Modulatory actions of light on the behavioural responses to magnetic fields by land snails probably occur at the magnetic field detection stage

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The attenuation of opioid peptide-mediated antinociception or analgesia is a well-established effect of extremely low frequency (ELF) magnetic fields. Results of prior studies indicated a modulatory role for light such that when the ELF exposures were carried out in the absence of light, the inhibitory effect on analgesia was reduced. Here, we investigated whether this modulatory effect of light occurs at either the magnetic field detection stage or is associated with a post-detection mechanism. We compared the effects of the presence and absence of light on the attenuation of opioid-induced analgesia in the land snail, *Cepaea nemoralis*, by (i) an ELF magnetic field (15 min, 60 Hz, 141 μ T peak), and (ii) the prototypic opiate antagonist, naloxone. Determinations were performed during the subjective 'day' and 'night' in the presence (1.9 W m⁻² and 1.0 mW m⁻², respectively) and absence of light (less than 10⁻⁶ W m⁻²). The inhibitory effects of the ELF magnetic fields and naloxone on opioid-induced analgesia were similar in the presence of light; whereas in the absence of light the inhibitory effects of the ELF magnetic fields as a percentage of sham were markedly reduced, while those of naloxone were unaffected. This indicates that the modulatory effects of light on the actions of the ELF magnetic fields probably affect the detection mechanism prior to its coupling to the opioid system.

Keywords: opioid; analgesia; ELF bioeffects; land snails

1. INTRODUCTION

A number of magnetic field exposures have been shown to reduce exogenous opiate (e.g. morphine) and endogenous opioid (e.g. enkephalin, endorphin) peptide-mediated antinociception ('analgesia') in a variety of species, including humans (Kavaliers & Ossenkopp 1988, 1991; Prato *et al.* 1995; Betancur *et al.* 1994; Del Seppia *et al.* 1995; Papi *et al.* 1995). Also, extremely low frequency (ELF) magnetic field exposures are reported to modify homing pigeon behaviour (Papi *et al.* 1992), and spatial learning in rodents (Kavaliers *et al.* 1996), in a manner consistent with alterations in opioid activity. Together, these results, from a number of different laboratories and species, indicate that ELF magnetic fields can affect opioid systems and their behavioural actions.

A variety of different mechanisms, including induced electric currents (Weaver & Astumian 1990; Greene *et al.* 1991; Polk 1992), magnetite (Kirschvink & Walker 1985), radical pair recombinations (Scaiano *et al.* 1994; Grissom 1995; Brocklehurst & McLauchlan 1996), and resonance interactions (e.g. parametric resonance model (PRM); Lednev 1991, 1994, 1996) have been proposed to explain magnetic field detection and effects. Using the well-defined

inhibitory effects which magnetic fields have on opioid-induced analgesia in the land snail, *Cepaea nemoralis*, we have addressed the possible detection mechanisms (Prato *et al.* 1995, 1996a). Through manipulations of both the static (B_{DC}) and ELF (B_{AC}) magnetic field exposure conditions, we presented evidence suggesting that magnetic field detection and/or its effects, follow a 'parametric resonance' related mechanism, rather than one being dependent on either induced currents or magnetite.

Recently, we observed that carrying out the ELF magnetic field exposures in the absence of light significantly reduced the inhibitory effects on opioid analgesia (Prato *et al.* 1996b, 1997, 1998). Both the light-dependent and light-independent behavioural effects of ELF magnetic fields were shown to be consistent with a parametric related mechanism (Prato *et al.* 1997). These effects were also consistent with the evidence for light-dependent magnetic compass orientation of several species of birds, salamanders and fruit flies (Phillips & Borland 1992; Phillips & Sayeed 1993; Wiltschko *et al.* 1993; Wiltschko & Wiltschko 1995).

