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Visual exposure to green light therapy reduces knee joint pain and alters the lipidome in osteoarthritic rats

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Abstract

Visual exposure to dim, green, light has been found to reduce pain levels in patients living with migraine, low back pain, and fibromyalgia. Preclinical studies discovered that the analgesic effect of green light was due to the central release of endogenous opioids and a reduction in inflammatory cytokines in the cerebrospinal fluid. The present study assessed the effect of green light therapy (GLT) on joint pain in a rat model of osteoarthritis (OA) and investigated the role of endolipids. Male and female Wistar rats (207-318 g) received an intra-articular injection of sodium monoiodoacetate (3 mg in 50 μL saline) into the knee to induce OA. On day 9, animals were placed in a room illuminated by either white (neutral-white 4000K; 20 lux) or green (wavelength: 525 nm; luminance: 20 lux) light for 5 days (8 hours per day). Joint nociception was assessed by von Frey hair algesiometry, dynamic weight bearing, and in vivo single unit extracellular recordings from knee joint mechanonociceptors. Compared to white light, GLT significantly reduced secondary mechanical hypersensitivity in both sexes and improved hindlimb weight bearing in females only. There was no effect of GLT on joint nociceptor activity in either sex. Serum lipidomics indicated an increase in circulating analgesic endolipids in response to GLT, particularly the *N*-acyl-glycines. Partial blockade of the endocannabinoid system with the G protein receptor-18/cannabinoid-1 receptor antagonist AM281 (500 μg/kg i.p.) attenuated GLT-induced analgesia. These data show for the first time that GLT acts to reduce OA pain by upregulating circulating analgesic endolipids, which then engage the endocannabinoid system.

Keywords: Animal model, Arthritis, Electrophysiology, Endocannabinoids, Green light therapy, Lipidomics, Pain behaviour

1. Introduction

The 2017 Global Burden of Disease Study identified musculoskeletal pain as one of the major causes of disability.[7] Out of all of the over 100 different arthritides, osteoarthritis (OA) of the hip and knee is by far the most common affecting around 250 million people worldwide, predominantly women.[11] Effective management of OA pain continues to evolve as we gain a better understanding of the mechanisms and mediators involved.[6,14] Long-term use of drugs such as opioids and nonsteroidal anti-inflammatory drugs to control chronic pain is often associated with safety concerns and diminishing benefits. Nonpharmacological therapies offer an attractive means of managing chronic pain as they tend to be safer and more cost-effective. Regular exercise, acupuncture, massage, and diet can all have a positive effect on pain relief[8,34,36,38] affirming that nonpharmacological approaches play an important role in pain management strategies.

Light therapy has recently gained traction as a nonpharmacological treatment for pain. While investigating photophobia in migraineurs, Noseda et al.[28] discovered that migraine attacks worsened when exposed to blue, red, and white light; however, low intensity green light reduced headache pain scores by about 15%. This phenomenon was investigated further by Martin et al.[20] who found that exposing migraineurs to green light for 1 to 2 hours per day for 10 weeks reduced their number of headache days by 60% and significantly improved their perceived quality of life. In contrast, white light had no beneficial effect in these patients. Visual exposure to green light has since been shown to be analgesic in other pain conditions such as low back pain[15] and fibromyalgia[17]; however, arthritis has yet to be tested.

Preclinically, light in the green wavelength range reduced nociception in naïve rats exposed to noxious mechanical and thermal stimuli.[12] Furthermore, in a model of neuropathic pain, rats receiving low level green light phototherapy at 4 lux for 8 hours per day for 5 days exhibited lower pain responses, which persisted for over 10 days after treatment.[12] The mechanism of phototherapeutic analgesia is obscure but appears to involve neural connections between the retina and pain processing areas in the brain. Blockade of green light photoreception in the retina using filtering contact lenses abolished the antinociceptive effects of green light therapy (GLT) suggesting that the green light must pass through the visual system to produce its analgesic effect.[12] Additional studies are required to elucidate the mechanism of

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action further and to determine whether other pain conditions can be similarly modulated by ambient green light.

The aim of this study was to investigate if exposure to green ambient light could alter pain behaviour in a rodent model of OA by affecting the peripheral nervous system. Because endocannabinoids have been shown to reduce joint pain preclinically,[13,22,30] the lipidome was also examined to identify a possible biochemical pathway responsible for the pain modulating effects of green light.

2. Methods

Studies were conducted on 50 male and 48 female Wistar rats (207-318 g; approximately 8-9 weeks old, Charles River, Canada). Upon arrival at the Dalhousie University Animal Care Facility, pairs of animals were housed in ventilated cages at room temperature (21 ±1°C) with a 12-hour light:12-hour dark cycle for 1 week before commencement of the study. Animals received standard rodent lab chow and had access to water ad libitum. All experimental protocols were preapproved by the Dalhousie University Committee on the Use of Laboratory Animals, which acts in accordance with the standards put forth by the Canadian Council for Animal Care.

2.1. Induction of arthritis

Osteoarthritis-like joint damage was induced by intra-articular injection of sodium monoiodoacetate (MIA: 3 mg in 50 μL of 0.9% saline) into the right stifle joint. Rats were deeply anaesthetised by gaseous inhalation of isoflurane (2% maintenance dose in 100% O_2 with a flow of 1 L/min), and depth of anaesthesia was confirmed by abolition of the hind paw pinch-withdrawal reflex. Injections were performed using a Hamilton syringe introduced anteriorly into the synovial cavity between the femoral condyle and tibial plateau via the patellar ligament. The joint was flexed and extended for 30 seconds to distribute the MIA throughout the joint space. Animals were allowed to recover for 9 days before being exposed to ambient white or green light.

2.2. Phototherapy

Separate cohorts of animals were exposed to either white light (neutral-white 4000K; 20 lux) or green light (525 nm; 20 lux) using IP65 LED Flex strips (LEDSupply, Randolph, VT). A mains timer was used to illuminate a darkened room for the first 8 hours of the regular dark phase on days 9 to 14 after arthritis induction. Animals were randomised to either white light therapy (WLT) or GLT.

2.3. Pain behaviour measurement

Pain behaviour experiments were conducted during the regular light phase of the cycle after treatment. Hindlimb secondary hypersensitivity was measured by von Frey hair algesiometry on day 0 (baseline), day 9 after MIA injection (control), and then on days 10 to 14 (test period). Naïve animals were not tested here as green light has been shown to have no effect on normal mechanosensitivity.[12] Animals were transferred to a Perspex chamber fitted with a wire mesh floor to enable access to the hind paws. After an acclimation period of at least 10 minutes, von Frey hair monofilaments of increasing bending force (2-15 g) were applied perpendicular to the volar surface of the ipsilateral hind paw for up to 3 seconds. Filaments of increasing force were applied until a positive response (withdrawal, licking, shaking of the paw) was observed. After a positive reaction, lower force filaments were subsequently applied, and the 50% withdrawal threshold was calculated using the following equation:

$$50\%\ \text{Threshold} = \frac{\left(10^{[Xf + \kappa\delta])}\right)}{10,000}$$

Where *Xf* is the bending force of the last von Frey filament used (in log units), κ is the tabular value for the pattern of the last 6 responses, and δ is the mean difference (in log units) between stimuli. The maximum cut-off was set to 15 g bending force.

Rat hindlimb weight distribution was measured using a dynamic incapacitance apparatus (Bioseb, Boulogne, France). Animals were placed in a clear, Perspex box (24 cm long × 24 cm wide × 30 cm high) equipped with a pressure-sensitive floor pad and a digital video camera facing from above. Video footage of the freely moving animal was captured over a 3-minute time period, and the concomitant hindlimb weight bearing was measured via the pressure-sensitive floor. The percentage of total weight borne on the ipsilateral hind paw during the recording session was then calculated.

