

Effects of 50 Hz Magnetic Fields on Circadian Rhythm Control in Mice

Louise Lundberg,^{1,2} Zenon Sienkiewicz,¹ Daniel C. Anthony,²
and Kerry A. Broom ^{1*}

¹Public Health England, Chilton, United Kingdom

²Department of Pharmacology, University of Oxford, Oxford, United Kingdom

Artificial light and power frequency magnetic fields are ubiquitous in the built environment. Light is a potent zeitgeber but it is unclear whether power frequency magnetic fields can influence circadian rhythm control. To study this possibility, 8–12-week-old male C57BL/6J mice were exposed for 30 min starting at zeitgeber time 14 (ZT14, 2 h into the dark period of the day) to 50 Hz magnetic fields at 580 μ T using a pair of Helmholtz coils and/or a blue LED light at 700 lux or neither. Our experiments revealed an acute adrenal response to blue light, in terms of increased adrenal *per1* gene expression, increased serum corticosterone levels, increased time spent sleeping, and decreased locomotor activity (in all cases, $P < 0.0001$) compared to an unexposed control group. There appeared to be no modulating effect of the magnetic fields on the response to light, and there was also no effect of the magnetic fields alone (in both cases, $P > 0.05$) except for a decrease in locomotor activity ($P < 0.03$). Gene expression of the cryptochromes *cry1* and *cry2* in the adrenals, liver, and hippocampus was also not affected by exposures (in all cases, $P > 0.05$). In conclusion, these results suggest that 50 Hz magnetic fields do not significantly affect the acute light response to a degree that can be detected in the adrenal response. Bioelectromagnetics. 2019;40:250–259. © 2019 Bioelectromagnetics Society.

Keywords: ELF; behavior; animals; biological clock; clock genes

INTRODUCTION

Modern lifestyles and behaviors, such as rapid travel across multiple time zones, shift work, or exposure to artificial light at night can cause disturbances to circadian rhythms. A number of studies have suggested that power frequency magnetic fields associated with the transmission, distribution, or use of electricity also may affect circadian rhythms, with effects on sleep quality [Stevens, 1987; Akerstedt et al., 1999; Lewczuk et al., 2014; Vanderstraeten et al., 2015].

Cryptochromes are central players in circadian rhythm control and have been proposed as a possible magnetosensitive candidate molecule in plants and animals [Gegeer et al., 2008; Gegeer et al., 2010; Fedele et al., 2014b]. This sensitivity to magnetic fields has been suggested to utilize a radical pair reaction [Wiltschko and Wiltschko, 2014].

Circadian rhythms are modulated by zeitgebers that can delay or advance the rhythm. Light is the most potent of these zeitgebers and it entrains the internal circadian rhythm to the external environment via photoreceptors in the eye [Roenneberg and Foster, 1997; Shigeyoshi et al., 1997; Roenneberg and Merrow, 2003; Saganich et al., 2006]. If light reaches the intrinsically photoreceptive retinal ganglion cells

(ipRGCs) during the dark period of the 12:12 light dark (LD) cycle, the cell membrane is depolarized, leading to glutamate excretion in the suprachiasmatic nucleus (SCN) via the retinohypothalamic tract. This induces phosphorylation of cAMP response element binding (cAMP cyclic adenosine monophosphate; CREB) and its binding to cAMP response element (CRE)-containing promotor regions in light sensitive genes, for example, *per1* and *per2*, in turn leading to a rapid increase in transcription of these genes [Albrecht et al., 1997; Shearman et al., 1997; Naruse et al., 2004]. Like the cryptochromes, the PERIOD

Grant sponsor: Public Health England.

Conflicts of interest: ZS is a member of the main Commission of ICNIRP, and has shares in the BT group.

*Correspondence to: Kerry A. Broom, Public Health England, Centre for Radiation, Chemical and Environmental Hazards, Chilton OX11 0RQ, UK. E-mail: kerry.broom@phe.gov.uk

Received for review 14 May 2018; Accepted 12 March 2019

DOI: 10.1002/bem.22188

Published online 04 April 2019 in Wiley Online Library (wileyonlinelibrary.com).

genes, *per1* and *per2*, are central players in circadian rhythm control.

Together with two other central core clock proteins, CLOCK (Circadian Locomotor Output Cycles Kaput) and BMAL1 (Brain and Muscle ARNT-Like1), cryptochrome and period (CRY and PER) form a transcriptional-translational feedback loop, the master clock, which has a self-sustained cycling of approximately 24 h [Hastings et al., 2014]. Signaling from the SCN neurons to target tissues cascade the rhythm of the master clock to the other cells in the body (such as the liver [Oishi et al., 2003; Liu et al., 2007]) and synchronizes their clocks. SCN and peripheral clock genes have been reported to be sensitive to modulation. For example, in chronically stressed rats, changes in clock gene expression has been reported in a number of brain regions including the hippocampus [Christiansen et al., 2016], and also in rats, altered feeding patterns has been reported to entrain liver clocks [Stokkan et al., 2001].

A well-established protocol to study the effects of light on circadian rhythms in mice consists of exposure to a 30 min light shock early in the dark period of the day, with assessment of the acute molecular response 30–60 min later, or the delayed behavioral response days afterwards [Hughes et al., 2012]. In addition to a strong response to the light shock in the SCN, the most rapid response is seen in the adrenal glands [Ishida et al., 2005]. Significant increases in SCN and adrenal *per1* expression and in serum corticosterone levels have been reported 30–60 min after light shock at zeitgeber time (ZT) 14–16 (2–4 h into the dark period of the day), when the baseline expression of both is low but not at its lowest [Shigeyoshi et al., 1997; Ishida et al., 2005; Loh et al., 2008; Kiessling et al., 2014; Pilonis et al., 2016]. In addition to the acute changes in *per1* expression and corticosterone levels, nocturnal light shock also induces sleep. This has been reported to occur within 2–20 min after the onset of light shock depending on the wavelength of the light and balance between the responses in the SCN and ventrolateral preoptic area [Pilonis et al., 2016]. Pilonis et al. [2016] demonstrated that green light at 530 nm induces sleep rapidly, while the sleep response induced by blue light at 470 nm is delayed, resulting in significantly longer sleep duration after light exposure during the dark phase of the day, in mice exposed to green light compared to mice exposed to blue light.