This modulatory effect of light may occur at the primary magnetic field detection mechanism (Prato *et al.* 1996b). However, it could be argued that light just acts as a 'post-detection amplifier', i.e. light could affect the responses of opioid systems to a fixed stimulus produced

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by the magnetic field exposure. The present study was designed to resolve these modes of action. We compared the effects of the presence and absence of light on the attenuation of opioid peptide-induced 'analgesia' in the snail, *Cepaea*, by (i) an ELF magnetic field, and (ii) the prototypic opiate antagonist, naloxone. If light affected the attenuation of opioid-induced analgesia equally for both these treatments, then the results would suggest that the light effects were post-ELF magnetic field detection. However, if light conditions did not affect the sensitivity of the endogenous opioid system to naloxone antagonism, then the modulatory effects of light on the actions of the ELF magnetic fields probably affect the magnetic field detection mechanism prior to its coupling to the opioid system.

2. METHODS

(a) *Animals*

Land snails (*Cepaea nemoralis*) were collected from old field sites (ambient 60 Hz magnetic field $<0.01 \mu\text{T}_{\text{rms}}$) and held in terraria (60 Hz magnetic field $<0.4 \mu\text{T}_{\text{rms}}$) under an approximate 12 h light:12 h dark cycle at $22 \pm 2^\circ\text{C}$. The snails had lettuce available *ad lib*.

(b) *Assessment of nociception*

Nociceptive sensitivity of individual snails was measured as the latency of their foot-lifting response to an aversive thermal stimulus (Kavaliers & Ossenkopp 1991; Dyakonova *et al.* 1995). Individual hydrated snails were placed on the thermal stimulus ('hotplate'; $40.0 \pm 0.2^\circ\text{C}$, based on previous studies (Kavaliers *et al.* 1983)) and the latency of their avoidance of the stimulus was determined. The avoidance behaviour was a characteristic elevation of the anterior portion of the foot. After displaying this aversive, or more appropriately, 'nociceptive' response, individual snails were quickly removed from the thermal surface. The appropriateness of the use of the term 'nociception' for describing the behavioural response of *Cepaea* is discussed in Kavaliers (1988).

After the electrically heated hotplate, i.e. aversive thermal stimulus, reaches operating temperatures, the heating cycles on and off for approximately 2 s every 12 s. During this heating, a maximum 60 Hz field of $2.8 \mu\text{T}_{\text{rms}}$ is reached. Separate experiments with a hotplate heated by water pumped from a circulating bath, which has no associated ELF magnetic field, have given similar results in prior investigations of the effects of ELF fields on opioid analgesia (Prato *et al.* 1996a,b, 1997, 1998).

(c) *Magnetic field conditions*

Two coils configured in a quasi-Helmholtz design (100 cm in diameter, spaced 103 cm apart) generated the 60 Hz ELF magnetic field. This coil consisted of a total of 200 turns of #24 motor enamel wire with a total resistance of 44Ω . The coil was covered with a resin coating to reduce vibration. Line current (60 Hz) from standard outlets in the laboratory equipped with surge suppressors (EMI/RF Filters, Fisher, Canada), and regulated with a variable transformer (PowerSlot 116b, Superior Electric, CT) was used, and produced a 60 Hz ELF magnetic field in the horizontal (east–west) direction. For the ELF magnetic field exposure conditions, snails were placed in the centre of the Helmholtz coil where B_{AC} was set to $141 \mu\text{T}$ peak linearly polarized in the west–east direction. This coil only generated the 60 Hz ELF field, however, there was an ambient

static field (B_{DC}) which was the same for both ELF and sham exposures: a $17 \mu\text{T}$ horizontal ($15 \mu\text{T}$, which was parallel to B_{AC} , west to east, and $7 \mu\text{T}$ north to south), and $28 \mu\text{T}$ vertical. In the sham exposure conditions, snails were placed in the centre of the Helmholtz coil and the ambient B_{AC} was less than $0.4 \mu\text{T}$ peak at 60 Hz. The 60 Hz fields were measured using a model ELF 66D meter and the DC fields were measured with a fluxgate magnetometer model FGM-3D (both Walker Scientific, Worcester, MA).