Area under the curve (AUC) calculations were performed for both von Frey algesiometry and dynamic incapacitance time-course experiments beginning with the onset of light treatment (day 9). Net area calculations are reported with both positive and negative peaks included in the analysis.

2.4. Assessment of endocannabinoid involvement

After 5 days of green or white light phototherapy, separate groups of male and female rats were treated with either the G-protein receptor-18 (GPR18)/cannabinoid-1 receptor (CB_1-R) antagonist AM281 (500 μg/kg i.p.) or vehicle (DMSO:cremaphor:saline, 1:1:8) 30 minutes before pain behaviour measurement. The effect of AM281 or vehicle on pain behaviour was measured over a 60-minute time course. To directly compare the effect of GPR18/CB_1-R blockade between light groups, relative effect sizes of AM281 are reported. These values were calculated by computing the difference in AUC values between AM281 and vehicle treatment over the 60-minute time courses. These data are reported as delta AUC.

2.5. Molecular analysis of endogenous pain-control systems

After 5 days of green or white light exposure, nerve cell bodies were harvested from animals for subsequent genetic and proteomic analysis.

To measure changes in the expression of genes involved in endogenous pain signalling, RNA sequencing was performed. After euthanization of the animal with pentobarbital (1 mL/kg), the ipsilateral L4 dorsal root ganglion was harvested, incubated in RNAlater (Thermo Fisher Scientific, Waltham, MA), and stored at −80°C. Frozen tissue was thawed, transferred into a 20% chloroform/TRIzol mixture, mechanically lysed with a tissue disruptor, and centrifuged at 12,000 × g for 15 minutes. The aqueous phase was transferred to a new tube with isopropanol, incubated for 10 minutes, and centrifuged at 12,000 × g for 10 minutes. An RNA pellet was harvested, washed, and stored in RNase-free water.

Two hundred fifty nanograms of RNA per sample was used to construct sequencing libraries using the Illumina Stranded Total RNA Prep with the Ribo-Zero Plus Kit according to the manufacturer's instructions (Illumina Inc, San Diego, CA). The size of the samples was verified using an Agilent Bioanalyzer

(Agilent Technologies, Inc, Santa Clara, CA), and the nucleic acid content was then quantified with a Qubit 4 Fluorometer using the DNA High Sensitivity Kit (Thermo Fisher Scientific). Samples were then each diluted to 40 nM and pooled for sequencing. Paired-end × 50 bp sequencing was performed on an Illumina NextSeq2000 (Illumina Inc). Libraries were pooled and sequenced using 2 lanes of 1 P2-100 flow cell to an average depth of 32 million reads per sample. All samples were run in triplicate at the Faculty of Medicine Genomics CORES facility at Dalhousie University. RNA sequencing analysis was performed using Illumina BaseSpace DRAGEN Analysis | Version: 1.3.0 and DRAGEN Differential Expression 4.0.3 (Illumina Inc) to provide a list of differentially expressed genes.

To measure levels of circulating bioactive lipids, including endocannabinoids, analysis of serum using HPLC tandem mass spectrometry was conducted. Animals were deeply anaesthetised with urethane (2 g/kg i.p.), and intracardiac blood samples were harvested. Blood was centrifuged at 5000 × g for 2 minutes, and serum was collected and stored at −80°C. Serum lipid analysis was performed as previously described.[1,2] In brief, serum was partially purified with a methanolic extract through C18 solid phase extraction columns, and then elutions of 100% methanol were analysed on an API 7500 triple quadrupole tandem mass spectrometer. Analysis was conducted as previously described and heatmaps generated with specific details on statistical analyses and fold-change generation.[16]

2.6. Electrophysiological evaluation of nociceptor activity

After the fifth day of light exposure, animals were deeply anaesthetised with urethane (2 g/kg i.p.) and placed supine on a thermostatically controlled heating blanket. The trachea was exposed by making a longitudinal skin incision in the neck, and the underlying sternohyoid muscles were reflected. The trachea was then isolated and cannulated to allow unobstructed airflow and artificial ventilation with 100% O_2 using a rodent respirator (Harvard Apparatus, MA; stroke volume: 2.5 mL; respiratory frequency: 52 breaths per minute). The cervical jugular vein was then isolated and cannulated so that gallamine triethiodide (50 mg/kg) could be administered to block skeletal neuromuscular activity. A skin incision was made in the medial aspect of the right hindlimb, and the resulting skin flaps were sutured to a metal "O" ring to create pool which was filled with warm (37°C) mineral oil. The saphenous nerve was gently isolated in the inguinal region and cut centrally to prevent spinal reflexes. Using watchmaker forceps, the perineurium was removed and the distal portion of the exposed nerve was placed on a microdissection platform. Thin axon bundles were then teased away from the nerve and placed on a platinum recording electrode to enable extracellular recording from joint afferents. The anteromedial aspect of the knee was probed with a blunt glass rod, and mechanosensitive afferents were identified when electrical burst activity was apparent. To mechanically stimulate the knee directly, the ankle was immobilised in a boot, and the hip joint was immobilized by clamping the femur in a stereotaxic frame. Rotational forces were then be applied to the knee, which were standardized by the inclusion of a torque meter (Data Track 244-1-R, Intertechnology, Toronto, Canada) oriented in series with the hindlimb.

The mechanosensitivity of knee joint afferents was determined by recording extracellularly from single units. Outward torque was gradually applied to the knee until an action potential was evoked; this determined the mechanical threshold of the unit. Noxious hyper-rotation of the knee was then held for 10 seconds, and the resulting firing events were recorded and subsequently counted offline. Noxious joint rotation was repeated a further 2 times (5 minutes apart), and the average firing frequency over the 3 movements was calculated using Spike2 software (Cambridge Electronic Design, Cambridge, United Kingdom). The presence of spontaneous neuronal activity was also examined. Joint afferent fibres that fired action potentials in the absence of mechanical stimuli where considered "spontaneously active." The percentage of fibres that were spontaneously active was calculated for each treatment group.

2.7. Drugs and reagents

Urethane, MIA, and gallamine triethiodide were obtained from Sigma-Aldrich (St. Louis, MO). AM281 (CB1 receptor antagonist; 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-4-morpholinyl-1H-pyrazole-3-carboxamide) was obtained from Cayman Chemicals (Ann Arbor, MI).

2.8. Statistics

All data were expressed as mean ± SEM and tested for Gaussian distribution. Pain behaviour and electrophysiological data were normally distributed and were, therefore, analysed using parametric statistics (1- or 2-way analysis of variance [ANOVA]). Repeated-measures ANOVA was used to analyse data from time courses. *Post hoc* analysis was conducted using Sidak or Tukey multiple comparison tests. Nonparametric data were analysed using a Chi-squared test. Lipidomic analysis of sex differences and treatment differences was performed using 2-tailed Student *t* tests as previously described.[16] All AUC data (GLT vs WLT) were analysed by 2-tailed Student *t* tests. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Green light therapy significantly attenuates nociceptive behaviour in both sexes

Nine days after administration of MIA, both sexes displayed enhanced nociceptive behaviour consistent with experimental OA pain. In male animals, paw withdrawal thresholds reduced from 14.91 ± 0.05 g at baseline to 5.55 ± 0.35 g at day 9 MIA ($P < 0.0001$, n = 16, **Fig. 1A**). Similar levels of secondary mechanical hypersensitivity were observed in females (baseline: 14.40 ± 0.31 g, day 9: 5.49 ± 0.39 g, $P < 0.0001$, n = 16, **Fig. 1B**). Both sexes also displayed weight-bearing deficits after MIA administration with similar responses observed in males (baseline: 51.78 ± 1.03%, day 9: 35.98 ± 2.81%, $P < 0.0001$, n = 16) and females (baseline: 49.28 ± 0.71%, day 9: 39.49 ± 2.24%, $P < 0.01$, n = 16).

