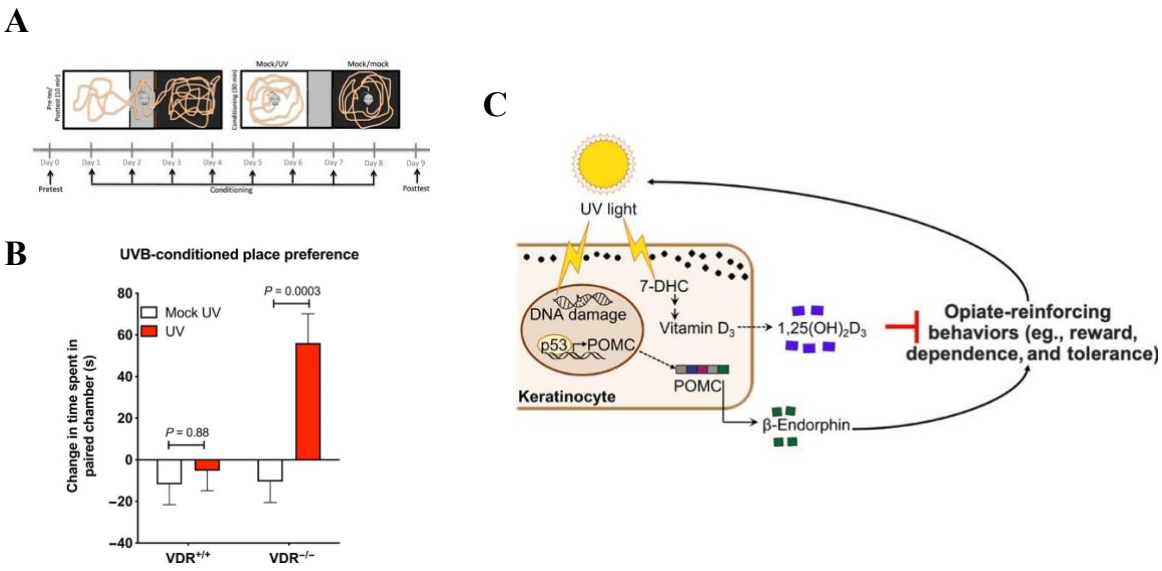


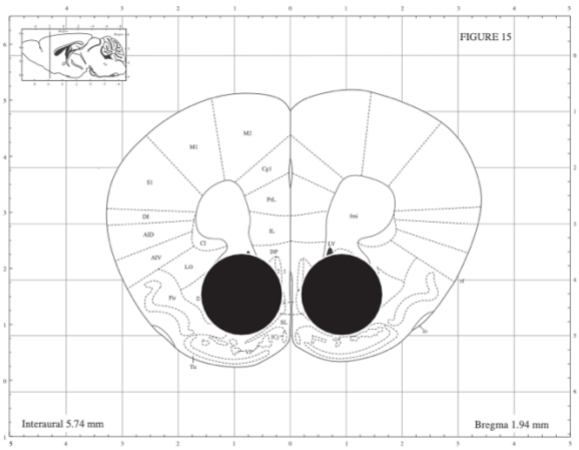
Fig. 7 The absence of VDR signaling increases UV radiation–induced reward.

*(**A**) Mice were conditioned to UVB (n = 12 and 9, for $VDR^{+/+}$ and $VDR^{-/-}$, respectively) or mock UV treatment (n = 12 and 11, for $VDR^{+/+}$ and $VDR^{-/-}$, respectively) in the white chamber and to mock UV in the black chamber, and place preferences were evaluated as change in time spent in the white chamber postconditioning versus preconditioning. (**B**) VDR represses chronic low-dose UV radiation–induced place preference. Data are represented as the means ± SEM, P values were obtained by two-way ANOVA with Sidak's multiple comparisons test. *P < 0.05 compared with the corresponding saline group. (**C**) Model depicting UV radiation–induced behavior changes that are inhibited by a negative feedback loop by VitD synthesis. POMC, proopiomelanocortin. 7-DHC, 7-Dehydrocholesterol. **P <0.01.*

Opioid-induced c-fos transcription in the nucleus accumbens is repressed by VDR

We hypothesized that if vitamin D deficiency sensitizes to opioid reward, then an increased neuronal activation may be present in central nervous system regions associated with rewarding behavior. Therefore, we investigated the kinetics of morphine-induced c-Fos mRNA and protein levels in the nucleus accumbens, where c-Fos has been shown to be required to elicit opioid reward[71]. For these experiments, we have obtained micropunches from frozen brain tissues obtained from mice treated with saline or morphine i.p. (Fig. 8.).

NACC, Bregma 1.94 mm, depth 2 mm



VTA, Bregma -2.92 mm, depth 1.0 mm

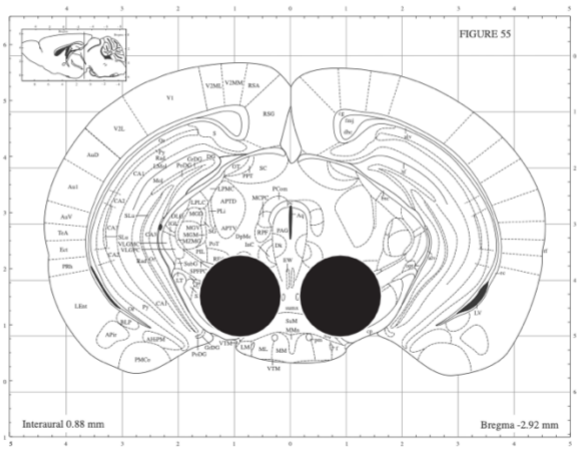


Fig. 8. Location of micropunches.

We found that maximal morphine induction of *c-Fos* mRNA was significantly higher in *Vdr*$^{-/-}$ mice compared with wild-type animals 30 and 60 min following injection (Fig. 9A). The greater *c-Fos* mRNA induction was accompanied by higher induction of c-FOS protein expression in *Vdr*$^{-/-}$ mice 90 min after morphine administration (Fig. 9B, C). The increased expression of *c-Fos* mRNA and protein suggest increased activity of the nucleus accumbens in response to morphine. Because of relatively low expression of VDR in RNA-seq data obtained from VTA, coupled with its very low/absent expression in the nucleus accumbens in human brain samples[70], these data suggest that VDR regulates morphine-induced nucleus accumbens activity via a noncell-autonomous pathway, such as increased activation of input to the nucleus accumbens.