It has been previously shown that this combination of B_{AC} and B_{DC} fields are effective in reducing opioid-induced analgesia (Kavaliers & Ossenkopp 1991). It is of interest that 60 Hz $141 \mu\text{T}$ peak fields are very effective in reducing opioid-induced analgesia almost independent of the strength (Prato *et al.* 1995) and direction of the static field, even though the relative directions of B_{AC} and B_{DC} have proven to be important for other amplitudes of B_{AC} . It remains for present theories like the PRM to be expanded and modified to allow for such geometries.

(d) *Light-dark procedures*

Basal nociceptive responses were measured and snails were then individually injected with a $2.0 \mu\text{l}$ microsyringe (#75, Hamilton, Nevada) into the haemocoel with the specific enkephalinase inhibitor SCH34826 (SCH; {(S)-N-(N-(((2,2-dimethyl-1,3-dioxolan-4-yl) methoxy)carbonyl)-2-phenylethyl)-L-phenylalanine)-B-alanine}) $2.0 \mu\text{g}$ total in $2.0 \mu\text{l}$ saline. SCH inhibits endopeptidase 24.11 activity and selectively increases methionine- and leucine-enkephalin activity (Chipkin *et al.* 1988). Both methionine- and leucine-enkephalin have been localized in *Cepaea* and shown to have analgesic effects (Kavaliers & Ossenkopp 1991; Sakharov *et al.* 1993; Dyakonova *et al.* 1995). Prior studies established that SCH induces maximum enkephalin-mediated analgesia in *Cepaea* 15–30 min after injection (Saksida *et al.* 1993).

The snails ($n=20$, per group) were then exposed to either the ELF or sham fields, in either a 'light' (presence of light) or 'dark' (absence of light) condition, for 15 min after which their thermal response latencies were again measured. Previous studies revealed no significant differences between the response latencies of saline treated and uninjected snails (Kavaliers *et al.* 1983). Exposure of only the SCH injection solution to the magnetic fields had no effects on its potency (Prato *et al.* 1995).

Other groups of snails ($n=10$, per group) were injected with either the opiate antagonist naloxone ($1.0 \mu\text{g}$ per $1.0 \mu\text{l}$ of saline), or $1.0 \mu\text{l}$ saline vehicle prior to the SCH treatment. This dose of naloxone has been shown previously to significantly reduce but not completely block opioid peptide-induced analgesia in *Cepaea* (Saksida *et al.* 1993). The snails were then maintained under the sham field conditions in either a 'light' or 'dark' condition for 15 min, after which their thermal response latencies were measured.

Determinations on separate groups of ELF-exposed and naloxone-treated snails and their controls were carried out during the midday and midnight portions of the ambient light–dark cycle. All testing was done in the same laboratory under fluorescent (daytime; Sylvania SuperSaver cool white 34W, GTE Canada), and red incandescent (night-time; Sylvania PhotoSafe Model 28A, GTE Canada) light conditions for the 'dark' and 'light' exposed snails.

In the light condition, snails were placed into translucent plastic containers made up of identical top and bottom halves ($12 \text{ cm square} \times 5 \text{ cm high}$, 250 ml weighing dishes, CanLab, Toronto Canada). For the 'dark' exposures, the top and bottom plastic containers were covered with black electrical tape.