Treatment with GLT rapidly attenuated nociceptive behaviour in both sexes compared to day 9 baseline responses. In male animals, GLT reversed hind paw mechanosensitivity after just 2 treatments (day 11: 12.60 ± 1.21 g, $P < 0.01$, n = 8, **Fig. 1A**) with sustained effects up to day 14. Female animals responded similarly to GLT, with significant improvements in withdrawal thresholds observed on days 11 and 14 (day 11: 10.58 ± 1.65 g and day 14: 12.49± 1.36 g, $P < 0.05$, n = 8, **Fig. 1B**). Treatment with WLT, in contrast, did not have any effect on mechanical hypersensitivity ($P > 0.05$, n = 8, **Fig. 1B**). Improvement in weight-bearing deficits was not observed in male animals treated with either colour of light ($P > 0.05$, n = 8, **Fig. 1C**); however, GLT reduced hindlimb incapacitance in female animals compared to those undergoing WLT ($P < 0.05$, n = 8, **Fig. 1D**).

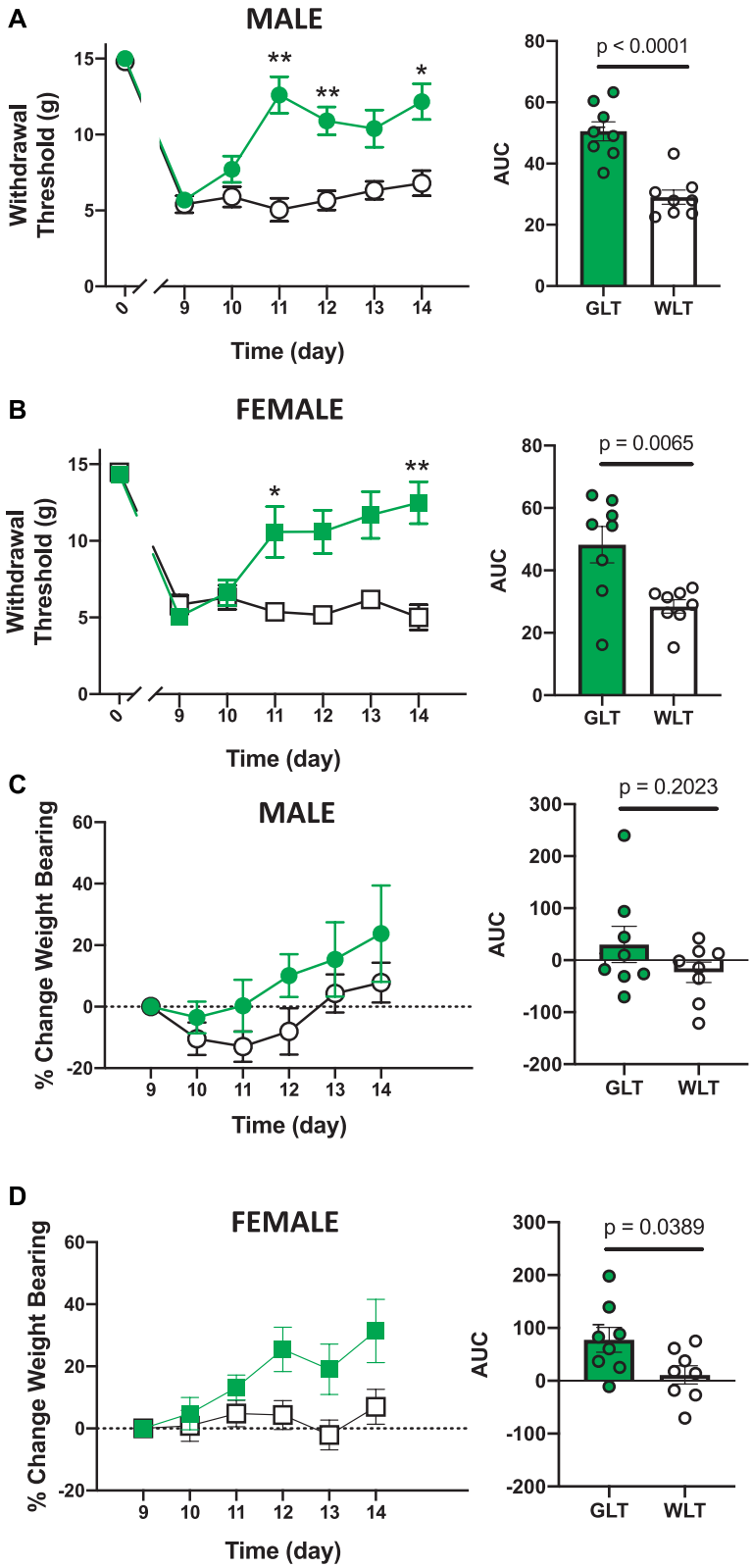

Figure 1. Green light therapy (GLT) reduces pain behaviour in experimental osteoarthritis (OA). (A) Treatment with green light significantly attenuated the secondary mechanical hypersensitivity associated with the MIA model in male animals (2-way repeated-measures analysis of variance [RM ANOVA], n = 8). (B) In female animals, green light reversed paw withdrawal deficits compared to white light (2-way RM ANOVA, n = 8). (C) Hindlimb weight-bearing deficits were observed in male animals on day 9 after induction of experimental OA (2-way RM ANOVA, n = 8), but treatment with green light did not significantly reduce these weight bearing deficits ($P > 0.05$, 2-way RM ANOVA, n = 8). (D) Female animals also experienced weight-bearing deficits; however, they did benefit from treatment with green light ($P < 0.05$, 2-way RM ANOVA, n = 8). Data are means ± SEM. Green symbols: animals treated with GLT; white symbols: animals treated with white light therapy. $^{**}P < 0.01$, $^{*}P < 0.05$, Sidak multiple comparisons test. Time course data are summarised as areas under the curve (AUC) and analysed by Student *t* test (right panels).

3.2. Green light therapy does not reduce joint nociceptor sensitization

Joint afferent fibres were classified based on their electrophysiological properties (mechanical threshold, evoked/spontaneous firing, electrical threshold, and conduction velocity; **Table 1**). Representative traces from each treatment group are depicted in **Figure 2**. Treatment with green light did not affect evoked joint afferent firing when compared with white light treated or untreated MIA animals ($P > 0.05$, n = 6-10, **Fig. 2C and D**). Green light also did not alter nociceptor sensitivity as measured by mechanical thresholds ($P > 0.05$, n = 6-10, **Fig. 2E and F**). Similarly, light treatment did not affect spontaneous firing, electrical thresholds, or the conduction velocity from recorded nerve fibres (**Table 1**). Sex differences were not observed in any of the electrophysiological parameters measured in MIA animals or those treated with either colour of light (Mechanical Threshold: $P = 0.26$; Evoked Firing: $P = 0.72$; Electrical Threshold: $P = 0.55$; Conduction Velocity: $P = 0.93$, 2-way ANOVA. Spontaneous Firing: MIA, $P > 0.99$; MIA + GLT, $P = 0.15$; MIA + WLT, $P = 0.60$, chi-square test, n = 6-10).

To discern the effects of GLT on molecular markers of nociception, RNA sequencing and proteomic analysis was conducted. In DRGs, proteomic analysis revealed 8 differentially expressed genes in male animals and 19 differentially expressed genes in females after green light treatment. Compared to DRGs from WLT animals, 4 genes were upregulated in males and 4 genes were downregulated in GLT animals (**Table 2**). In female animals that received GLT, 11 genes were upregulated and 8 were downregulated in DRG tissue when compared to WLT-treated animals (**Table 3**). Upon further investigation of these genes with modified expression, none have been implicated in nociceptive or inflammatory processes thus far. Furthermore, genes that are involved in nociceptor excitability were similarly expressed in the DRGs of GLT and WLT-treated animals.