Long-term exposure to power frequency magnetic fields (50 Hz) has been reported to potentiate the effects of known carcinogenic or mutagenic chemical or physical agents in both experimental and human

studies [Juutilainen, 2008; Turner et al., 2017]. An opinion from a European Union scientific committee in 2015 concluded that while ELF fields appear to interact with some chemical and physical agents, the interaction is not unitary, and sometimes there appears an increased response, and other times a decreased response. However, this opinion was based on limited data with few animal studies, and few studying short-term exposures to 50 Hz magnetic fields. Nevertheless, it highlights the potential complexity of possible interactions between ELF fields and other agents [SCENIHR, 2015].

The light shock protocol described above was used to investigate if an acute exposure to a 50 Hz magnetic field could have effects on *per1* expression and corticosterone levels, or have effects on sleep, and thereby influence circadian rhythm control both in the presence and absence of light shock. Blue light was used for induction of light shock, since it is known to increase the involvement of the retinal photopigment melanopsin [Hughes et al., 2012].

MATERIALS AND METHODS

Animals

Male C57BL/6J mice (Envigo, Bicester, UK) at 8–12 weeks of age were group-housed in polycarbonate cages in a ventilated cabinet (SCANBUR, Copenhagen, Denmark) under controlled environmental conditions (19–21 °C; RH 45–65%) with a light cycle from 00:00 to 12:00 h. Standard laboratory diet and water were freely available, and bedding was provided by commercial aspen wood shavings and shredded tissue paper. Animals were kept under these conditions for at least two weeks before any procedure to ensure entrainment to this light cycle.

All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986, and all protocols were approved by the Animal Welfare Ethical Review Body at the Center for Radiation, Chemical and Environmental Hazards, Public Health England.

Experimental Protocols

Individual mice were placed within each compartment of the exposure cages at the start of the dark phase (at ZT12) and kept under constant dark (DD 12:12) conditions. To assess the effects of magnetic fields on the nocturnal light response, after 26 h in the dark, animals were exposed for 30 min starting at ZT14 to 50 Hz magnetic fields at 580 μ T and/or a blue LED light at 700 lux or neither (Fig. 1A). Thus there were four different

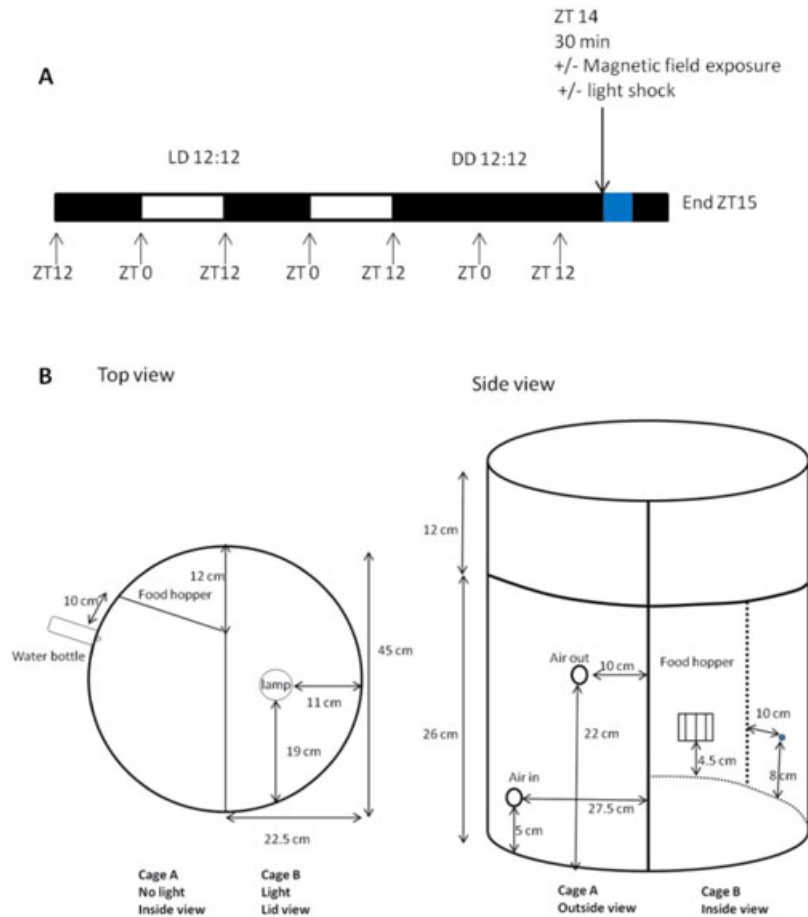


Fig. 1. **(A)** Protocol used to study the nocturnal light response. **(B)** Exposure cages with light and camera mounted on one side of the lid (Cage B).

treatment groups ($n=6$ for each group): a non-exposed group that was not exposed to the magnetic field or blue light shock (MF-/LS-); another group was exposed to the blue light shock but not to the magnetic field (MF-/LS+); a further group was exposed to the magnetic field but not exposed to the blue light shock (MF+/LS-); and the final group was exposed to both the magnetic field and the blue light shock (MF+/LS+). Mice were free to move during exposures and were not restrained.

All mice were euthanized in the exposure cages 30 min after the end of exposure (at ZT15) by increasing the concentration of CO₂ using appropriate equipment (VTS ANO44, Vet-Tech Solutions, Congleton, UK). Animals were under observation using CCD cameras (NC1358WA, Sony, Tokyo, Japan) during this procedure. The euthanized mice were moved to a procedure room and blood was collected by cardiac puncture prior to serum preparation. The adrenal glands, liver, and dorsal hippocampi were collected for gene expression analysis and stored at -80°C .