Previous studies revealed no significant effect on response latencies because of the electrical tape itself and exposing the snails in the absence of light (daytime) did not alter response latencies in saline-injected snails (Prato *et al.* 1997). Once the snails were placed in the darkened containers the two halves were taped together to prevent light leakage. During the day and night phases 'light' levels were approximately 1.9 W m^{-2} and 1.0 mW m^{-2} , respectively, as measured with a spectroradiometer (Li-Cor, Lincoln, Nebraska). In both 'dark' conditions light levels were below the detection limits (less than 10^{-6} W m^{-2}). Results of previous control studies established that the ELF fields had no significant effects on the temperature in the container or that of the snails. In addition, results of previous studies have established that slight changes in temperature have no significant effects on the nociceptive responses of *Cepaea* (Kavaliers 1987). Molluscs such as *Cepaea* display ocular or cephalic photosensitivity at about 475–500 nm (Menzel 1979; Land 1984). Lower threshold, broader spectrum, extra-ocular photosensitivity is also evident in molluscs (Land 1984).

(e) Data analysis

Because the data tended to be non-homogeneous for group variances, non-parametric testing of the sham and exposed groups for each treatment condition was carried out using a Kruskal–Wallis one-way ANOVA on ranks, and all pairwise comparisons were done post hoc using the Dunn's test. When only two groups were compared, a Mann–Whitney *U*-test was used. To evaluate the interaction between magnetic field exposure or naloxone and the presence of light, a parametric two-way ANOVA with a post hoc Student–Neuman–Keuls test was used to compare latency times within treatment groups. In this ANOVA, the first factor had two levels corresponding to the light conditions (exposure in the presence of light and exposure in the absence of light), and the second factor had two levels (either sham and B_{AC} -exposed or naloxone- and saline-injected). The pre-SCH latency values were compared using a one-way ANOVA on ranks. The comparison was made for the eight groups tested during the day and the eight tested at night. A significance level of $p < 0.05$ (α level) was used throughout.

To quantify the effect of the magnetic field exposure as a per cent inhibition of sham, the difference in the means of the sham group and the exposed group were divided by the mean of the sham group and then multiplied by 100, i.e.

$$((\text{sham latency} - B_{AC} \text{ exposed latency}) / \text{sham latency}) \times 100.$$

Error propagation for this quantity assumed parametric statistics and formulation as proposed by Bevington (1969).

3. RESULTS

The basal (pre-injection) values for the ELF magnetic field/sham-exposed and naloxone/saline-injected snails during the day were not significantly different ($H_7 = 5.21$, $p = 0.63$; the range of mean values was 5.1–5.65 s). Similarly, basal response latencies at night were also not different ($H_7 = 11.8$, $p = 0.11$; the range of mean values was 8.6–11.2 s). However, as is evident from figures 1 and 2, the basal response latencies at night were significantly greater than in the day ($T = 7.405$, $p < 0.0001$).

During both day and night, administration of SCH resulted in a significant increase in response latency,

Figure 1. Thermal (40°C) response latencies of snails injected with the enkephalinase inhibitor SCH34826 (SCH; $2.0 \mu\text{g}$) and exposed for 15 min to either a 60 Hz (ELFMF, $B_{AC} = 141 \mu\text{T}$ peak, $B_{DC} = \mu\text{T}$ parallel to B_{AC}) or a sham 60 Hz (Sham), in either the presence of light ('LIGHT EXPOSED') or absence of light ('DARK EXPOSED') in either the (a) day or (b) night. Response latencies recorded prior to (Pre) injection of SCH are shown combined for both the Sham and ELFMF groups since they were not significantly different. In all cases, $n = 20$. Vertical lines denote ± 1 s.e.m.

indicative of the induction of analgesia ($T > 55$, $p < 0.001$ in all cases), with a significantly greater level of analgesia evident at night than during the day, if raw latency scores are compared ($T > 65$, $p < 0.002$ in all cases). (However, if basal latency scores are subtracted from post-SCH values, then the increase in latency time induced at night is not significantly greater than that in the daytime.)