3.3. The effect of green light therapy on the serum lipidome

The effect of phototherapy on the systemic lipidome was determined by HPLC tandem mass spectrometry (summarized in **Fig. 3A**). **Figures 3B-G** highlight examples from each of the 3 families of serum lipids tested. Endogenous *N*-acyl ethanolamines (NAEs) were predominantly lower in female OA rats after WLT compared to males, while GLT increased 3 of the NAEs tested in females and none in males (**Fig. 3A**). For example, the analgesic fatty acid amide *N*-palmitoyl ethanolamine (PEA) was elevated in females treated with GLT, while in male animals, PEA levels were unaltered (**Fig. 3B**). In addition, the classic endocannabinoid anandamide (*N*-arachidonoyl ethanolamine) was unaffected by GLT in either sex (**Fig. 3C**). Overall, the *N*-acyl glycines were significantly elevated in both male and female OA rats after GLT (**Figs. 3A, D, and E**). There were no changes with GLT treatment in any of the 2-acyl-sn-glycerol lipids examined including the classic endocannabinoid 2-arachidonyl glycerol (2-AG) (**Figs. 3A, F, and G**).

3.4. Green light therapy–induced analgesia is mediated through the endocannabinoid system

To test the involvement of endogenous biolipids in GLT-induced analgesia, the effect of GPR18 and CB_1 receptor blockade on pain behaviour was evaluated. Administration of AM281 (500 μg/kg i.p.) after 5 days of GLT reversed the antinociceptive effects of light treatment but had no effect in WLT-treated animals (**Fig. 4**). Thirty minutes after treatment with AM281, withdrawal thresholds were significantly decreased compared to vehicle in both male and female GLT animals ($P < 0.05$, n = 8, **Fig. 4A and B**). This pronociceptive response was sustained in both sexes for the duration of the time course. In contrast, AM281 did not affect withdrawal thresholds in WLT-treated animals of either sex ($P > 0.05$, n = 8, **Fig. 4A and B**). Relative effect sizes of GPR18/CB_1-R blockade was significantly greater in GLT animals compared with WLT ($P < 0.0001$, n = 8, **Fig. 4A and B**). In GLT animals, GPR18/CB_1-R blockade also significantly reduced ipsilateral hindlimb weight bearing 60 minutes after administration in males ($P < 0.05$, n = 8, **Fig. 4C**) but had no overall effect on weight bearing in female animals ($P > 0.05$, n = 8, **Fig. 4D**). Treatment with AM281 did not affect hindlimb incapacitance in white light treated animals of either sex ($P < 0.05$, n = 8, **Fig. 4C and D**).

4. Discussion

The pharmacological control of arthritis pain is generally hindered by limited efficacy of the available drugs and concerns over safety. Nonpharmacological approaches offer a promising adjunct to medications and can themselves provide significant pain relief. Exercise, cognitive-based therapy, and diet have, to varying degrees, been shown to improve pain symptoms and overall

Table 1

Electrophysiological properties of joint nociceptors after light treatment.

Fibre type	Mechanical threshold (mNm)	Evoked baseline firing (action potentials/rotation)	Spontaneous fibres (%)	Electrical threshold (V)	Conduction velocity (m/s)	n
Male						
MIA	13 ± 2 (2-20)	41 ± 9 (11-100)	50	3 ± 0.4 (2.0-4.0)	3.1 ± 1.2 (1.9-4.7)	10
MIA + GLT	10 ± 2 (3-18)	51 ± 10 (25-88)	28.6	3 ± 0.3 (2.25-4)	1.64 ± 0.3 (0.8-2.8)	7
MIA + WLT	12 ± 3 (4-23)	45 ± 10 (12-94)	14.3	3 ± 0.5 (1.75-3.75)	1.41 ± 0.2 (0.9-1.7)	7
Female						
MIA	14 ± 2 (4-23)	38 ± 7 (18-69)	50	3 ± 0.4 (2.5-4.0)	3.5 ± 1.0 (1.7-6.1)	8
MIA + GLT	18 ± 3 (5-28)	55 ± 19 (29-80)	0	3 ± 0.3 (2.0-3.0)	2.40 ± 0.3 (1.9-3.0)	6
MIA + WLT	14 ± 3 (3-23)	58 ± 39 (24-112)	25	3 ± 0.3 (2.5-4.0)	2.53 ± 0.5 (1.3-3.3)	8

Single unit joint afferent recordings were performed in male and female rats on day 15 of the MIA model. Separate cohorts of animals received green or white light treatment for 5 d preceding electrophysiological recordings. Electrophysiological properties of joint afferents including mechanical threshold, electrical threshold, and conduction velocity are summarised herein. In addition, both evoked firing rates and the percentage of fibres that exhibited spontaneous firing are shown. Data presented as means (range), or percentages.