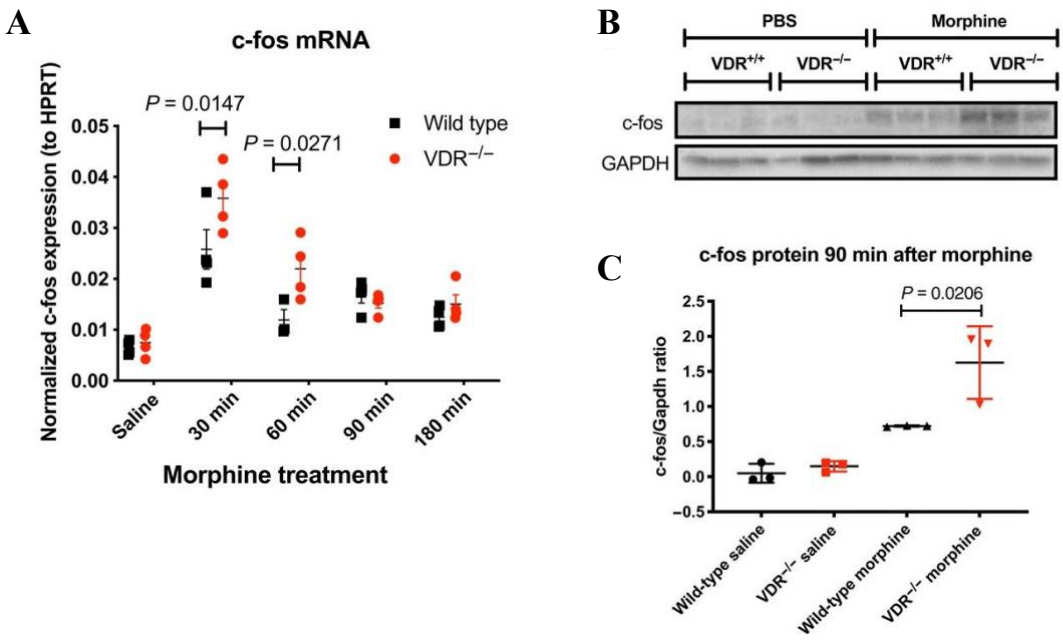


Fig. 9. Morphine dependence and morphine-induced accumbal c-fos expression is greater in the absence of VitD signaling.

*(**A**) Morphine induced significantly higher c-Fos mRNA induction in the nucleus accumbens in Vdr$^{-/-}$ mice compared with wild-type mice. HPRT, hypoxanthine-guanine phosphoribosyltransferase. (**B** and **C**) Morphine-induced c-Fos protein expression is significantly higher in Vdr$^{-/-}$ mice compared with wild-type mice. Data are represented as the means ± SEM; P values were obtained by two-way ANOVA with Sidak's multiple comparisons test. *P < 0.05 compared with the corresponding saline group. GAPDH, glyceraldehyde phosphate dehydrogenase. **P < 0.01 and ****P < 0.0001.*

3.3. MC1R signaling alters central nociception in the periaqueductal grey area

Central opioid dependent nociceptive thresholds are observed in redhaired mice

First, we have used in *Mc1r*$^{e/e}$ and *Mc1r*$^{wt/wt}$ (C57Bl6/J) mice to demonstrate that MC1R signaling deficiency results in increased nociceptive thresholds (compare pre-naltrexone groups on Fig. 10). First, we asked whether the increased thresholds are opioid dependent and if such dependency exists, whether the opioid modulation of nociception is central or peripheral. The nociceptive difference observed between black and red-haired mice was diminished after peripheral (intraperitoneal) administration of naltrexone, an opioid receptor antagonist that is able to cross the blood-brain barrier (BBB) (Fig. 10A). However, peripheral administration of methylated naltrexone, a BBB-impermeable opioid antagonist, did not diminish the nociceptive differences between black and red-haired mice (Fig. 10B), suggesting minimal peripheral influence. This suggests that the relative increase in opioid signaling in red-haired mice occurs centrally, not peripherally, and suggests the possible role of α-MSH in balancing opioid receptor–mediated regulation of central nociception.

Previous studies have demonstrated a role for cAMP signaling in modulating opioid analgesia[72]. We therefore measured the impact of antagonism between melanocortin and opioid signaling on cAMP content in rat primary hypothalamic neurons (RPHNs). We observed that the melanocortin agonist [Nle4,D-Phe7]-α-MSH increased cAMP content, whereas the opioid agonist morphine significantly inhibited melanocortin-induced cAMP elevation (Fig. 10C), consistent with previous observations[73]. These data suggest that melanocortin and opioid signaling may antagonize each other in a cell-autonomous manner.