In both the 'day' and 'night' exposure to the ELF field, there resulted a significant reduction in the level of SCH-induced analgesia. When these exposures occurred in the absence of light (i.e. darkness), the inhibitory effect of the ELF magnetic field on opioid-induced analgesia was significantly reduced. The interaction of ELF magnetic field with the light conditions when experiments were performed during the day cycle are shown in figure 1a and when performed during the night cycle are shown in figure 1b. For the experiments during the day cycle, the two-way ANOVA indicated that after correcting for

Figure 2. Thermal (40°C) response latencies of snails injected with the enkephalinase inhibitor SCH34826 (SCH; $2.0\text{ }\mu\text{g}$), and either the opiate antagonist, naloxone ($1.0\text{ }\mu\text{g}$ per $1.0\text{ }\mu\text{l}$ saline) or saline vehicle ($1.0\text{ }\mu\text{l}$) and maintained for 15 min in either the presence of light ('LIGHT EXPOSED') or absence of light ('DARK EXPOSED') in either the (a) day or (b) night. Response latencies prior to (Pre) injection of SCH are shown combined for both the Sham and ELFMF groups since they were not significantly different. In all cases, $n=10$. Vertical lines denote ± 1 s.e.m.

differences in ELF magnetic field exposure, there was a significant effect of light conditions ($F_{1,76}=107$, $p<0.001$), and that after correcting for differences in light exposure, there was a significant effect of ELF magnetic field exposure ($F_{1,76}=200.5$, $p<0.0001$). Furthermore, there was a significant interaction of light conditions and ELF magnetic field exposure conditions ($F_{1,176}=16.8$, $p<0.0001$). For experiments conducted during the night (figure 1b), results of the two-way ANOVA were similar (for variable light, $F_{1,76}=35.21$, $p<0.0001$; for variable ELF magnetic field, $F_{1,76}=203.27$, $p<0.0001$; for

Figure 3. Effect of ELF magnetic field exposure and naloxone treatments on per cent inhibition of sham (error bars correspond to ± 1 s.e.m.). Note that for naloxone treatment, the reduction of sham values was constant, independent of the day or night cycle and independent of the presence or absence of light between the injection of naloxone and the latency testing approximately 15 min later. However, the inhibition of opioid-induced analgesia by 60 Hz ELF magnetic fields ($141\text{ }\mu\text{T}$ peak) was dependent on light conditions during exposure.

interaction, $F_{1,176}=5.71$, $p<0.02$). Post hoc testing indicated that all four groups were significantly different during the day and also during the night. Note that in all four cases, the ELF magnetic field significantly reduced but did not completely block the level of SCH-induced analgesia as determined by ANOVA on ranks followed by post hoc testing, i.e. $H_3>55$, $p<0.0001$ in all cases.

Naloxone resulted in a significant reduction in the level of SCH-induced analgesia in both the day and night. Naloxone had equivalent effects in either the presence or absence of light. The interactions of naloxone treatment with the light condition when experiments were performed during the day are shown in figure 2a and when performed during the night cycle are shown in figure 2b. For experiments conducted during the day, there was a significant but weak effect of light after accounting for the effect of the naloxone/saline treatment ($F_{1,36}=5.72$, $p<0.02$), and a large effect associated with the naloxone/saline treatment after accounting for the light effect ($F_{1,36}=597.46$, $p<0.0001$). However, there was no significant interaction between light condition and saline/naloxone treatment ($F_{1,136}=1.13$, $p<0.30$). A similar statistical trend was observed for the night experiments shown in figure 2b (for variable light, $F_{1,36}=26.91$, $p<0.0001$; for variable saline/naloxone, $F_{1,36}=299.77$, $p<0.0001$; for interaction, $F_{1,136}=2.4$, $p<0.13$). Note that in all four cases, naloxone significantly reduced but did not completely block the level of SCH-induced analgesia as determined by ANOVA on ranks followed by post hoc testing, i.e. $H_3>26$, $p<0.001$ in all cases.