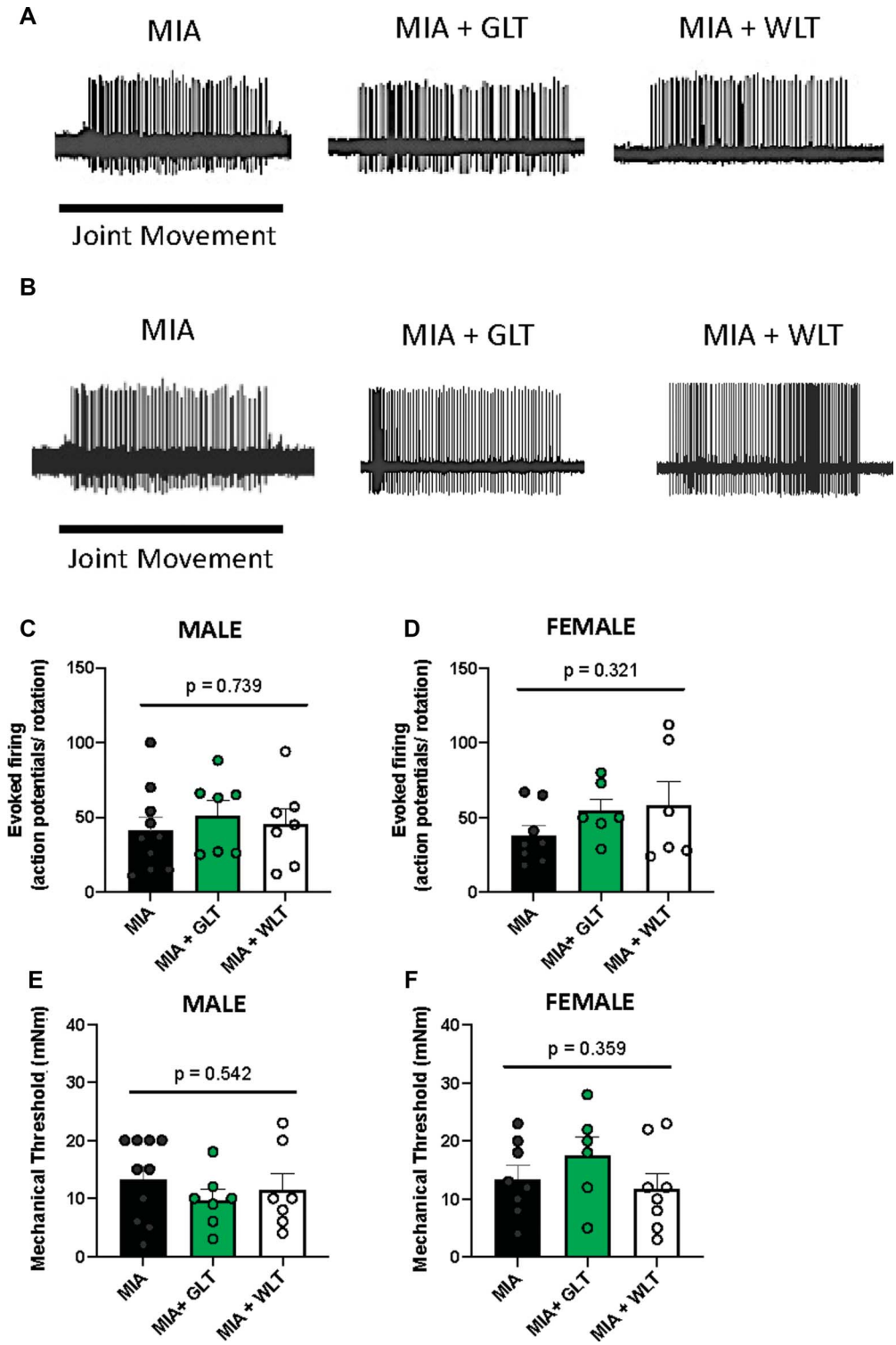

Figure 2. Electrophysiological properties of knee joint nociceptors are not affected by treatment with green light therapy (GLT). (A) Representative traces of single unit joint nociceptor recordings in day 15 male MIA animals. (B) Representative traces of single unit joint nociceptor recordings in day 15 female MIA animals. (C) Evoked firing of joint nociceptors in male animals are not affected by green or white light (1-way analysis of variance [ANOVA], n = 7-10). (D) In female animals, evoked firing of joint nociceptors is not impacted by treatment with either green or white light (1-way ANOVA, n = 6-8). (E) Mechanosensitivity of joint afferents in male animals is not affected by green or white light treatment (1-way ANOVA, n = 7-10). (F) The mechanical thresholds of joint nociceptors are similarly not significantly affected by treatment with green or white light treatment (1-way ANOVA, n = 6-8). Black bars: untreated MIA animals; green bars: MIA animals treated with GLT; white bars: MIA animals treated with white light therapy (WLT). Data are means ± SEM.

health of patients with chronic arthritis. Using a preclinical model of OA, the current study found that visual exposure to low level green light in rats was able to reduce joint pain via engagement of the endocannabinoid system. The analgesic effect of GLT appeared to function centrally without affecting peripheral nociception.

Table 2

Differentially expressed genes in male dorsal root ganglia after green light therapy.

Gene name	ENSMBL gene ID	Log fold-change	Adjusted *P* value
Significantly upregulated genes in GLT-treated animals			
XRCC5	ENSG00000079246.16	1.41	6.04E-03
TET2	ENSG00000168769.13	1.17	2.09E-02
SIPA1	ENSG00000213445.10	1.09	2.91E-02
TATDN2	ENSG00000157014.11	2.76	2.76E+00
Significantly downregulated genes in GLT-treated animals			
POLE	ENSG00000177084.17	−2.05	9.91E-04
MTF1	ENSG00000188786.10	−0.94	6.04E-03
KLF6	ENSG00000067082.15	−0.67	8.71E-03
PLXNC1	ENSG00000136040.9	−0.74	3.13E-02

Summary of differentially expressed genes identified through RNA sequencing of GLT-treated male MIA animals.
GLT, green light therapy.

Daily exposure of OA rats to 8 hours of ambient green light produced by low power LEDs significantly reduced mechanical secondary hypersensitivity as measured by von Frey hair algesiometry. This analgesic effect was not apparent in a control cohort of animals exposed to white light over a similar time course. There was no statistical difference in green light responses on hind paw withdrawal threshold between the sexes. Induction of OA also resulted in a shift in weight bearing away from the affected hindlimb, and this incapacitance was also ameliorated by green light, but in females only. A possible explanation for this sex difference in incapacitance is that female rats are generally more active and the dynamic incapacitance meter may have been unable to detect any changes in weight-bearing improvements in less mobile males. A further limitation of the pain behaviour experiments is that the dark phase was altered by the introduction of 8 additional hours of dim light, which could impact the sleep/wake cycle of the rats and thereby differentially affect their activity. Other studies have also found that visual exposure to green light can reduce pain produced by acute, noxious physical stimuli, peripheral neuropathy, and postsurgical incision.[12,18] These analgesic effects were devoid of any anxiolysis or alteration in motor function. Light in the blue range of the visual spectrum (ie, 472 nm wavelength) had a moderate effect on acute pain responses in previous studies; however, this response was not as pronounced as green light.[12]

Opsins are photosensitive receptors that have been localised in skin and peripheral neurones where they can modulate nociceptor activity and aversive behaviours by a nonvisual mechanism.[3,21] To test whether ambient green light was having an effect in the periphery, therefore, single unit recordings from joint nociceptors were performed in OA rats. The heightened firing frequency of joint primary afferents in response to noxious knee movement was unchanged by green light and was not significantly different compared to white light exposure. This lack of effect on joint peripheral sensitization was consistent between male and female animals. It seems, therefore, that green light reduced joint pain centrally via transmission through the visual system. Ibrahim et al.[12] tested this hypothesis by developing

Table 3

Differentially expressed genes in female dorsal root ganglia after green light therapy.

Gene name	ENSMBL gene ID	Log fold-change	Adjusted *P* value
Significantly upregulated genes in GLT-treated animals			
AC093010.3	ENSG00000259976.3	9.29	2.42E-13
7SK	ENSG00000202198.1	23.72	1.01E-08
MYBPC1	ENSG00000196091.14	5.00	1.71E-07
AC097658.1	ENSG00000228981.3	1.83	3.54E-06
RPPH1	ENSG00000277209.1	1.16	3.73E-03
MYOZ1	ENSG00000177791.12	4.96	4.09E-03
TIMM10B	ENSG00000132286.12	6.09	4.87E-03
SCARNA10	ENSG00000239002.3	1.18	6.29E-03
C1QL3	ENSG00000165985.10	1.22	9.73E-03
RPL10L	ENSG00000165496.5	0.67	9.78E-03
TPM2	ENSG00000198467.15	1.55	9.78E-03
Significantly downregulated genes in GLT-treated animals			
ABLIM1	ENSG00000099204.20	−1.14	1.19E-09
GTF2IP1	ENSG00000277053.4	−3.47	1.71E-07
AC011603.2	ENSG00000258017.2	−0.74	2.54E-03
MAP3K12	ENSG00000139625.13	−0.71	4.37E-03
SPTBN4	ENSG00000160460.16	−0.93	1.59E-02
BAHCC1	ENSG00000266074.8	−1.93	2.46E-02
LRFN5	ENSG00000165379.13	−1.34	4.00E-02

Summary of differentially expressed genes identified through RNA sequencing of GLT-treated female MIA animals.
GLT, green light therapy.