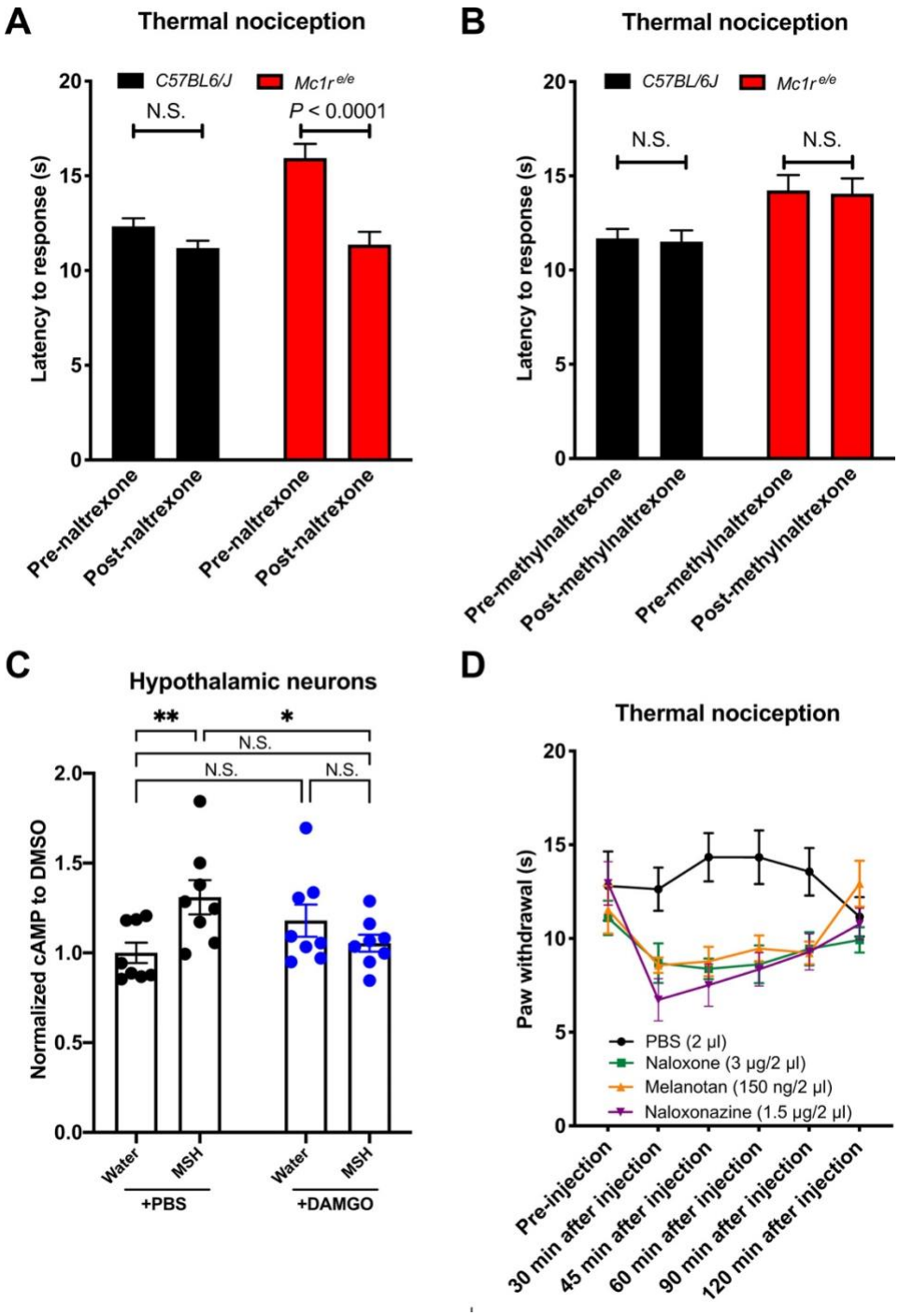


Fig. 10. Increased central opioid tone regulates basal nociception in red-haired mice. *Blood-brain barrier (BBB)–impermeable methylnaltrexone (10 mg/kg) cannot reverse increased nociception in red Mc1r$^{e/e}$ mice when injected peripherally (**A**) (n = 20 for all groups), whereas BBB-permeable naltrexone (10 mg/kg) can (**B**) (n = 20 per group), suggesting the involvement of CNS regions in the increased nociception. (**C**) In line with the coexpression of MC4R and OPRM1, melanocortin and opioid signaling can antagonistically modulate cAMP levels in primary rat hypothalamic neurons. DMSO, dimethyl sulfoxide. (**D**) Local administration of naloxone, naloxonazine, or melanotan to the periaqueductal gray area (PAG) reduces nociceptive thresholds in red Mc1r$^{e/e}$ mice (n = 9 using the same mice for all treatments). (**E**) Summary of the antagonistic opioid-melanocortin model in black and red-haired contexts. Graphs show means ± SEM; numbers in parentheses = n per group.*

Central opioid-melanocortin antagonism determines nociceptive thresholds in redhaired mice

Costaining MC4R-GFP (green fluorescent protein) neurons with OPRM1 in mice has suggested the existence of neurons expressing both receptors in the periaqueductal gray area (PAG)[74]. We investigated the potential role of the PAG in modulating nociception and found that local antagonism of opioid receptors by naloxone, by the μ-opioid receptor–specific naloxonazine[75], or agonism of melanocortin receptors (independently of *Mc1r*) in PAG-cannulated $Mc1r^{e/e}$ mice significantly decreases nociceptive thresholds (Fig. 10D). These results are consistent with the possibility that melanocortin and opioid signaling may antagonize each other in a cell-autonomous manner. However, it is also possible that other indirect or non–cell-autonomous mechanisms may additionally contribute to increased opioid tone in red-haired $Mc1r^{e/e}$ mice. For example, heterodimerization of G protein–coupled receptors can affect receptor function[76] and OPRM1 has been previously shown to interact with MC3R[77]; therefore, it is possible that formation of different heterodimers might also contribute to the increased opioid tone.

The data presented here suggest that elevated nociceptive thresholds found in the red-haired genetic background arise from a reduction in α-MSH levels caused by decreased POMC production in melanocytes, resulting in diminished MC4R signaling. Lower MC4R signaling, in turn, decreases its antagonism of opioid signaling within the CNS, which, despite diminished β-endorphin production, exhibits no discernible differences in other endogenous opioid ligands in the red hair background. Collectively, this produces a net melanocortin deficiency relative to opioid signaling, which alters the balance in favor of μ-opioid receptor–induced analgesia within the red hair background.

4. DISCUSSION

4.1. Radiation therapy induced fatigue and the role of skin

This study provides mechanistic insight into a significantly debilitating side effect of cancer radiation therapy[50]. 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[78,79], but not others[80]. Additional research has suggested that radiation-induced fatigue may arise from the emotional and psychological toll of having cancer[81], however a correlation between presence of clinical anxiety or depression and fatigue has not been consistently observed[80]. 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[82–85] , as this model was utilized not to explain a unifying mechanism behind radiation induced fatigue, but to demonstrate a novel 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 in animal models. Prior studies investigating peripheral irradiation in mice investigate a likely different mechanism that is supported by (1) different irradiation protocols and (2) 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[86–88], 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 which 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 [16], which is in line with our observations that skin-derived signal mediate fatigue-like behavior.

Importantly, 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 timeframe (days) and the fatigue-like behavior is observed quite rapidly within days of the last radiation[82,84] - 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 build up 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 prior 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 [16]. 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 b-endorphin[20,21]. Additional variables regulating plasma endorphin levels (circadian rhythm, stressors from blood draw, UV exposure, etc.) might make it difficult to measure radiation induced endorphin elevations in the plasma in man, similar to UV radiation[21]. 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 of the lack of demonstrated endorphin elevation upon tanning[89,90], naloxone has been shown to elicit behavior effects[29,91].

Fatigue-like symptoms have been measured by running wheel[52], treadmill[92], and open field[51,93–98] experimental methods. While all three assays are potential indicators of fatigue, we utilized 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 utilized a variety of genetic models to elucidate the underlying mechanism connecting ionizing radiation to upregulation 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[20,21]. 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[20] and the addiction-like effects of UV radiation[21]. 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[56].

