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

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**PhD Thesis**

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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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## 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). 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. 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.

### 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. 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. 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. 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. Disruptions in POMC processing or receptor signaling pathways have been associated with metabolic disorders, including obesity and adrenal insufficiency.

Collectively, these insights underscore the multifaceted roles of POMC-derived peptides across various physiological domains. 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. 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. 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.

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. Systemic increases in β-endorphin after UV exposure produce opiate-like behaviors and phenotypes, indicating systemic β-endorphin effects following local UV exposure. Opiate drugs can cause sedation, a common symptom associated with fatigue, prompting us to ask whether radiation-induced increases in β-endorphin might contribute to the fatigue associated with radiation therapy.

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

Human studies have suggested that ultraviolet (UV) tanning may be addictive, exhibiting characteristics highly reminiscent of opioid addiction. Recent preclinical data have identified an endogenous opioid-mediated addiction-like pathway triggered by UV-induced cutaneous synthesis of β-endorphin. Maintenance of UV-dependent vitamin D (VitD) synthesis has been suggested as a driver for the evolution of light skin pigmentation. 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. 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. MC1R mutant red-haired mice are less sensitive to noxious thermal, mechanical, and chemical stimuli. 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.

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 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, to test for changes in pain thresholds with tail radiation. To ablate keratinocyte specific p53 expression, mice with floxed alleles of p53 were crossed with a strain expressing Cre recombinase driven by the Keratin 14 promoter. *Vdr*$^{-/-}$ mice were provided by M. Damay.

For open-field actimetry experiments using rats, 8-week-old male Sprague-Dawley rats (Jackson Laboratories, Bar Harbor, ME) were used. All animal experiments were performed in accordance with institutional policies and Institutional Animal Care and Use Committee-approved protocols.

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.

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. 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 prior to tail irradiation for the day. β-endorphin was measured by radioimmunoassay (Phoenix Pharmaceuticals). Straub Tail was measured as previously described.

Mice underwent mechanical threshold testing in the von Frey test and the hot plate test as previously described.

*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.

*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 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 testing procedures were followed as described.

*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.

*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*

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.

## 3. RESULTS

In order to introduce all projects in a meaningful manner in this Results section, I am referring to 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 results in this Thesis that were obtained 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. 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. We observed that, following irradiation, rats became progressively sedate, as quantified by movement measurements calculated using open field actimetry.

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. 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 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 and Straub tail, 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 as well as an elevation in nociceptive threshold.

Given the requirement for peripheral β-endorphin synthesis in these opiate-like phenotypes, 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, 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), 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.

*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, in line with prior observations, in contrast to rats 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.

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. 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 tail irradiation.

Although, we have seen consistent hyperpigmentation in mice tails upon radiation, we did not observe hyperpigmentation of rat tails upon radiation, because Sprague-Dawley rats carry a mutation in the tyrosinase gene that makes them albino. This missense mutation (R299H) is conserved across all albino rat strains and has been described in patients with oculocutaneous albinism type I. 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. 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.

*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. 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. 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. 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 using the tail radiation regimen. 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. These studies suggest that radiation-induced increases in β-endorphin produce changes in opioid receptor dependent phenotypes.

Since upregulation of POMC and production of cutaneous β-endorphin in response to UV exposure has been shown to be p53-dependent, 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, 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 and did not display Straub tail. 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, 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. 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. 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, 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.

*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. For these experiments, we have obtained micropunches from frozen brain tissues obtained from mice treated with saline or morphine i.p..

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. The greater *c-Fos* mRNA induction was accompanied by higher induction of c-FOS protein expression in $Vdr^{-/-}$ mice 90 min after morphine administration. 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, 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.

### 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). 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). However, peripheral administration of methylated naltrexone, a BBB-impermeable opioid antagonist, did not diminish the nociceptive differences between black and red-haired mice, 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. 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, consistent

with previous observations. These data suggest that melanocortin and opioid signaling may antagonize each other in a cell-autonomous manner.

*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). 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, or agonism of melanocortin receptors (independently of *Mc1r*) in PAG-cannulated $Mc1r^{e/e}$ mice significantly decreases nociceptive thresholds. 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 and OPRM1 has been previously shown to interact with MC3R; 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. 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, but not others. Additional research has suggested that radiation-induced fatigue may arise from the emotional and psychological toll of having cancer, however a correlation between presence of clinical anxiety or depression and fatigue

has not been consistently observed. 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, 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 different irradiation protocols and the different kinetics of development of fatigue-like behavior.

The precise mechanism through which β-endorphin may induce sedation is uncertain, but it is notable that UV radiation also elevates circulating β-endorphin 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, although this point remains debated and inconclusive given small sample sizes in reporting studies.

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, 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. 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.

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. 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) 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, 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.

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. Individuals with *MC4R* polymorphisms may also have elevated pain thresholds and altered sensitivities to analgesics similar to those reported in red-haired individuals. This study has revealed previously unknown and unexpected insights into the molecular and signaling determinants of basal nociceptive thresholds.

## 5. Summary

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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