A

	Between Sex Treatment		Within Sex Treatment Analysis	
	Male WLT→ Female WLT	Male GLT → Female GLT	Male WLT→ Male GLT	Female WLT→ Female GLT
N-acyl ethanolamine				
N-palmitoyl ethanolamine (PEA)	↓↓	↑		↑↑
N-stearoyl ethanolamine (SEA)	↓↓			
N-oleoyl ethanolamine (OEA)	↓↓			↑↑
N-linoleoyl ethanolamine (LEA)				↑↑
N-arachidonoyl ethanolamine (AEA)				
N-docosahexaenoyl ethanolamine (DEA)	↓↓			
N-acyl glycine				
N-palmitoyl glycine (PalGly)			↑	↑↑
N-stearoyl glycine (SteGly)	↓		↑	↑↑
N-oleoyl glycine (OlGly)			↑↑↑	↑↑↑
N-linoleoyl glycine (LinGly)	↓↓↓		↑↑↑	↑↑↑↑
N-arachidonoyl glycine (NAGly)	↓↓↓↓	↓↓↓	↑↑↑	↑↑
N-docosahexaenoyl glycine (DocGly)				↑↑
2-acyl-sn-glycerol				
2-palmitoyl-sn-glycerol (2-PG)				
2-oleoyl-sn-glycerol (2-OG)				
2-linoleoyl-sn-glycerol (2-LG)				
2-arachidonoyl-sn-glycerol (2-AG)	↑↑↑			

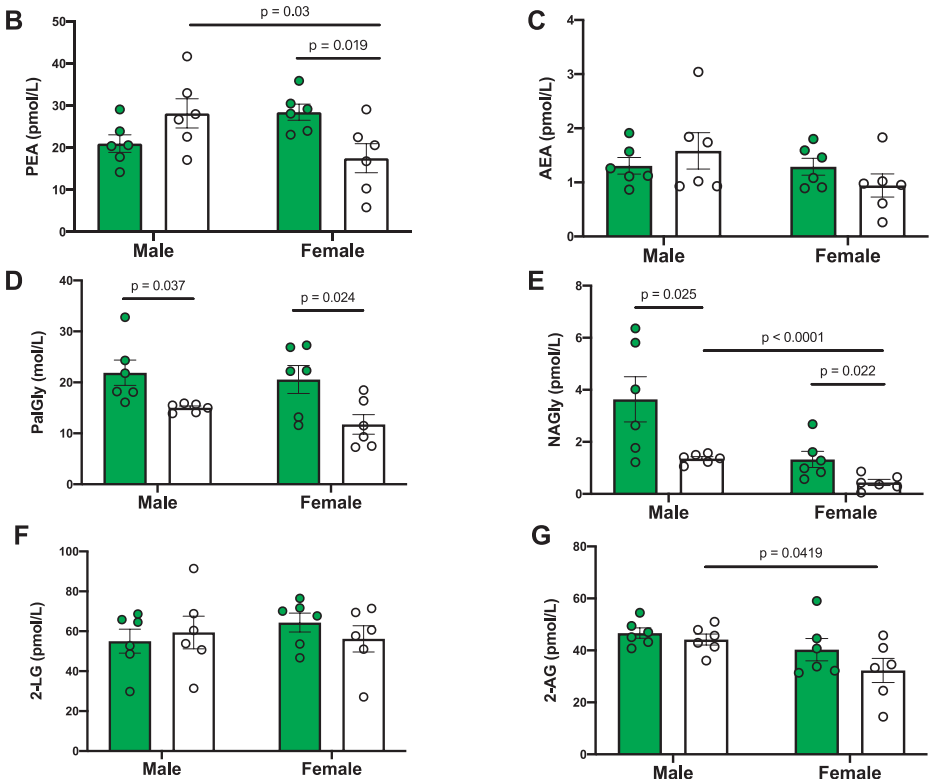

Figure 3. Serum lipidome in osteoarthritis (OA) model is modulated by green light therapy (GLT). (A) Heatmap of changes in endogenous lipids (see Methods for additional details on analysis). Orange indicates significant decreases, green indicates significant increases (light hue $P < 0.1$-0.051; dark hue $P < 0.05$), and arrows indicate relative fold change. Subject group listed second is the direction of change. (B-G) Bar graphs of exemplar endolipids from each of the 3 lipid groups to provide mathematical context for the heatmaps.