Exposure System

Magnetic field exposures were performed using a pair of custom-built horizontal Helmholtz coils with an internal diameter of 0.8 m. Each coil consisted of 180 turns of 1 mm diameter, bifilar copper wire (Wires.co.uk, Great Dunmow, UK) wound around a circular frame of Bear Brand TUFNOL resin (fabricated by Engineering Solutions & Supply, Newbury, UK). In order to minimize noise and vibration, the wires were impregnated with epoxy resin (RP-522, PRF Composite Materials, Poole, UK) and the assembly stood on anti-vibration rubber mounts.

The sinusoidal 50 Hz 580 μT magnetic field was generated using a waveform generator (33502B, Keysight Technologies, Santa Rosa, CA) and two bipolar operational power amplifiers (BOP 50-8M, Kepco, Flushing, NY) connected to the coils, and controlled using a personal computer running custom-made software. Each power amplifier was connected to one of the bifilar pairs. The coils were calibrated using

bespoke software (Low Frequency Magnetic Field Exposure System V2, Public Health England, Oxfordshire, UK) that adjusted the output of the amplifiers while measuring the field generated using a three-axis fluxgate magnetometer (MAG-03MC1000, Bartington Instruments, Witney, UK). The magnetic field was found to be homogenous ($\pm 10\%$ of the nominal value) within a diameter of 40 cm from the center of the coils. The background time-varying magnetic field in the laboratory was measured using an EMDEX II field meter (ENERTECH, Patterson, CA) and found to be 0.1–0.2 μT .

Exposure Cages

The experiments were carried out using cylindrical exposure cages (0.45 m diameter, 0.38 m height) made from lightproof, black, polymethyl methacrylate material. Each cage was divided into two equal compartments (Fig. 1B). Both compartments were provided with standard diet and water ad libitum and with aspen shavings as bedding. A narrowband blue LED light (GU10, Interlux, Pazin, Croatia) with a peak at 457 nm was mounted into the lid of one compartment. When energized, the LED produced 700 lux at the level of the floor in the center of the compartment, but had no measurable value in the other compartment. Because the cages were largely closed to the outside, compressed air was provided to both compartments at 2 L/min: this also produced a constant background noise in the cages. One cage was placed centrally between the coils, while the other was placed away from the coils where the 50 Hz magnetic field was at background levels. Use of the coils did not measurably increase the ambient temperature in the exposure cages.

Recording of Activity and Sleep

The behavior of the mice in the exposure cages was recorded using a digital IR CCD camera (NC1358WA, Sony, Tokyo, Japan) connected to a DVR (SRD-476D, Samsung, Seoul, South Korea). Sleep (latency and duration) was measured manually as immobility, when the mice had both eyes closed for more than 5 s (this method has been validated for assessment of sleep behavior in mice [Fisher et al., 2012]); distance moved was measured using EthoVision XT 9 software (Noldus Information Technologies, Wageningen, the Netherlands).

Corticosterone Enzyme Immunoassay

Serum corticosterone levels were measured using an enzyme immunoassay kit (ADI-900-097,

Enzo Life-Sciences, Farmingdale, NY), following the manufacturer's protocol. Samples were initially mixed with serum displacement reagent and diluted 40 times in assay buffer. The optical density was measured at 430 nm using a Synergy HT plate reader and KC4 software (BioTek, Swindon, UK). All samples were run in duplicate ($\text{CV} < 5\%$).

qPCR

RNA extraction was performed using the RNeasy minikit (74106, Qiagen, Hilden, Germany) following the manufacturer's protocols. Before extraction, tissues were lysed in RLT lysis buffer (74106, Qiagen) from the RNeasy kit with 1% beta-mercaptoethanol (M6250, Sigma-Aldrich, Gillingham, UK) and homogenized using a pestle homogenizer (47748–370, VWR International, Lutterworth, UK). The lysate was then homogenized using the QIAshredder system (M3148, Qiagen). Sample purity was evaluated from the 260/280 wavelength ratio. Ratios of 1.8–2.2 were considered acceptable.

cDNA conversion of RNA was performed using High-Capacity cDNA Reverse Transcription Kit (4368814, Applied Biosystems, Foster City, CA) following the manufacturer's protocol.

qPCR was carried out using PerfeCTa SYBR Green FastMix (95072, QuantaBio, Beverly, MA) and a LightCycler 480 (Roche Diagnostics, Burgess Hill, UK). The $\Delta\Delta\text{Ct}$ method was used to estimate differences in expression level between groups [Livak and Schmittgen, 2001]. Expression of the gene of interest was normalized to the geometric mean of expression of two reference genes (*gapdh*, *β -actin*, Primerdesign, Chandlers Ford, Hampshire, UK): *per1* (accession no: NM_011065, F: 5'TCCTCCTCCTACACTGCCTCT3', R: 5'TGGCTGACGACGGATCTTT3' [Loh et al., 2008], *cry1* (accession no: NM_007771, F: 5'GATC CACCATTAGCCAGACAC3', R: 5' ACAGCCAC ATCCAACCTTCCA3', Primerdesign), *cry2* (accession no: NM_009963, F: 5' CAAGCACTTGGAACGG AAGG3', R: 5' GAAGAGGCGGCAGGAGAG3', Primerdesign). Primers were used at a concentration of 300 nM and 25 ng template was used.

Statistical Analyses

All statistical analyses were carried out using Minitab 17 (Minitab, Coventry, UK). Comparison of experimental groups was performed using two-way ANOVA for the effects of magnetic field exposure and blue light shock. Post hoc testing was performed using Bonferroni's post hoc test; $P < 0.05$ was considered significant.