Previously, the role of cancer cells and cell death-induced changes in fatigue-like behavior have been investigated [99]. Some studies have implicated the role of cytokines released by cancer cells in promoting fatigue in rodents and in humans [78,79], but in some there was no evidence for the role of systemic inflammation [80]. 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 [100,101]; however, their role in regulating behavior warrants further investigation. In this manuscript, we attempted to de-couple cancer-derived signals from skin-derived signals, due to prior observations that the exposed skin area is correlated with the development of fatigue. Indeed, 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 beta endorphin ultimately act through the 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 DRG neurons. In that case, the chronic beta 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[21] 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[102], although this point remains debated[103] 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. It should be noted that in humans UV exposure has been associated with greater levels of outdoor activity, extended life expectancy, decreases in multiple causes of mortality independently of vitamin D [104], 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 endpoints shortly after injections, naltrexone might be a better choice clinically due to its longer duration of action as well as oral administration. Patients who require opiates for pain management would clearly 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.

4.2. The role of vitamin D in promoting UV/endorphin seeking behavior

Evolutionarily, developing addiction to ultraviolet B (UVB) radiation, a ubiquitous carcinogen whose effects manifest mainly in postreproductive years, could increase fitness of a population if accompanied by positive effects in childhood or early adulthood. The detrimental health effects of VitD deficiency have been suggested to contribute as an evolutionary driver for light skin pigmentation in humans[30], and it is plausible that additional mechanisms, like UV-seeking behavior, may further contribute to maintenance of VitD levels in humans and other species. Panther chameleons optimize natural sunlight exposure by fine-tuning basking behavior, depending on their VitD status[105]. VitD deficiency–associated modulation of endogenous opioid sensitivity and reward might have evolved to promote sun-seeking behavior that replenishes VitD levels that are essential during development and growth, despite late negative consequences of accelerated skin photoaging and skin cancer. However, consumption of exogenous opioids does not trigger VitD synthesis and consequent suppression of opioid sensitivity but may instead produce an amplifying cycle of dependence.

Mechanistically, increased c-fos activation in the nucleus accumbens in the absence of VitD signaling is in line with our observations that opioids induce greater CPP and opioid dependence in VitD-deficient backgrounds. While the exact mechanism that causes greater nucleus accumbens activation remains unclear, several possible mechanisms exist. VDR is ubiquitously expressed in multiple brain regions; therefore, it is plausible that VDR represses opioids simultaneously in multiple regions (i.e., VTA, BLA, etc.). Future functional studies modulating VDR signaling in these regions could clarify in which regions VDR signaling is necessary and sufficient to modulate opioid reward.

Although dopamine signaling may be altered in the VTA of *Vdr*$^{-/-}$ mice, in line with previous observations[106], it is possible that there are compensatory mechanisms downstream of dopamine receptor signaling that contribute to increased opioid reward in the VitD–deficient (and dopamine deficient) setting. Alternatively, nondopaminergic mechanisms (i.e., glutamatergic transmission from the BLA) might contribute to the phenotype.

The roles of VDR and VitD in regulating the immune system and redox homeostasis are well established. Therefore, future studies are warranted to explore the role of VitD signaling

in various immune cell populations, like microglial cells, to investigate their potential role in opioid addiction. The inverse dose-response relationship between VitD levels and likelihood of opioid use, coupled with available preclinical data, is consistent with a model in which even modest rescue of VitD deficiency could be beneficial in the prevention and treatment of opioid addiction, especially considering that VitD is generally inexpensive, accessible, and safe.

It is an important clinical question to determine which patient subpopulation(s) would benefit most from VitD supplementation. It is unclear whether the critical VitD level, above which no additional benefit could be observed, might differ between species. The range of normal VitD levels in humans is quite wide, and we believe that future studies should examine this question within a clinical context because there exists a possibility that patients with low-normal VitD levels might benefit from additional VitD supplementation.

Although effective medication treatments for OUD exist, in real world, evaluations treatment retention remains challenging, and further interventions to augment the efficacy of these existing therapies are needed. In addition, limited data exist on effective interventions to prevent the development of OUD. Our findings offer several different therapeutic opportunities: Our results imply that VitD-deficient individuals may be at risk for developing tolerance and physiologic opioid dependence more rapidly, experiencing more significant withdrawal, and experiencing greater reward from opioid exposure. VitD supplementation might have a preventative benefit by decreasing opioid reward and possibly diminishing the risk of OUD. VitD supplementation may also improve the beneficial effects of medications for OUD. The alarming prevalence and toll of untreated OUD warrants timely clinical studies to test these therapeutic approaches directly, especially given the safety and availability of VitD supplementation.

4.3. Red hair alters nociception through modulating central melanocortin/opioid signaling

Our observations regarding high nociceptive thresholds in red-haired *Mc1r*$^{e/e}$ mice match previous findings of genotype-driven and sex-independent human and mouse nociceptive patterns[34]. We provide mechanistic insights for these differential nociceptive thresholds by identifying the role of MC1R variant (red-hair) loss of function in altering the balance of physiologic antagonism between OPRM1 and melanocortin signaling. However, other mechanisms could also contribute to the observed differences. Heterodimerization of OPRM1 with melanocortin receptors (possibly MC3)[77] or additional sex-dependent mechanisms could also contribute to the nociceptive differences observed in melanocortin-deficient mice. Alternatively, melanocortin signaling may directly influence nociception by modifying basal OPRM1 receptor activities such as ion channel activities, G protein coupling, or cAMP inhibition. These possibilities warrant future investigations.

POMC induction causes β-endorphin–mediated analgesia after UV exposure[21,70], whereas in the absence of UV exposure, a reduction in POMC products results in melanocortin deficiency–mediated analgesia in *Mc1r*$^{e/e}$ mice, as reported here. That both an increase and decrease in POMC can increase analgesia reflects the circumstance wherein a single gene encodes different peptides that activate two opposing pathways. The net effect of POMC depends on the balance of signaling between these two pathways and appears to be affected by differences in basal activities and the presence of other (non–POMC-derived) endogenous ligands. In the red hair background, the decrease in MC4R ligands with low POMC is proportionally large (as MC4R has no known ligands beyond POMC-derived MSH/ACTH) relative to μ-opioid receptor ligands where dynorphin, endomorphin, and enkephalin are unchanged (as illustrated[107]).

While our study focused on the red hair phenotype, the underlying melanocortin/opioid signaling balance may also account for pain variations among non–red-haired individuals. For example, we observed that *Mc4r* null mice exhibit high nociceptive thresholds like *Mc1r*$^{e/e}$ mice, but are not red haired, reflecting an alternative means to alter the melanocortin-opioid balance[107]. Individuals with *MC4R* polymorphisms may also have elevated pain thresholds and altered sensitivities to analgesics similar to those reported in red-haired individuals[34]. This study has revealed previously unknown and unexpected insights into the molecular and signaling determinants of basal nociceptive thresholds.