The post hoc analysis which followed the two-way ANOVA, as well as one-way ANOVA on ranks followed by post hoc testing, indicated that for the experiments performed during the day (figure 2a), all latencies were significantly different except the two naloxone-treated

groups; whereas for the experiments performed during the night (figure 2b), all six possible comparisons were significantly different.

Figure 3 compares the effects of per cent inhibition of sham produced by the 60 Hz ELF magnetic field and naloxone treatment during the day and night. The per cent inhibition of opioid-induced analgesia produced by naloxone for both light conditions and for both day and night cycles falls within one standard error of the mean ($52 \pm 4\%$, $53 \pm 3\%$, $52 \pm 6\%$, $51 \pm 3\%$); whereas there was a large difference during the day between light- and dark-exposed groups ($50 \pm 3\%$ versus $23 \pm 4\%$, respectively, i.e. $27 \pm 5\%$ difference), and a smaller difference in the groups exposed during the night ($43 \pm 4\%$ versus $28 \pm 3\%$, respectively, i.e. $15 \pm 5\%$ difference).

4. DISCUSSION

The results of the present study show that brief exposure to an ELF magnetic field results in a significant reduction in the level of enkephalin-mediated analgesia, with the extent of this reduction being dependent on the presence of light. Both the daytime and night-time exposure to the magnetic fields elicited a markedly greater inhibition of opioid-induced analgesia in the presence of light. The prototypic opiate antagonist, naloxone, also elicited significant reductions in the levels of daytime and night-time opioid peptide-induced analgesia. In the presence of light, naloxone at $1 \mu\text{g}$ and the ELF magnetic fields similarly reduced, but did not completely block, opioid-induced analgesia. However, in contrast to the ELF magnetic fields, the effects of naloxone as a per cent inhibition of sham were independent of the lighting conditions, being equivalent in either the presence or absence of light.

These findings confirm and extend prior observations of significant inhibitory effects of a variety of magnetic fields on the analgesic responses of a number of different species, including that of humans (Kavaliers *et al.* 1994; Betancur *et al.* 1994; Del Seppia *et al.* 1995; Papi *et al.* 1995). They also agree with previous observations of a significant light-dependency of the inhibitory effects of ELF magnetic fields on opioid analgesia in *Cepaea* (Prato *et al.* 1996b, 1997, 1998), and the report that inhibitory effects of magnetic fields upon morphine-induced analgesia in laboratory mice may be affected by ambient light exposure (Betancur *et al.* 1994). This work extends our prior findings, demonstrating that both the inhibitory effects of ELF magnetic fields and their light dependency are similar during the day and night. The present results, expressed as per cent inhibition of sham, further show that the functioning of endogenous opioid systems, as exemplified by their sensitivity to antagonism by naloxone, is unaffected by the absence of light. Since the ELF magnetic field effects are light-dependent, light probably affects the detection mechanism prior to its coupling to the opioid system.

The present results with *Cepaea* also indicate the existence of significant day–night rhythms in nociception and SCH-induced, enkephalin-mediated analgesia, as well as the inhibitory effects of ELF magnetic fields. They are also consistent with prior reports that relatively low doses of naloxone can be used to selectively reduce, but not

completely block, enkephalin-mediated analgesic responses (Crain & Shen 1996; Saksida *et al.* 1993; Sarne *et al.* 1996). Similar day–night rhythms in nociception and morphine-induced analgesia, as well as sensitivity to magnetic fields have been previously indicated in *Cepaea* and nocturnally active rodents (Frederickson & Edwards 1977; Kavaliers & Hirst 1983; Kavaliers *et al.* 1990). These rhythms are consistent with the nocturnal–crepuscular activity pattern of *Cepaea* and other molluscs and may be related to the day–night rhythms of behavioural thermoregulation and temperature preference that have been recorded from molluscs (e.g. Ford & Cook 1987).