RESULTS

The effects of magnetic field exposure or blue light shock on *per1* gene expression in the adrenals, liver, and hippocampus are shown in Figure 2, along with serum corticosterone levels. Except in the hippocampus, the blue light shock tended to increase *per1* expression or serum corticosterone levels, while exposure to the magnetic field caused a very slight decrease in *per1* expression or serum corticosterone. However, only some of the changes caused by exposure to the blue light shock were statistically significant.

In the adrenals, exposure to the blue light shock caused a significant increase in *per1* expression ($F(1,26)=26.52$, $P=0.000$) both on its own (MF-/LS- vs. MF-/LS+ (95% CI [0.117, 0.808] $P=0.004$)) and in combination with the magnetic field (MF+/LS- vs. MF+/LS+ (95%CI [0.081, 0.827] $P=0.011$)). Exposure to the magnetic field resulted in a significant increase on *per1* expression ($F(1,26)=5.26$, $P=0.03$). This was attributed to differences between the MF-/LS+ and MF+/LS- groups (95%CI [0.317, 1.008] $P=0.000$) since any changes caused by exposure to the field alone (MF-/LS- vs. MF+/LS- (95%CI [-0.545, 0.145] $P=0.661$)) or in combination with the blue light shock (MF-/LS+ vs. MF+/LS+ (95%CI [-0.581, 0.165] $P=0.737$)) were not significant. There was no significant interaction between the light shock and magnetic field ($F(1,26)=0$, $P=0.963$).

In the liver, *per1* expression was significantly increased by exposure to blue light shock ($F(1,18)=4.798$, $P=0.0419$), but this could only be attributed to differences between the two groups exposed to blue light shock and the two groups that had not been exposed to the blue light shock (comparing MF-/LS- plus MF+/LS- with MF-/LS+ plus MF+/LS+ (95%CI [0.014, 0.660] $P=0.042$)). No significant changes were caused by exposure to the light shock alone (MF-/LS- vs. MF-/LS+ (95%CI [-0.392, 1.012] $P=1.00$)) or in combination with the magnetic field (MF+/LS- vs. MF+/LS+ (95%CI [-0.218, 0.946] $P=0.481$)). Exposure to the magnetic field had no significant effect on *per1* expression ($F(1,18)=1.261$, $P=0.2762$). There was no significant interaction between the light shock and magnetic field ($F(1,18)=0.03$, $P=0.862$).

In the hippocampus, *per1* expression was not significantly changed by exposure to blue light shock ($F(1,19)=0.12$, $P=0.732$) or the magnetic field ($F(1,19)=2.42$, $P=0.136$). There was no significant interaction between the light shock and magnetic field ($F(1,27)=0.12$, $P=0.732$).

The serum corticosterone levels were significantly increased by exposure to the blue light shock ($F(1,28)=22.21$, $P=0.000$) but only in the absence of the magnetic field (MF-/LS- vs. MF-/LS+ (95% CI [17.3, 92.1] $P=0.002$); MF+/LS- vs. MF+/LS+ (95%CI [-4.4, 70.4] $P=0.110$)). Exposure to the magnetic field had no significant effect ($F(1,28)=1.37$, $P=0.251$) and there was no significant

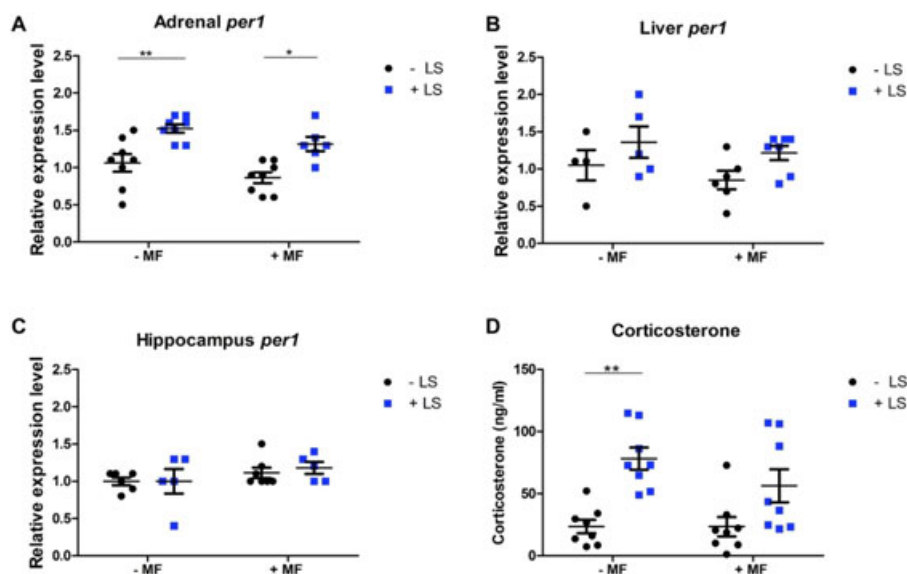


Fig. 2. Magnetosensitivity of the light shock response. (A–D) Exposure to the magnetic field (MF) had no significant effect in any tissue, but exposure to the blue light shock (LS) resulted in significant increases in *per1* expression in the adrenals (A) and in serum corticosterone levels (D). Results are presented as mean \pm SEM; $n=4-8$. * $P < 0.05$, ** $P < 0.01$.

interaction between the light shock and magnetic field ($F(1,28) = 1.36$, $P = 0.253$).

The effects of magnetic field exposure or blue light shock on sleep behavior are shown in Figure 3 in terms of latency to sleep and sleep duration both during the 30 min exposure period to either the blue light shock and/or magnetic field and the 30 min post-exposure period.

The latency to sleep was not significantly changed by exposure to either the blue light shock ($F(1,27) = 1.90$, $P = 0.179$) or the magnetic field ($F(1,27) = 0.32$, $P = 0.575$). There was no significant interaction between the blue light shock and magnetic field ($F(1,27) = 0.41$, $P = 0.526$). However, there were behavioral differences between the groups. In the MF-/LS- group only four of the eight mice went to sleep during the exposure period, and in the MF+/LS- group only two of the eight mice went to sleep. In comparison, all eight mice in the MF-/LS+ group went to sleep during exposure, with average sleep latency of 17.2 ± 2.2 min, and in the MF+/LS+ group seven out of eight mice went to sleep, with an average sleep latency of 16.9 ± 3.1 min. In addition, it was observed that mice that were asleep at the onset of the exposure period were aroused by the blue light shock, and not by the magnetic field.