5. CONCLUSION

This thesis explores previously unrecognized roles of **POMC-derived opioid signaling** beyond pigmentation, focusing on its involvement in radiation-induced fatigue, opioid addiction susceptibility, and pain modulation. While POMC has long been associated with melanogenesis, its broader physiological functions remain underappreciated.

A key discovery of this work is that **skin-derived β-endorphin contributes to radiation-induced fatigue**, revealing a direct link between peripheral opioid production and systemic fatigue symptoms. This study provides evidence that radiation therapy elevates circulating β-endorphin levels, leading to behavioral effects that mimic opioid exposure. Importantly, these effects were reversed by opioid antagonists and were absent in β-endorphin knockout models, suggesting a **potential therapeutic avenue using opioid receptor blockers** to mitigate cancer therapy-related fatigue.

Beyond fatigue, this work identifies **vitamin D as a modulator of endogenous opioid reward pathways**, showing that **vitamin D deficiency amplifies UV/β-endorphin-seeking behavior**. This provides new insights into the **intersection between sunlight exposure, opioid signaling, and addiction vulnerability**, with potential implications for opioid use disorder prevention.

Finally, this study uncovers a **functional antagonism between melanocortin and opioid signaling in pain perception**, explaining why individuals with MC1R mutations (red-haired phenotype) exhibit heightened pain thresholds and increased opioid sensitivity.

Collectively, these findings expand our understanding of **POMC-derived opioid biology**, bridging dermatology, oncology, addiction science, and pain research. Future investigations may refine these mechanisms in humans and explore targeted interventions that leverage opioid signaling to improve patient outcomes in **cancer-related fatigue, opioid dependence, and pain management**.

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7. REFERENCES

1. Harno, E., Gali Ramamoorthy, T., Coll, A. P. & White, A. POMC: The Physiological Power of Hormone Processing. *Physiological Reviews* **98**, 2381–2430 (2018).
2. Clark, A. J. L. & Lowry, P. 60 YEARS OF POMC: POMC: the consummate peptide hormone precursor. *Journal of Molecular Endocrinology* **56**, E1–E2 (2016).
3. Hsiao, J. J. & Fisher, D. E. The roles of Microphthalmia Transcription Factor and pigmentation in melanoma. *Arch Biochem Biophys* **563**, 28–34 (2014).
4. Chakraborty, A. K. *et al.* Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: regulation by ultraviolet B. *Biochim Biophys Acta* **1313**, 130–8 (1996).
5. Rousseau, K. *et al.* Proopiomelanocortin (POMC), the ACTH/melanocortin precursor, is secreted by human epidermal keratinocytes and melanocytes and stimulates melanogenesis. *FASEB J.* **21**, 1844–1856 (2007).
6. Ascsillán, A. A. & Kemény, L. V. The Skin-Brain Axis: From UV and Pigmentation to Behaviour Modulation. *Int J Mol Sci* **25**, 6199 (2024).
7. Sinno, M. H. *et al.* Regulation of feeding and anxiety by α-MSH reactive autoantibodies. *Psychoneuroendocrinology* **34**, 140–149 (2009).
8. Gregoric, N. *et al.* Two Cases With an Early Presented Proopiomelanocortin Deficiency—A Long-Term Follow-Up and Systematic Literature Review. *Front. Endocrinol.* **12**, 689387 (2021).
9. Stasi, R., Abriani, L., Beccaglia, P., Terzoli, E. & Amadori, S. Cancer-related fatigue. *Cancer* **98**, 1786–1801 (2003).
10. Greenberg, D. B., Sawicka, J., Eisenthal, S. & Ross, D. Fatigue syndrome due to localized radiation. *J Pain Symptom Manage* **7**, 38–45 (1992).

11. Jacobsen, P. B. & Thors, C. L. Fatigue in the radiation therapy patient: current management and investigations. *Seminars in Radiation Oncology* **13**, 372–380 (2003).
12. Hickok, J. T., Morrow, G. R., Roscoe, J. A., Mustian, K. & Okunieff, P. 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).
13. Back, M. *et al.* Absence of adverse early quality of life outcomes of radiation therapy in breast conservation therapy for early breast cancer. *Australasian radiology* **49**, 39–43 (2005).
14. Mock, V. *et al.* NCCN Practice Guidelines for Cancer-Related Fatigue. *Oncology (Williston Park, N.Y.)* **14**, (2000).
15. Barbarino, R. *et al.* Fatigue in patients undergoing radiation therapy: an observational study. *Minerva medica* **104**, 185–91 (2013).
16. Taunk, N. K. *et al.* Comparison of radiation-induced fatigue across 3 different radiotherapeutic methods for early stage breast cancer. *Cancer* **117**, 4116–4124 (2011).
17. Jereczek-Fossa, B. A., Marsiglia, H. R. & Orecchia, R. Radiotherapy-related fatigue. *Critical reviews in oncology/hematology* **41**, 317–25 (2002).
18. Hofman, M., Ryan, J. L., Figueroa-Moseley, C. D., Jean-Pierre, P. & Morrow, G. R. Cancer-related fatigue: the scale of the problem. *The oncologist* **12 Suppl 1**, 4–10 (2007).
19. Schwartz, A. L. *et al.* Fatigue patterns observed in patients receiving chemotherapy and radiotherapy. *Cancer investigation* **18**, 11–9 (2000).
20. Cui, R. *et al.* Central role of p53 in the suntan response and pathologic hyperpigmentation. *Cell* **128**, 853–64 (2007).
21. Fell, G. L., Robinson, K. C., Mao, J., Woolf, C. J. & Fisher, D. E. Skin β-endorphin mediates addiction to UV light. *Cell* **157**, 1527–34 (2014).