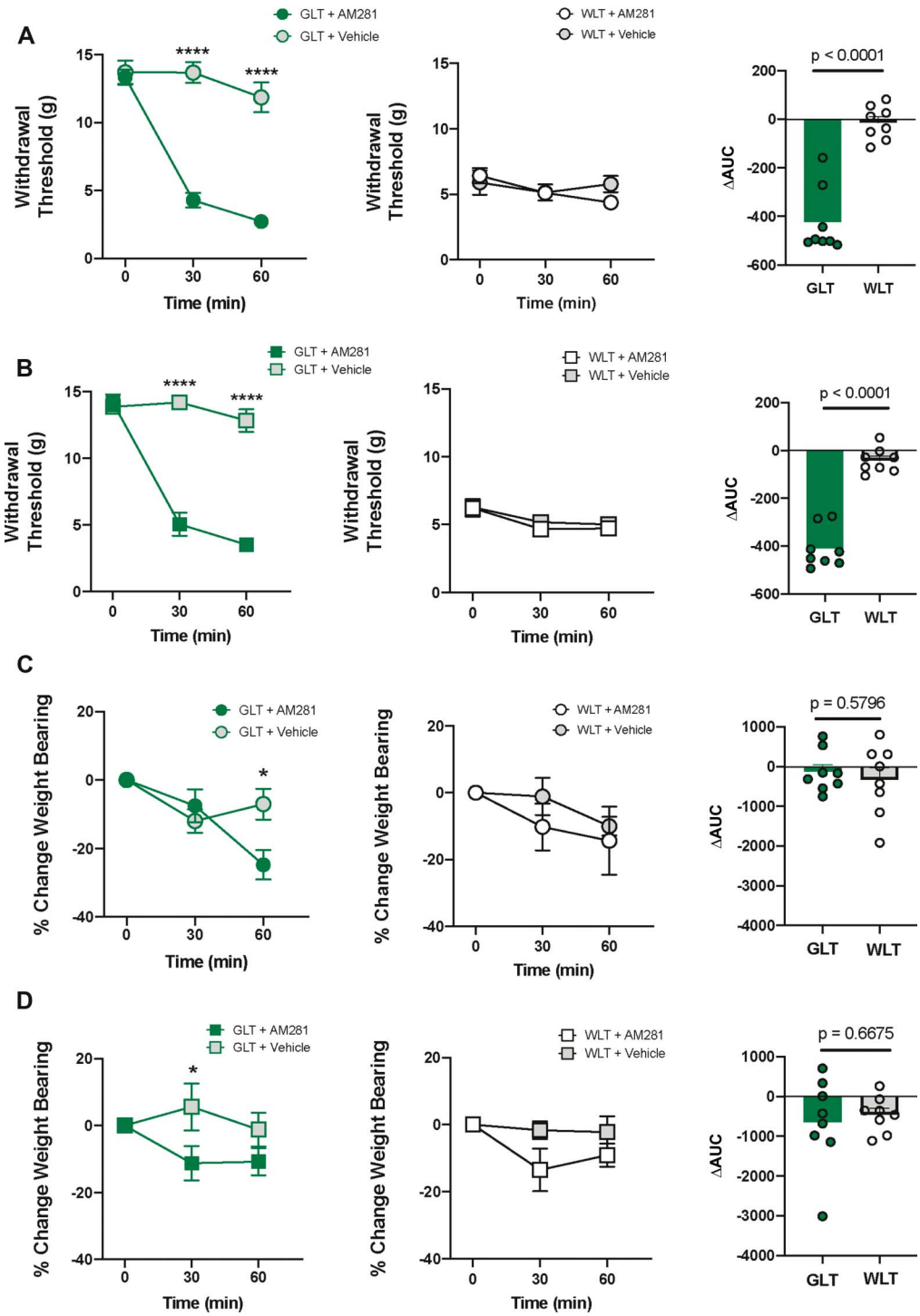

Figure 4. Blocking the CB1 receptor attenuates the analgesic effect of green light therapy (GLT). (A) Treatment with the GPR18/CB_1 receptor antagonist, AM281, after 5 days of green light treatment, significantly reduced hind paw withdrawal thresholds in male animals (2-way repeated-measures analysis of variance [RM ANOVA], n = 8, left panel). AM281 did not significantly alter withdrawal thresholds in male animals after 5 days of WLT treatment (2-way RM ANOVA, n = 8, middle panel). The relative effect size of AM281 in GLT vs WLT-treated animals is significantly different as summarised by the change in area under the curve (ΔAUC) (Student *t* test, n = 8, right panel). (B) Significant reversal of green light–induced mechanical antinociception was observed in female animals after administration of AM281 (2-way RM ANOVA, n = 8, left panel). AM281 did not significantly alter withdrawal thresholds in WLT female animals (2-way RM ANOVA, n = 8, middle panel). AM281 treatment significantly reduced withdrawal thresholds in GLT vs WLT-treated animals (Student *t* test, n = 8, right panel). (C) Blockade of GPR18/CB_1 receptors significantly reduced weight bearing on the ipsilateral hind paw in male GLT animals (2-way RM ANOVA, n = 8, left panel) but had no significant effect in WLT male animals (2-way RM ANOVA, n = 8, middle panel). The relative effect size of AM281 in GLT vs WLT-treated animals was not significantly different (Student *t* test, n = 8, right panel). (D) In contrast, weight bearing was not significantly affected overall by treatment with AM281 in female GLT (2-way RM ANOVA, n = 8, left panel) or WLT animals (2-way RM ANOVA, n = 8, middle panel). The relative effect size of AM281 treatment was not significantly different between light treatment groups (Student *t* test, n = 8, right panel). Data are means ± SEM. $^{****}P < 0.0001$, $^{*}P < 0.05$, Sidak multiple comparisons test.

opaque contact lenses, which were worn by rats during green light exposure. By blocking light from entering the visual system, the analgesic properties of ambient green light were abolished. Furthermore, placement of green contact lenses over the eyes resulted in antinociception compared to clear, transparent lenses. Thus, light in the green range of the visual spectrum must enter the eyes in order for an analgesic response to be achieved, although this was not directly assessed here. Neural processes from the visual cortex to pain control regions of the brain have been identified[3]; however, the precise circuitry and associated pain neuromodulation in the brain requires further investigation.

Previous studies have demonstrated that the neuropharmacological mechanism for GLT involves, in part, activation of the central endogenous opioid system. Intrathecal injection of naloxone has been shown to reduce green light antinociception, while analysis of cerebrospinal fluid revealed that β-endorphin and proenkephalin levels were elevated in treated animals.[12,18,19] Interestingly, opioid receptor levels remained unchanged suggesting that changes in the local concentration of the endogenous ligands is responsible for modulating pain. It is likely that other neuromodulators are involved in this process, the identity of which have yet to be determined. In the present study, analysis of peripheral molecular markers in serum and at the level of the DRG were conducted to identify potential pathways in GLT-induced analgesia. Examination of differentially expressed genes in peripheral neurones did not identify any genes known to be associated with inflammation or pain. There were, however, changes in genes involved in regulating axon guidance and cytoskeletal architecture (PLXNC1, SPTBN4), but their relationship to pain is unknown.

Evaluation of the serum lipidome provided a novel insight into a potential mechanism for GLT-induced analgesia by upregulating various *N*-acyl ethanolamines and *N*-acyl glycines, but not 2-acyl-sn-glycerols. While there were no serum changes in some endolipids, including the classic endocannabinoids anandamide and 2-AG, it is possible that lipid levels may still be altered in the cerebrospinal fluid, brain tissue, and dorsal horn of the spinal cord. The bioactive lipids *N*-palmitoyl ethanolamine (PEA) and *N*-linoleoyl ethanolamine (LEA) are able to elevate endocannabinoid levels by inhibiting degradative endolipases and promoting anabolic lipase activity.[4,5,29] The current study found that GLT increased serum PEA and LEA in females, which could account for some of the analgesia observed in this sex.

Dramatic increases in serum *N*-acyl glycines were observed in the serum demonstrating a very strong relationship between the concentration of this family of endolipids and GLT in both males and females. Previous data have shown that *N*-arachidonoyl glycine (NAGly) acts on the abnormal cannabidiol receptor GPR18, and its effects can be blocked by AM281.[23–25] The GPR18-NAGly signalling system has also been implicated in central pain control through the periaqueductal gray,[27] leading to interrogation of this pathway further. The role of the endocannabinoid system in GLT-induced analgesia was, therefore, examined by treating animals with the dual GPR18/CB_1-R antagonist AM281. These experiments revealed a significant reduction in mechanical antinociception confirming the importance of this pathway, although more selective antagonists need to be tested. Selective blockade of CB_1-receptors has been shown to increase peripheral and central sensitization in models of OA indicating that endocannabinoids are key regulators of OA pain.[31,32] The role of CB_2-receptors in OA pain is less clear with reports of no effect, decrease, and even an increase in joint nociception.[26,33] More recently, GPR18 has been identified as an important receptor in the proresolution aspect of sensory and immune function.[9,10,37] Again, the ability of the dual GPR18/CB_1-R antagonist AM281 to block GLT-induced analgesia suggests that green light may also engage the actions of specialized proresolving mediators. The *N*-acyl glycines *N*-oleoyl glycine and *N*-arachidonyl glycine were also upregulated in GLT OA rats of both sexes, and these mediators are believed to be analgesic by inhibiting the glycine transporter-2 leading to enhanced inhibitory neurotransmission.[35] The role of endogenous lipids in GLT-induced analgesia requires further investigation, particularly in the CNS, but the data presented here support the involvement of these diverse signalling molecules in the antinociceptive responses observed after green light exposure.

In summary, this study showed for the first time that visual exposure to ambient green light reduced OA pain in male and female rats by a centrally driven mechanism. Circulating lipids with putative analgesic properties were upregulated after GLT suggesting involvement of the endocannabinoid system. Future studies are required to unravel the neural pathways between the retina and pain control areas of the central nervous system as well as further characterize the mediators responsible for GLT-induced analgesia.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

[1] Bashashati M, Bradshaw HB, Johnson CT, Zuckerman MJ, Sarosiek J, McCallum RW, Sarosiek I. Plasma endocannabinoids and cannabimimetic fatty acid derivatives are altered in cyclic vomiting syndrome: the effects of sham feeding. J Investig Med 2023;71:821–9.
[2] Bradshaw HB, Johnson CT. Measuring the content of endocannabinoid-like compounds in biological fluids: a critical overview of sample preparation methodologies. Methods Mol Biol 2023;2576:21–40.
[3] Cheng K, Martin LF, Slepian MJ, Patwardhan AM, Ibrahim MM. Mechanisms and pathways of pain photobiomodulation: a narrative review. J Pain 2021;22:763–77.
[4] Di Marzo V, Melck D, Orlando P, Bisogno T, Zagoory O, Bifulco M, Vogel Z, De Petrocellis L. Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells. Biochem J 2001;358:249–55.
[5] di Tomaso E, Beltramo M, Piomelli D. Brain cannabinoids in chocolate. Nature 1996;382:677–8.
[6] Fu K, Robbins SR, McDougall JJ. Osteoarthritis: the genesis of pain. Rheumatology (Oxford) 2018;57:iv43–50.
[7] GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392:1789–858.