Sleep duration was significantly increased by exposure to the blue light shock ($F(1,27) = 44.51$, $P = 0.000$) both on its own (MF-/LS- vs. MF-/

LS+ (95%CI [10.88, 43.53] $P = 0.000$)) or in combination with the magnetic field (MF+/LS- vs. MF+/LS+ (95%CI [10.97, 44.77] $P = 0.000$)). Exposure to the magnetic field had no significant effect on sleep duration ($F(1,27) = 0.07$, $P = 0.796$) and there was no significant interaction between the light shock and magnetic field ($F(1,27) = 0.01$, $P = 0.936$).

There were significant differences between treatment groups in locomotor activity as measured by distance moved in the cage over 60 min ($F(3,376) = 19.85$, $P = 0.000$) with significant differences between the non-exposed group and the other three groups (MF-/LS- vs. MF-/LS+ (95%CI [-285.7, -128.2] $P = 0.000$); MF-/LS- vs. MF+/LS- (95%CI [-195.3, -378] $P = 0.001$); MF-/LS- vs. MF+/LS+ (95%CI [-278.7, -121.2] $P = 0.000$), and between the group exposed only to the magnetic field and both groups exposed to the blue light shock (MF+/LS- vs. MF-/LS+ (95%CI [-169.1, -11.6] $P = 0.013$; (MF+/LS- vs. MF+/LS+ (95%CI [-1621, -4.6] $P = 0.029$)).

The effects of magnetic field exposure and/or blue light shock on *cry1* and *cry2* gene expression in the adrenals, liver, and hippocampus are shown in Figure 4. There were no significant effects of blue light shock or magnetic field exposure on gene expression of *cry1* and *cry2* in any of the three tissues.

However, in the liver, for *cry1* expression, there was a near-significant interaction between the blue

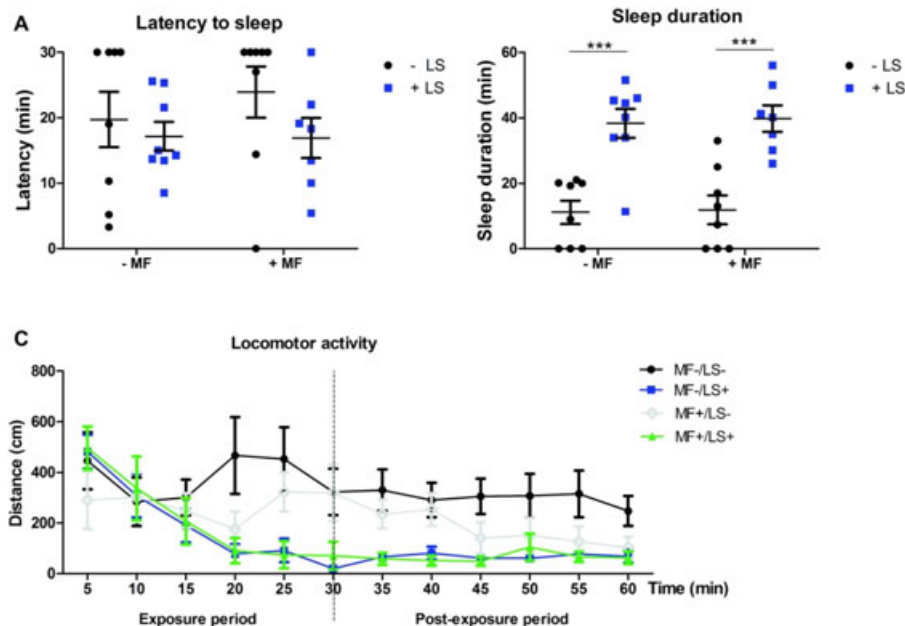


Fig. 3. Behavioral responses to blue light shock and magnetic field exposure. (A) LS and MF had no significant effects on sleep latency. (B) LS had a significant effect on sleep duration (C) Both LS and MF had a significant effect on locomotion compared to the non-exposed group ($P < 0.03$ in all cases). Data are presented as mean \pm SEM; $n = 7-8$. *** $P < 0.001$.