22. Reissig, J. E. & Rybarczyk, A. M. Pharmacologic treatment of opioid-induced sedation in chronic pain. *The Annals of pharmacotherapy* **39**, 727–31 (2005).
23. O'Mahony, S., Coyle, N. & Payne, R. Current management of opioid-related side effects. *Oncology (Williston Park, N.Y.)* **15**, 61–73, 77; discussion 77-8, 80–2 (2001).
24. Key Substance Use and Mental Health Indicators in the United States: Results from the 2018 National Survey on Drug Use and Health | SAMHSA Publications. https://store.samhsa.gov/product/Key-Substance-Use-and-Mental-Health-Indicators-in-the-United-States-Results-from-the-2018-National-Survey-on-Drug-Use-and-Health/PEP19-5068 (2019).
25. Dasgupta, N., Beletsky, L. & Ciccarone, D. Opioid Crisis: No Easy Fix to Its Social and Economic Determinants. *Am J Public Health* **108**, 182–186 (2018).
26. Volkow, N. D. & Collins, F. S. The Role of Science in Addressing the Opioid Crisis. *New England Journal of Medicine* **377**, 391–394 (2017).
27. Feldman, S. R. *et al.* Ultraviolet exposure is a reinforcing stimulus in frequent indoor tanners. *J Am Acad Dermatol* **51**, 45–51 (2004).
28. Zeller, S., Lazovich, D., Forster, J. & Widome, R. Do adolescent indoor tanners exhibit dependency? *J Am Acad Dermatol* **54**, 589–96 (2006).
29. Kaur, M. *et al.* Induction of withdrawal-like symptoms in a small randomized, controlled trial of opioid blockade in frequent tanners. *J Am Acad Dermatol* **54**, 709–11 (2006).
30. Jablonski, N. G. & Chaplin, G. Colloquium paper: human skin pigmentation as an adaptation to UV radiation. *Proc Natl Acad Sci U S A* **107 Suppl 2**, 8962–8 (2010).
31. Xing, Y., Sonner, J. M., Eger, E. I., Cascio, M. & Sessler, D. I. Mice with a Melanocortin 1 Receptor Mutation Have a Slightly Greater Minimum Alveolar Concentration than Control Mice. *Anesthes* **101**, 544–546 (2004).

32. Mogil, J. S. *et al.* The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 4867–4872 (2003).

33. Liem, E. B. *et al.* Anesthetic requirement is increased in redheads. *Anesthesiology* **101**, 279–283 (2004).

34. Mogil, J. S. *et al.* Melanocortin-1 receptor gene variants affect pain and mu-opioid analgesia in mice and humans. *J. Med. Genet.* **42**, 583–587 (2005).

35. Liem, E. B., Joiner, T. V., Tsueda, K. & Sessler, D. I. Increased sensitivity to thermal pain and reduced subcutaneous lidocaine efficacy in redheads. *Anesthesiology* **102**, 509–514 (2005).

36. Andresen, T., Lunden, D., Drewes, A. M. & Arendt-Nielsen, L. Pain sensitivity and experimentally induced sensitisation in red haired females. *Scandinavian Journal of Pain* **2**, 3–6 (2011).

37. Valverde, P., Healy, E., Jackson, I., Rees, J. L. & Thody, A. J. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat. Genet.* **11**, 328–330 (1995).

38. D'Orazio, J. A. *et al.* Topical drug rescue strategy and skin protection based on the role of *Mc1r* in UV-induced tanning. *Nature* **443**, 340–344 (2006).

39. Mitra, D. *et al.* An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. *Nature* **491**, 449–53 (2012).

40. Rubinstein, M. *et al.* Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 3995–4000 (1996).

41. Marino, S., Vooijs, M., van der Gulden, H., Jonkers, J. & Berns, A. 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).

42. Dassule, H. R., Lewis, P., Bei, M., Maas, R. & McMahon, A. P. Sonic hedgehog regulates growth and morphogenesis of the tooth. *Development* **127**, 4775–4785 (2000).

43. Bilbey, D. L., Salem, H. & Grossman, M. H. The anatomical basis of the straub phenomenon. *British journal of pharmacology and chemotherapy* **15**, 540–3 (1960).

44. Kwan, K. Y. *et al.* TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* **50**, 277–89 (2006).

45. Mogil, J. S. *et al.* Heritability of nociception II. 'Types' of nociception revealed by genetic correlation analysis. *Pain* **80**, 83–93 (1999).

46. Li, Y. C. *et al.* Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9831–9835 (1997).

47. Kopec, A. M., Rivera, P. D., Lacagnina, M. J., Hanamsagar, R. & Bilbo, S. D. Optimized solubilization of TRIzol-precipitated protein permits Western blotting analysis to maximize data available from brain tissue. *J. Neurosci. Methods* **280**, 64–76 (2017).

48. Robbins, L. S. *et al.* Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell* **72**, 827–834 (1993).

49. Eddy, N. B. & Leimbach, D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J. Pharmacol. Exp. Ther.* **107**, 385–393 (1953).

50. Hermann, A. L. *et al.* β-Endorphin mediates radiation therapy fatigue. *Sci Adv* **8**, eabn6025 (2022).

51. Lira, F. S. *et al.* Sleep pattern and locomotor activity are impaired by doxorubicin in non-tumor-bearing rats. *Sleep Sci* **9**, 232–235 (2016).

52. Goh, J. & Ladiges, W. Voluntary Wheel Running in Mice. *Curr Protoc Mouse Biol* **5**, 283–290 (2015).

53. Dougherty, J. P., Springer, D. A. & Gershengorn, M. C. The Treadmill Fatigue Test: A Simple, High-throughput Assay of Fatigue-like Behavior for the Mouse. *J. vis. exp.* (2016) doi:10.3791/54052.

54. Matthes, H. W. *et al.* Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature* **383**, 819–23 (1996).

55. Katz, R. J. & Gelbart, J. Endogenous opiates and behavioral responses to environmental novelty. *Behavioral Biology* **24**, 338–348 (1978).

56. Kuschinsky, K. & Hornykiewicz, O. 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).

57. Brase, D. A., Loh, H. H. & Way, E. L. 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**, 368–74 (1977).

58. Filibeck, U., Castellano, C. & Oliverio, A. Development of morphine-induced changes of activity in the mouse. *Brain research* **254**, 602–5 (1981).

59. Gwynn, G. J. & Domino, E. F. Genotype-dependent behavioral sensitivity to mu vs. kappa opiate agonists. I. Acute and chronic effects on mouse locomotor activity. *J Pharmacol Exp Ther* **231**, 306–11 (1984).

60. Marona-Lewicka, D. & Vetulani, J. 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**, 431–8 (1989).

61. Mizuo, K. *et al.* Prenatal and neonatal exposure to bisphenol-A affects the morphine-induced rewarding effect and hyperlocomotion in mice. *Neuroscience Letters* **356**, 95–98 (2004).