Results of prior investigations on ELF detection mechanisms associated with the modulation of opioid-induced analgesia strongly supports a parametric resonance mechanism. However, this model as originally proposed by Lednev (1991, 1994; see also comments by Adair 1992; Engstrom 1996), may need to be modified to account for the light effects reported here. The tendency for the ‘dark’ sham values to be greater than the ‘light’ sham values confirms earlier results (Prato *et al.* 1997, 1998), where it has been found that sham values are also dependent on the magnitude of the static fields. Again, this points to the need for expanding the present PRM to explain the effects of light, perhaps in the form of optical pumping of ions to excited states (Prato *et al.* 1996b, 1997). In fact, if the excitation of an ion in the hypothesized ‘protein well’ required a light photon, and if its oscillation could only be disturbed by another light photon or thermal collision of the same energy, then the lifetime of the excitation would be sufficiently long to integrate/detect the ELF signals. This would help resolve one of the outstanding issues with respect to the PRM which has been raised by Adair (1992) and Engstrom (1996).

The exact mechanisms underlying the light-independent effects of magnetic fields and their relations to the light-dependent actions remain to be clarified. The relative roles of tissue/cellular light responses and how they modulate magnetic field sensitivity also remain to be determined. These light-dependent effects may relate to the ‘optical pumping’ mechanism (Leask 1977) evoked, at least in part, to explain some of the light-dependent orientational effects of magnetic fields (Phillips & Borland 1992; Phillips & Sayeed 1993; Wiltchko *et al.* 1993). This optical pumping mechanism bears some commonalities to the PRM (Prato *et al.* 1996b; theoretical figure 3), and raises the possibility of similar underlying mechanisms for the expression of a number of different light-dependent and light-independent behavioural effects of magnetic fields.

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REFERENCES

- Adair, R. K. 1992 Criticism of Lednev’s mechanism for the influence of weak magnetic fields on biological systems. *Bioelectromagnetics* **13**, 231–235.