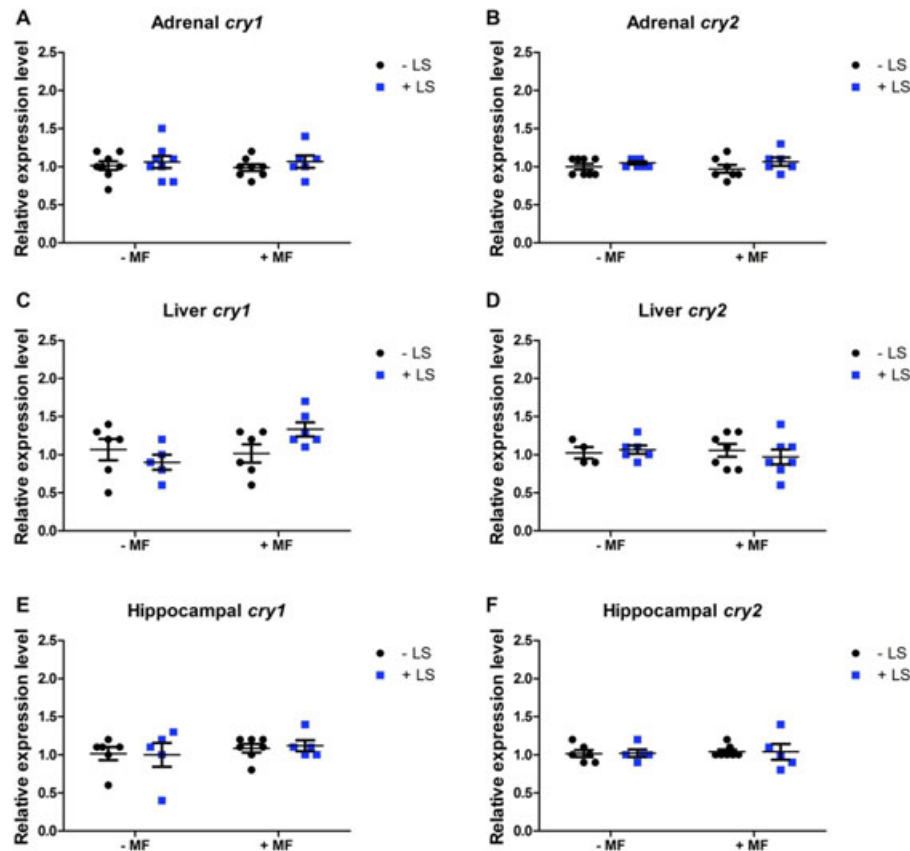


Fig. 4. Changes in cryptochrome gene expression after blue light shock and magnetic field exposure. (A–F) Exposure to the MF and/or LS had no significant effects on *cry1* and *cry2* in the adrenal glands, liver and hippocampus (A–F). Data are presented as means \pm SEM; $n = 4$ –8.

light shock and magnetic field exposure ($F(1,19) = 4.3$, $P = 0.052$), but post hoc testing could not attribute this difference to a specific treatment group comparison.

DISCUSSION

To the best of our knowledge, this is the first in vivo study to investigate the potential modulating effects of 50 Hz magnetic fields on the molecular mechanisms of the adrenal light response. Significant increases in both corticosterone levels and *per1* expression were seen following combined exposure to a magnetic field at 580 μ T and blue light, but these changes were largely attributable to an effect of the light shock since comparable increases were seen using the blue light alone. Interestingly, exposure to the magnetic field reduced adrenal *per1* expression in both groups that were exposed to the magnetic field compared to the groups that were not exposed to this field. However, the size of these decreases was small and differences between treatment groups were not

statistically significant. The possibility that magnetic field exposure might have an inhibitory effect on *per1* expression was also suggested by the results of a pilot experiment (data not shown) that used a much lower magnetic flux density of 300 μ T. In this experiment, a non-significant 30% reduction in *per1* expression was found between the group exposed to the magnetic field exposure group without light shock and the group that was not exposed to either the magnetic field or light shock. Nevertheless, it seemed reasonable to investigate at approximately double the flux density, (at 580 μ T), and, given the present results, It would be interesting to investigate the effects of an even more intense field (although such fields may not be relevant outside some occupational settings). In addition, no significant effects on corticosterone levels were seen either at 300 μ T in the pilot experiments or at 580 μ T in the main experiment, and it was not possible to see any potential trends due to the relatively low levels of corticosterone measured in the animals without light shock, and the variability in the data.

To further investigate the impact of the change in adrenal *per1* expression after magnetic field exposure in other tissues, gene expression was measured in the liver and dorsal hippocampus. The liver is particularly important for controlling metabolism and, as with any organ, the circadian clock rhythmically regulates genomic and epigenetic processes to anticipate and adapt to changes [Hastings et al., 2008]. Disturbances to the circadian clocks in the liver (set by daily feeding) and brain (set by light) are known to affect metabolic and cognitive functions and thereby general health [Lamont et al., 2007; Hastings et al., 2008; Zwighaft et al., 2016]. No changes in *per1* expression were found after magnetic field exposure or light shock, either alone or together.

Although the effect of power frequency magnetic field exposure on the nocturnal light response has not been investigated in rodents, a number of studies in other non-mammalian models, such as transgenic *Drosophila*, have investigated light-dependent magnetosensitivity, focusing on the cryptochromes. Wild type (WT) *Drosophila* express type I CRY, a photoreceptor that is sensitive to magnetic fields under blue light [Yoshii et al., 2009]. Mammalian CRY1 and CRY2, both type II CRY, are involved in circadian rhythm control, but not photoreception [Vanderstraeten et al., 2012]. Foley et al. [2011] showed that *Drosophila* expressing human CRY2 were behaviorally sensitive to magnetic field exposure (at 10–500 μ T), and their response was also light-dependent. Fedele et al. [2014a] also studied light-dependent magnetosensitivity of *Drosophila* expressing human CRY1 or CRY2, and found that only flies expressing CRY2 were behaviorally sensitive to magnetic field exposure at 300 μ T and blue light. Based on the magnetosensitivity of human CRY, but with the limitations for studying this in vivo, magnetosensitivity of rodent CRY has been investigated in mice SCN slice cultures expressing luminescent PER2. No change in *per2* expression level or rhythm was observed after 50 Hz magnetic field exposure at 50–500 μ T under blue light. It was therefore concluded that the cellular environment plays an important role in both the light and magnetic field response. It is, however, possible that magnetic fields can affect mammalian CRY in its native environment in a manner that could not be detected in these assays. Since these studies were published, no evidence for magnetic field sensitivity of mammalian CRY has been found, and the precise mechanisms underlying the reported behavioral responses remain unknown.

In the experiments reported here, cryptochrome gene expression was measured in the adrenal glands,

liver, and dorsal hippocampus after magnetic field exposure in the presence or absence of blue light to investigate the acute effects of magnetic fields on *cry* transcription. No acute sensitivity to light was observed, which is in accordance with the lack of photoreceptor properties in mammals [Owens et al., 2012]. Also, no effect of magnetic field exposure was detected. However, a near-significant interaction between the magnetic field and blue light was detected on *cry1*, but only in the liver. This was due to an increase in *cry1* expression in the group exposed to both magnetic field and blue light compared to the three other exposure groups, indicating a possible additive effect of the two exposures. However, it is possible that this result is due to chance, as it seems unlikely that the combined exposure should have an acute effect on *cry1* expression in the liver but not in the adrenal glands where the light response is first detected.