62. Kitanaka, N., Kitanaka, J. & Takemura, M. Modification of morphine-induced hyperlocomotion and antinociception in mice by clorgyline, a monoamine oxidase-A inhibitor. *Neurochem. Res.* **31**, 829–837 (2006).
63. Mickley, G. A., Stevens, K. E., Burrows, J. M., White, G. A. & Gibbs, G. L. Morphine tolerance offers protection from radiogenic performance deficits. *Radiation research* **93**, 381–7 (1983).
64. Browne, R. G. & Segal, D. S. Behavioral activating effects of opiates and opioid peptides. *Biol. Psychiatry* **15**, 77–86 (1980).
65. Kuramoto, T. *et al.* Origins of Albino and Hooded Rats: Implications from Molecular Genetic Analysis across Modern Laboratory Rat Strains. *PLoS One* **7**, e43059 (2012).
66. Blaszczyk, W. M., Arning, L., Hoffmann, K.-P. & Epplen, J. T. A Tyrosinase missense mutation causes albinism in the Wistar rat. *Pigment Cell Res* **18**, 144–145 (2005).
67. Tripathi, R. K., Strunk, K. M., Giebel, L. B., Weleber, R. G. & Spritz, R. A. Tyrosinase gene mutations in type I (tyrosinase-deficient) oculocutaneous albinism define two clusters of missense substitutions. *Am J Med Genet* **43**, 865–871 (1992).
68. Lowe, S. W. *et al.* p53 status and the efficacy of cancer therapy in vivo. *Science* **266**, 807–810 (1994).
69. Ziegler, A. *et al.* Sunburn and p53 in the onset of skin cancer. *Nature* **372**, 773–776 (1994).
70. Kemény, L. V. *et al.* Vitamin D deficiency exacerbates UV/endorphin and opioid addiction. *Sci Adv* **7**, eabe4577 (2021).
71. Tolliver, B. K., Sganga, M. W. & Sharp, F. R. Suppression of c-fos induction in the nucleus accumbens prevents acquisition but not expression of morphine-conditioned place preference. *Eur. J. Neurosci.* **12**, 3399–3406 (2000).
72. Pierre, S., Eschenhagen, T., Geisslinger, G. & Scholich, K. Capturing adenylyl cyclases as potential drug targets. *Nature Reviews Drug Discovery* **8**, 321–335 (2009).

73. René, F. *et al.* Melanocortin receptors and delta-opioid receptor mediate opposite signalling actions of POMC-derived peptides in CATH.a cells. *Eur. J. Neurosci.* **10**, 1885–1894 (1998).

74. Chen, M., He, Z.-G., Liu, S.-G. & Xiang, H.-B. Motor cortex-periaqueductal gray-rostral ventromedial medulla neuronal circuitry may involve in modulation of nociception by melanocortinergic-opioidergic signaling. *Int J Clin Exp Pathol* **9**, 7897–7907 (2016).

75. Smith, C. M. *et al.* Endogenous central amygdala mu-opioid receptor signaling promotes sodium appetite in mice. *Proc Natl Acad Sci U S A* **113**, 13893–13898 (2016).

76. Jordan, B. A. & Devi, L. A. G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* **399**, 697–700 (1999).

77. Rediger, A. *et al.* Heterodimerization of Hypothalamic G-Protein-Coupled Receptors Involved in Weight Regulation. *Obes Facts* **2**, 3–3 (2009).

78. De Sanctis, V. *et al.* Cytokines, fatigue, and cutaneous erythema in early stage breast cancer patients receiving adjuvant radiation therapy. *BioMed research international* **2014**, 523568 (2014).

79. Greenberg, D. B., Gray, J. L., Mannix, C. M., Eisenthal, S. & Carey, M. Treatment-related fatigue and serum interleukin-1 levels in patients during external beam irradiation for prostate cancer. *Journal of pain and symptom management* **8**, 196–200 (1993).

80. Geinitz, H. *et al.* Fatigue, serum cytokine levels, and blood cell counts during radiotherapy of patients with breast cancer. *International journal of radiation oncology, biology, physics* **51**, 691–8 (2001).

81. Jacobsen, P. B. *et al.* Fatigue after treatment for early stage breast cancer: a controlled comparison. *Cancer* **110**, 1851–9 (2007).

82. Renner, M. *et al.* A murine model of peripheral irradiation-induced fatigue. *Behav. Brain Res.* **307**, 218–226 (2016).

83. Wolff, B. S., Renner, M. A., Springer, D. A. & Saligan, L. N. A Mouse Model of Fatigue Induced by Peripheral Irradiation. *J Vis Exp* (2017) doi:10.3791/55145.

84. Wolff, B. S., Raheem, S. A. & Saligan, L. N. Comparing passive measures of fatigue-like behavior in mice. *Sci Rep* **8**, 1–12 (2018).

85. Wolff, B. S. *et al.* Induction of fatigue-like behavior by pelvic irradiation of male mice alters cognitive behaviors and BDNF expression. *PLOS ONE* **15**, e0235566 (2020).

86. Bower, J. E. *et al.* Inflammatory Biomarkers and Fatigue during Radiation Therapy for Breast and Prostate Cancer. *Clin Cancer Res* **15**, 5534–5540 (2009).

87. Bower, J. E. & Lamkin, D. M. Inflammation and cancer-related fatigue: Mechanisms, contributing factors, and treatment implications. *Brain, Behavior, and Immunity* **30**, S48–S57 (2013).

88. Xiao, C. *et al.* 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).

89. Gambichler, T. *et al.* Plasma levels of opioid peptides after sunbed exposures. *Br J Dermatol* **147**, 1207–1211 (2002).

90. Kaur, M., Liguori, A., Fleischer, A. B., Jr. & Feldman, S. R. Plasma beta-endorphin levels in frequent and infrequent tanners before and after ultraviolet and non-ultraviolet stimuli. *J Am Acad Dermatol* **54**, 919–20 (2006).

91. Kaur, M., Liguori, A., Fleischer, A. B., Jr. & Feldman, S. R. Side effects of naltrexone observed in frequent tanners: could frequent tanners have ultraviolet-induced high opioid levels? *J Am Acad Dermatol* **52**, 916 (2005).

92. Dougherty, J. P., Springer, D. A. & Gershengorn, M. C. The Treadmill Fatigue Test: A Simple, High-throughput Assay of Fatigue-like Behavior for the Mouse. *J Vis Exp* (2016) doi:10.3791/54052.

93. Chi, A. *et al.* Immunomodulating and antioxidant effects of polysaccharide conjugates from the fruits of Ziziphus Jujube on Chronic Fatigue Syndrome rats. *Carbohydrate Polymers* **122**, 189–196 (2015).

94. Chi, A., Zhang, Y., Kang, Y. & Shen, Z. Metabolic mechanism of a polysaccharide from Schisandra chinensis to relieve chronic fatigue syndrome. *International Journal of Biological Macromolecules* **93**, 322–332 (2016).