[8] Gwinnutt JM, Wieczorek M, Rodriguez-Carrio J, Balanescu A, Bischoff-Ferrari HA, Boonen A, Cavalli G, de Souza S, de Thurah A, Dorner TE, Moe RH, Putrik P, Silva-Fernandez L, Stamm T, Walker-Bone K, Welling J, Zlatkovic-Svenda M, Guillemin F, Verstappen SMM. Effects of diet on the outcomes of rheumatic and musculoskeletal diseases (RMDs): systematic review and meta-analyses informing the 2021 EULAR recommendations for lifestyle improvements in people with RMDs. RMD Open 2022;8:e002167.
[9] Hao JW, Liu TT, Qiu CY, Li XM, Qiao WL, Li Q, Qin QR, Hu WP. Lipid mediator resolvin D2 inhibits ATP currents in rat primary sensory neurons. J Neurochem 2023.
[10] Honkisz-Orzechowska E, Lazewska D, Baran G, Kiec-Kononowicz K. Uncovering the power of GPR18 signalling: how RvD2 and other ligands could have the potential to modulate and resolve inflammation in various health disorders. Molecules 2024;29:1258.
[11] Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. Lancet 2019;393:1745–59.
[12] Ibrahim MM, Patwardhan A, Gilbraith KB, Moutal A, Yang X, Chew LA, Largent-Milnes T, Malan TP, Vanderah TW, Porreca F, Khanna R. Long-lasting antinociceptive effects of green light in acute and chronic pain in rats. PAIN 2017;158:347–60.
[13] Krustev E, Reid A, McDougall JJ. Tapping into the endocannabinoid system to ameliorate acute inflammatory flares and associated pain in mouse knee joints. Arthritis Res Ther 2014;16:437.
[14] Krustev E, Rioux D, McDougall JJ. Mechanisms and mediators that drive arthritis pain. Curr Osteoporos Rep 2015;13:216–24.
[15] Leichtfried V, Matteucci Gothe R, Kantner-Rumplmair W, Mair-Raggautz M, Bartenbach C, Guggenbichler H, Gehmacher D, Jonas L, Aigner M, Winkler D, Schobersberger W. Short-term effects of bright light therapy in adults with chronic nonspecific back pain: a randomized controlled trial. Pain Med 2014;15:2003–12.
[16] Leishman E, Mackie K, Luquet S, Bradshaw HB. Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain. Biochim Biophys Acta 2016; 1861:491–500.
[17] Martin L, Porreca F, Mata El, Salloum M, Goel V, Gunnala P, Killgore WDS, Jain S, Jones-MacFarland FN, Khanna R, Patwardhan A, Ibrahim MM. Green light exposure improves pain and quality of life in fibromyalgia patients: a preliminary one-way crossover clinical trial. Pain Med 2021;22: 118–30.
[18] Martin LF, Cheng K, Washington SM, Denton M, Goel V, Khandekar M, Largent-Milnes TM, Patwardhan A, Ibrahim MM. Green light exposure elicits anti-inflammation, endogenous opioid release and dampens synaptic potentiation to relieve post-surgical pain. J Pain 2023;24: 509–29.
[19] Martin LF, Moutal A, Cheng K, Washington SM, Calligaro H, Goel V, Kranz T, Largent-Milnes TM, Khanna R, Patwardhan A, Ibrahim MM. Green light antinociceptive and reversal of thermal and mechanical hypersensitivity effects rely on endogenous opioid system stimulation. J Pain 2021;22: 1646–56.
[20] Martin LF, Patwardhan AM, Jain SV, Salloum MM, Freeman J, Khanna R, Gannala P, Goel V, Jones-MacFarland FN, Killgore WD, Porreca F, Ibrahim MM. Evaluation of green light exposure on headache frequency and quality of life in migraine patients: a preliminary one-way cross-over clinical trial. Cephalalgia 2021;41:135–47.
[21] Matynia A, Nguyen E, Sun X, Blixt FW, Parikh S, Kessler J, Perez de Sevilla Muller L, Habib S, Kim P, Wang ZZ, Rodriguez A, Charles A, Nusinowitz S, Edvinsson L, Barnes S, Brecha NC, Gorin MB. Peripheral sensory neurons expressing melanopsin respond to light. Front Neural Circuits 2016;10:60.
[22] McDougall JJ, Muley MM, Philpott HT, Reid A, Krustev E. Early blockade of joint inflammation with a fatty acid amide hydrolase inhibitor decreases end-stage osteoarthritis pain and peripheral neuropathy in mice. Arthritis Res Ther 2017;19:106.
[23] McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM, Bradshaw HB. N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci 2010;11:44.
[24] McHugh D, Page J, Dunn E, Bradshaw HB. Δ(9)-Tetrahydrocannabinol and N-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. Br J Pharmacol 2012;165:2414–24.
[25] McHugh D, Wager-Miller J, Page J, Bradshaw HB. siRNA knockdown of GPR18 receptors in BV-2 microglia attenuates N-arachidonoyl glycine-induced cell migration. J Mol Signal 2012;7:10.
[26] Mlost J, Kostrzewa M, Borczyk M, Bryk M, Chwastek J, Korostynski M, Starowicz K. CB2 agonism controls pain and subchondral bone degeneration induced by mono-iodoacetate: implications GPCR functional bias and tolerance development. Biomed Pharmacother 2021;136:111283.
[27] Narasimhan M, Mahimainathan L, Reddy PH, Benamar K. GPR18-NAGly system in periaqueductal gray and chronic neuropathic pain. Biochim Biophys Acta Mol Basis Dis 2020;1866:165974.
[28] Noseda R, Bernstein CA, Nir RR, Lee AJ, Fulton AB, Bertisch SM, Hovaguimian A, Cestari DM, Saavedra-Walker R, Borsook D, Doran BL, Buettner C, Burstein R. Migraine photophobia originating in cone-driven retinal pathways. Brain 2016;139:1971–86.
[29] Petrosino S, Schiano Moriello A, Verde R, Allara M, Imperatore R, Ligresti A, Mahmoud AM, Peritore AF, Iannotti FA, Di Marzo V. Palmitoylethanolamide counteracts substance P-induced mast cell activation in vitro by stimulating diacylglycerol lipase activity. J Neuroinflammation 2019;16:274.
[30] Philpott HT, McDougall JJ. Combatting joint pain and inflammation by dual inhibition of monoacylglycerol lipase and cyclooxygenase-2 in a rat model of osteoarthritis. Arthritis Res Ther 2020;22:9.
[31] Sagar DR, Staniaszek LE, Okine BN, Woodhams S, Norris LM, Pearson RG, Garle MJ, Alexander SP, Bennett AJ, Barrett DA, Kendall DA, Scammell BE, Chapman V. Tonic modulation of spinal hyperexcitability by the endocannabinoid receptor system in a rat model of osteoarthritis pain. Arthritis Rheum 2010;62:3666–76.
[32] Schuelert N, McDougall JJ. Cannabinoid-mediated antinociception is enhanced in rat osteoarthritic knees. Arthritis Rheum 2008;58:145–53.
[33] Schuelert N, Zhang C, Mogg AJ, Broad LM, Hepburn DL, Nisenbaum ES, Johnson MP, McDougall JJ. Paradoxical effects of the cannabinoid CB2 receptor agonist GW405833 on rat osteoarthritic knee joint pain. Osteoarthritis Cartilage 2010;18:1536–43.
[34] Uthman OA, van der Windt DA, Jordan JL, Dziedzic KS, Healey EL, Peat GM, Foster NE. Exercise for lower limb osteoarthritis: systematic review incorporating trial sequential analysis and network meta-analysis. BMJ 2013;347:f5555.
[35] Vandenberg RJ, Ryan RM, Carland JE, Imlach WL, Christie MJ. Glycine transport inhibitors for the treatment of pain. Trends Pharmacol Sci 2014; 35:423–30.
[36] Witt C, Brinkhaus B, Jena S, Linde K, Streng A, Wagenpfeil S, Hummelsberger J, Walther HU, Melchart D, Willich SN. Acupuncture in patients with osteoarthritis of the knee: a randomised trial. Lancet 2005; 366:136–43.
[37] Zhao M, Zheng Z, Yin Z, Zhang J, Qin J, Wan J, Wang M. Resolvin D2 and its receptor GPR18 in cardiovascular and metabolic diseases: a promising biomarker and therapeutic target. Pharmacol Res 2023;195:106832.
[38] Zhu B, Ba H, Kong L, Fu Y, Ren J, Zhu Q, Fang M. The effects of manual therapy in pain and safety of patients with knee osteoarthritis: a systematic review and meta-analysis. Syst Rev 2024;13:91.