As another measure of light response, in the current study, the behavior of the mice was tracked during and for 30 min after exposure to magnetic field and/or light shock. Exposure to light during the early dark phase of the day has previously been found to induce sleep in mice [Pilorz et al., 2016]. Here, the latency to sleep by the blue light shock at 700 lux, either alone or with magnetic field exposure (of about 17 min for both), was in line with that reported by Pilorz et al. [2016] for blue light shock at approximately 200 lux (17.5 ± 1.64 min). Magnetic fields alone did not affect sleep latency or the cumulative sleep duration. However, exposure to the magnetic field alone resulted in a significant difference in locomotory behavior. This effect was not as obvious as that caused by the blue light shock, and was most apparent around 15 min after the exposure period, in contrast to blue light shock where changes were seen during the exposure period. This might suggest that the magnetic field was acting as a weak environmental stimulus, but since this was the only behavioral indication in this experiment that the mice could sense or respond to the magnetic field, additional work is required to explore this suggestion.

Circadian clock disruption appears to have a role in neurodegenerative diseases [Charrier et al., 2017] and disruption of peripheral clocks, such as those in the liver have a role in metabolic diseases [Lamia et al., 2008; Jagannath et al., 2017], although the cause-and-effect relationship remains unclear [Lamia et al., 2008; Musiek, 2015; Jagannath et al., 2017]. However, it is accepted that alterations in circadian clocks affect metabolism and behavior [Karatsoreos et al., 2011]. In this study, although we observed no evidence that power frequency magnetic

fields alone do affect gene expression, sleep, or behavior, we did demonstrate some evidence that alterations in clock genes by blue light, and in combination with power frequency magnetic fields, could manifest in altered sleep and activity in mice. Although the mechanism(s) is not clear, or what the cause-and-effect relationship might be, it is interesting to consider whether such combined exposures could occur in the built environment and if they could have any implications for public health.

ACKNOWLEDGMENTS

LL was awarded a PhD studentship by Public Health England and these studies form part of her thesis submitted for a D Phil degree to the University of Oxford. We are also extremely grateful to Prof. Jim Metcalfe and the EMF Biological Research Trust for funding preliminary work on the light shock experiment; to Tony Hickman, Department of Physics, University of Oxford for advice regarding construction of the Helmholtz coils; and to Dr. Marina Khazova, Public Health England Chilton, for the optical measurements.

REFERENCES

- Akerstedt T, Arnetz B, Ficca G, Paulsson LE, Kallner A. 1999. A 50-Hz electromagnetic field impairs sleep. *J Sleep Res* 8:77–81.
- Albrecht U, Sun ZS, Eichele G, Lee CC. 1997. A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light. *Cell* 91:1055–1064.
- Charrier A, Olliac B, Roubertoux P, Tordjman S. 2017. Clock genes and altered sleep-wake rhythms: Their role in the development of psychiatric disorders. *Int J Mol Sci* 18:938.
- Christiansen SL, Bouzinova EV, Fahrenkrug J, Wiborg O. 2016. Altered expression pattern of clock genes in a rat model of depression. *Int J Neuropsychopharmacol* 19:1–13.
- Fedele G, Edwards MD, Bhutani S, Hares JM, Murbach M, Green EW, Dissel S, Hastings MH, Rosato E, Kyriacou CP. 2014a. Genetic analysis of circadian responses to low frequency electromagnetic fields in *Drosophila melanogaster*. *PLoS Genet* 10:e1004804.
- Fedele G, Green EW, Rosato E, Kyriacou CP. 2014b. An electromagnetic field disrupts negative geotaxis in *Drosophila* via a CRY-dependent pathway. *Nat Commun* 5:4391.
- Fisher SP, Godinho SI, Potheary CA, Hankins MW, Foster RG, Peirson SN. 2012. Rapid assessment of sleep-wake behavior in mice. *J Biol Rhythms* 27:48–58.
- Foley LE, Gegear RJ, Reppert SM. 2011. Human cryptochrome exhibits light-dependent magnetosensitivity. *Nat Commun* 2:356.
- Gegear RJ, Casselman A, Waddell S, Reppert SM. 2008. Cryptochrome mediates light-dependent magnetosensitivity in *Drosophila*. *Nature* 454:1014–1018.
- Gegear RJ, Foley LE, Casselman A, Reppert SM. 2010. Animal cryptochromes mediate magnetoreception by an unconventional photochemical mechanism. *Nature* 463:804–807.
- Hastings MH, Brancaccio M, Maywood ES. 2014. Circadian pacemaking in cells and circuits of the suprachiasmatic nucleus. *J Neuroendocrinol* 26:2–10.
- Hastings MH, Maywood ES, Reddy AB. 2008. Two decades of circadian time. *J Neuroendocrinol* 20:812–819.
- Hughes S, Hankins MW, Foster RG, Peirson SN. 2012. Melanopsin phototransduction: slowly emerging from the dark. *Prog Brain Res* 199:19–40.
- Ishida A, Mutoh T, Ueyama T, Bando H, Masubuchi S, Nakahara D, Tsujimoto G, Okamura H. 2005. Light activates the adrenal gland: Timing of gene expression and glucocorticoid release. *Cell Metab* 2:297–307.
- Jagannath A, Taylor L, Wakaf Z, Vasudevan SR, Foster RG. 2017. The genetics of circadian rhythms, sleep and health. *Hum Mol Genet* 26:R128–R138.
- Juutilainen J. 2008. Do electromagnetic fields enhance the effects of environmental carcinogens? *Radiat Prot Dosimetry* 132:228–231.
- Karatsoreos IN, Bhagat S, Bloss EB, Morrison JH, McEwen BS. 2011. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc Natl Acad Sci USA* 108:1657–1662.
- Kiessling S, Sollars PJ, Pickard GE. 2014. Light stimulates the mouse adrenal through a retinohypothalamic pathway independent of an effect on the clock in the suprachiasmatic nucleus. *PLoS ONE* 9:e92959.
- Lamia KA, Storch KF, Weitz CJ. 2008. Physiological significance of a peripheral tissue circadian clock. *Proc Natl Acad Sci USA* 105:15172–15177.
- Lamont EW, James FO, Boivin DB, Cermakian N. 2007. From circadian clock gene expression to pathologies. *Sleep Med* 8:547–556.
- Lewczuk B, Redlarski G, Zak A, Ziolkowska N, Przybylska-Gornowicz B, Krawczuk M. 2014. Influence of electric, magnetic, and electromagnetic fields on the circadian system: current stage of knowledge. *Biomed Res Int* 2014:169459.
- Liu S, Cai Y, Sothorn RB, Guan Y, Chan P. 2007. Chronobiological analysis of circadian patterns in transcription of seven key clock genes in six peripheral tissues in mice. *Chronobiol Int* 24:793–820.
- Loh DH, Abad C, Colwell CS, Waschek JA. 2008. Vasoactive intestinal peptide is critical for circadian regulation of glucocorticoids. *Neuroendocrinology* 88:246–255.
- Musiek ES. 2015. Circadian clock disruption in neurodegenerative diseases: Cause and effect? *Front Pharmacol* 6:1–6.
- Naruse Y, Oh-hashii K, Iijima N, Naruse M, Yoshioka H, Tanaka M. 2004. Circadian and light-induced transcription of clock gene *Per1* depends on histone acetylation and deacetylation. *Mol Cell Biol* 24:6278–6287.
- Oishi K, Miyazaki K, Kadota K, Kikuno R, Nagase T, Atsumi G, Ohkura N, Azama T, Mesaki M, Yukimasa S, Kobayashi H, Iitaka C, Umehara T, Horikoshi M, Kudo T, Shimizu Y, Yano M, Monden M, Machida K, Matsuda J, Horie S, Todo T, Ishida N. 2003. Genome-wide expression analysis of mouse liver reveals CLOCK-regulated circadian output genes. *J Biol Chem* 278:41519–41527.
- Owens L, Buhr E, Tu DC, Lamprecht TL, Lee J, Van Gelder RN. 2012. Effect of circadian clock gene mutations on nonvisual photoreception in the mouse. *Invest Ophthalmol Vis Sci* 53:454–460.
- Pilorz V, Tam SK, Hughes S, Potheary CA, Jagannath A, Hankins MW, Bannerman DM, Lightman SL, Vyazovskiy