95. Chi, A. *et al.* Characterization of a protein-bound polysaccharide from Herba Epimedii and its metabolic mechanism in chronic fatigue syndrome. *Journal of Ethnopharmacology* **203**, 241–251 (2017).

96. Shao, C. *et al.* Detection of Urine Metabolites in a Rat Model of Chronic Fatigue Syndrome before and after Exercise. *Biomed Res Int* **2017**, (2017).

97. Shao, C. *et al.* Therapeutic Effect and Metabolic Mechanism of A Selenium-Polysaccharide from Ziyang Green Tea on Chronic Fatigue Syndrome. *Polymers (Basel)* **10**, (2018).

98. Yi, T. *et al.* Moxibustion upregulates hippocampal progranulin expression. *Neural Regen Res* **11**, 610–616 (2016).

99. Yang, S. *et al.* A Narrative Review of Cancer-Related Fatigue (CRF) and Its Possible Pathogenesis. *Cells* **8**, 738 (2019).

100. White, A. *et al.* Pro-opiomelanocortin gene expression and peptide secretion in human small-cell lung cancer cell lines. *Journal of Molecular Endocrinology* **3**, 65–70 (1989).

101. Ray, D. W., Littlewood, A. C., Clark, A. J., Davis, J. R. & White, A. Human small cell lung cancer cell lines expressing the proopiomelanocortin gene have aberrant glucocorticoid receptor function. *J. Clin. Invest.* **93**, 1625–1630 (1994).

102. Scott, L. V, Burnett, F., Medbak, S. & Dinan, T. G. Naloxone-mediated activation of the hypothalamic-pituitary-adrenal axis in chronic fatigue syndrome. *Psychological medicine* **28**, 285–93 (1998).

103. Inder, W. J., Prickett, T. C. R. & Mulder, R. T. Normal opioid tone and hypothalamic-pituitary-adrenal axis function in chronic fatigue syndrome despite marked functional impairment. *Clinical endocrinology* **62**, 343–8 (2005).

104. Weller, R. B. Sunlight Has Cardiovascular Benefits Independently of Vitamin D. *Blood Purif* **41**, 130–134 (2016).

105. Karsten, K. B., Ferguson, G. W., Chen, T. C. & Holick, M. F. Panther chameleons, Furcifer pardalis, behaviorally regulate optimal exposure to UV depending on dietary vitamin D3 status. *Physiol. Biochem. Zool.* **82**, 218–225 (2009).

106. Trinko, J. R. *et al.* Vitamin D3: A Role in Dopamine Circuit Regulation, Diet-Induced Obesity, and Drug Consumption. *eNeuro* **3**, (2016).

107. Robinson, K. C. *et al.* Reduced MC4R signaling alters nociceptive thresholds associated with red hair. *Sci. Adv.* **7**, eabd1310 (2021).

8. ANNEX

8.1. Magyar nyelvű összefoglaló

Jelen tézis célja, hogy kiterjessze a proopiomelanocortin (POMC) hagyományosan a pigmentáció szabályozásában ismert szerepét, és feltárja annak bőr által termelt derivátumainak – különösen a β-endorphinnak – jelentőségét a sugárterápiát követő fáradtság érzésben, az opioidfüggőségben, valamint a fájdalomérzés modulációjában. A POMC egy olyan prekurzor, amely több aktív peptiddé bomlik, többek között a melanocita-stimuláló hormonná (MSH), mely a melanogenezist indítja el, valamint a β-endorphinná, mely az endogén opioid rendszer kulcsfontosságú komponense, és a fájdalomcsillapításban, illetve a jutalmazási mechanizmusokban játszik szerepet.

Kísérleti állatmodellekkel kimutattuk, hogy a bőr sugárzás által kiváltott károsodása p53 aktivációját eredményezi, ami fokozza a POMC transzkripcióját, ezáltal megnöveli a vér β-endorphin szintjét. Ezen emelkedés opioid-szerű viselkedési jelenségekkel, például a megnövekedett fájdalomküszöbbel és a Straub-féle farokreakcióval társul, valamint fáradtság-szerű tünetek formájában jelentkezik. Fontos, hogy ezen hatások az opioid receptor blokkolásával visszafordíthatók, és nem figyelhetők meg β-endorphin hiányában (Hermann et al, 2022).

Továbbá, kutatásaink rámutattak arra is, hogy a D-vitamin hiány fokozza az UV-sugárzás által kiváltott β-endorphin okozta eufóriát, ami új mechanisztikai összefüggést tár fel az opioidfüggőség kialakulása és a vitamin D állapot között. Ezen túlmenően, a melanokortin jelátvitel zavara – különösen a vörös hajú, MC1R mutációval rendelkező egyének esetében – központi opioid útvonalakon keresztül modulálja a fájdalomérzékelést, ami magasabb fájdalomküszöbben nyilvánul meg.

Összességében eredményeink azt sugallják, hogy a bőr képes befolyásolni POMC termelésen keresztül számos viselkedési mintázatot. Vizsgálataink új lehetőségeket nyithatnak meg a sugárterápiás fáradtság, az opioidfüggőség és a fájdalom kezelésének optimalizálásában, klinikai szempontból releváns terápiás intervenciók kifejlesztését célozva meg.

8.2. Co-author certification form

Co-author certification

I, myself as a corresponding author of the following publication(s) declare that the authors have no conflict of interest, and Andrea Hermann, Ph.D. candidate had significant contribution to the jointly published research(es). The results discussed in her thesis were not used and not intended to be used in any other qualification process for obtaining a PhD degree.

David E. Fisher, MD, PhD

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2025.Feb.28. Prof. Dr. David E. Fisher

The publication(s) relevant to the applicant's thesis:

Kemény LV, Robinson KC, **Hermann AL**, Walker DM, Regan S, Yew YW, Lai YC, Theodosakis N, Rivera PD, Ding W, Yang L, Beyer T, Loh YE, Lo JA, van der Sande AAJ, Sarnie W, Kotler D, Hsiao JJ, Su MY, Kato S, Kotler J, Bilbo SD, Chopra V, Salomon MP, Shen S, Hoon DSB, Asgari MM, Wakeman SE, Nestler EJ, Fisher DE. Vitamin D deficiency exacerbates UV/endorphin and opioid addiction. Sci Adv. 2021.

Robinson KC, Kemény LV, Fell GL, **Hermann AL**, Allouche J, Ding W, Yekkirala A, Hsiao JJ, Su MY, Theodosakis N, Kozak G, Takeuchi Y, Shen S, Berenyi A, Mao J, Woolf CJ, Fisher DE. Reduced MC4R signaling alters nociceptive thresholds associated with red hair. Sci Adv. 2021.