- VV, Nolan PM, Foster RG, Peirson SN. 2016. Melanopsin regulates both sleep-promoting and arousal-promoting responses to light. *PLoS Biol* 14:e1002482.
- Roenneberg T, Foster RG. 1997. Twilight times: Light and the circadian system. *Photochem Photobiol* 66:549–561.
- Roenneberg T, Merrow M. 2003. The network of time: Understanding the molecular circadian system. *Curr Biol* 13: R198–R207.
- Saganich MJ, Schroeder BE, Galvan V, Bredesen DE, Koo EH, Heinemann SF. 2006. Deficits in synaptic transmission and learning in amyloid precursor protein (APP) transgenic mice require C-terminal cleavage of APP. *J Neurosci* 26:13428–13436.
- SCENIHR. 2015. Potential Health Effects Of Exposure To Electromagnetic Fields (EMF), Scientific Committee on Emerging and Newly Identified Health Risks.
- Shearman LP, Zylka MJ, Weaver DR, Kolakowski LF, Jr, Reppert SM. 1997. Two period homologs: Circadian expression and photic regulation in the suprachiasmatic nuclei. *Neuron* 19:1261–1269.
- Shigeyoshi Y, Taguchi K, Yamamoto S, Takekida S, Yan L, Tei H, Moriya T, Shibata S, Loros JJ, Dunlap JC, Okamura H. 1997. Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. *Cell* 91:1043–1053.
- Stevens RG. 1987. Electric power use and breast cancer: A hypothesis. *Am J Epidemiol* 125:556–561.
- Stokkan KA, Yamazaki S, Tei H, Sakaki Y, Menaker M. 2001. Entrainment of the circadian clock in the liver by feeding. *Science* 291:490–493.
- Turner MC, Benke G, Bowman JD, Figuerola J, Fleming S, Hours M, Kincl L, Krewski D, McLean D, Parent ME, Richardson L, Sadetzki S, Schlaefel K, Schlehofer B, Schuz J, Siemiatycki J, Tongeren MV, Cardis E. 2017. Interactions between occupational exposure to extremely low frequency magnetic fields and chemicals for brain tumour risk in the INTEROCC study. *Occup Environ Med* 74:802–809.
- Vanderstraeten J, Burda H, Verschaeve L, De Brouwer C. 2015. Could magnetic fields affect the circadian clock function of cryptochromes? Testing the basic premise of the cryptochrome hypothesis (ELF magnetic fields). *Health Phys* 109:84–89.
- Vanderstraeten J, Verschaeve L, Burda H, Bouland C, de Brouwer C. 2012. Health effects of extremely low-frequency magnetic fields: Reconsidering the melatonin hypothesis in the light of current data on magnetoreception. *J Appl Toxicol* 32:952–958.
- Wiltshko R, Wiltshko W. 2014. Sensing magnetic directions in birds: Radical pair processes involving cryptochrome. *Biosensors (Basel)* 4:221–242.
- Yoshii T, Ahmad M, Helfrich-Forster C. 2009. Cryptochrome mediates light-dependent magnetosensitivity of *Drosophila*'s circadian clock. *PLoS Biol* 7:e1000086.
- Zwighaft Z, Reinke H, Asher G. 2016. The liver in the eyes of a chronobiologist. *J Biol Rhythms* 31:115–124.