- Betancur, C., Dell'Omo, G. & Allera, E. 1994 Magnetic field effects on stress-induced analgesia in mice: modulation of light. *Neurosci. Lett.* **182**, 147–150.
- Bevington, P. R. 1969 *Data reduction and error analysis for the physical sciences*. New York: McGraw-Hill.
- Brocklehurst, B. & McLauchlan, K. A. 1996 Free radical mechanism for the effects of environmental electromagnetic fields on biological systems. *Int. J. Radiat. Biol.* **69**, 3–24.
- Chipkin, R. E., Berger, J. G., Billard, W., Iorio, L. C., Chapman, R. & Barnett, A. 1988 Pharmacology of SCH34826, an orally active enkephalinase inhibitor analgesic. *J. Pharmacol. Exp. Therapeutics* **245**, 829–838.
- Crain, S. M. & Shen, K.-F. 1996 Modulatory effects of Gs-coupled excitatory opioid receptor functions on opioid analgesia, tolerance and dependence. *Neurochem. Res.* **21**, 1347–1351.
- Del Seppia, C., Ghione, S., Luschi, P. & Papi, F. 1995 Exposure to oscillating magnetic fields influences sensitivity to electrical stimuli. I. Experiments on pigeons. *Bioelectromagnetics* **16**, 290–294.
- Dyakonova, V., Carlberg, M., Sakharov, D. & Elofsson, R. 1995 Anatomical basis for interactions of enkephalins with other transmitters in the CNS of a snail. *J. Comp. Neurol.* **361**, 38–47.
- Engstrom, S. 1996 Dynamic properties of Lednev's parametric resonance mechanism. *Bioelectromagnetics* **17**, 58–70.
- Ford, D. J. G. & Cook, A. 1987 The effects of temperature and light on the circadian activity of the pulmonate slug, *Limax pueuglavus* Evans. *Anim. Behav.* **35**, 1754–1765.
- Frederickson, R. & Edwards, J. D. 1977 Hyperalgesia induced by naloxone follows diurnal rhythm in responsivity to painful stimuli. *Science* **198**, 756–758.
- Greene, J. J., Skowronski, J., Mullins, J. M. & Nardone, R. M. 1991 Delineation of electric and magnetic field effects of extremely low frequency electromagnetic radiation on transcription. *Biochem. Biophys. Res. Commun.* **174**, 742–749.
- Grissom, C. B. 1995 Magnetic field effects in biology: a survey of possible mechanisms with emphasis on radical-pair recombination. *Chem. Rev.* **95**, 3–24.
- Kavaliers, M. 1987 Evidence for opioid and non-opioid forms of stress-induced analgesia in the snails, *Cepaea nemoralis*. *Brain Res.* **410**, 111–115.
- Kavaliers, M. 1988 Evolutionary and comparative aspects of nociception. *Brain Res. Bull.* **21**, 923–931.
- Kavaliers, M. & Hirst, M. 1983 Daily rhythms of analgesia in mice: effects of age and photoperiod. *Brain Res.* **279**, 387–393.
- Kavaliers, M. & Ossenkopp, K.-P. 1988 Day-night rhythms of opioid and non-opioid stress-induced analgesia: Differential inhibitory effects of exposure to magnetic fields. *Pain* **32**, 223–229.
- Kavaliers, M. & Ossenkopp, K.-P. 1991 Opioid systems and magnetic field effects in the land snail, *Cepaea nemoralis*. *Biol. Bull.* **180**, 301–309.
- Kavaliers, M., Hirst, H. & Teskey, G. C. 1983 A functional role for an opiate system in snail thermal behaviour. *Science* **220**, 99–101.
- Kavaliers, M., Ossenkopp, K.-P. & Lipa, S. M. 1990 Day-night rhythms in the inhibitory effects of 60 Hz magnetic fields on opiate-mediated 'analgesic' behaviours of the land snail, *Cepaea nemoralis*. *Brain Res.* **517**, 276–282.
- Kavaliers, M., Ossenkopp, K.-P., Prato, F. S. & Carson, J. J. L. 1994 Opioid systems and the biological effects of magnetic fields. In *On the nature of electromagnetic field interactions with biological systems* (ed. A. H. Frey), pp. 181–190. Austin, TX: R. G. Landes Co.
- Kavaliers, M., Ossenkopp, K.-P., Prato, F. S., Innes, D. G. L., Galea, L. A. M. & Kinsella, D. M. 1996 Spatial learning in deer mice: sex differences and the effects of endogenous opioids and 60 Hz magnetic fields. *J. Comp. Physiol. A* **179**, 715–724.
- Kirschvink, J. L. & Walker, M. M. 1985 Particle size considerations for magnetite-based magnetoreceptors. In *Magnetite biomineralisation and magnetoreception in organisms: a new biomagnetism*. (ed. J. L. Kirschvink, D. S. Johnes & B. J. MacFadden), pp. 243–256. New York: Plenum.
- Land, M. E. 1984 Molluscs. In *Photoreception and vision in invertebrates*. (ed. M. A. Ali), pp. 699–726. New York: Plenum.
- Leask, M. J. M. 1977 A physicochemical mechanism for magnetic field detection by migratory birds and homing pigeons. *Nature* **267**, 144–145.
- Lednev, V. V. 1991 Possible mechanisms for the influence of weak magnetic fields on biological systems. *Bioelectromagnetics* **12**, 71–75.
- Lednev, V. V. 1994 Interference with the vibrational energy sublevels of ions bound in calcium-binding proteins as the basis for the interaction of weak magnetic fields with biological systems. In *On the nature of electromagnetic field interactions with biological systems*. (ed. A. H. Frey), pp. 59–72. Austin, TX: R. G. Landes Co.
- Lednev, V. V. 1996 Bioeffects of weak combined static and alternating magnetic fields. *Biofizika* **41**, 224–232.
- Menzel, R. 1979 Spectral sensitivity and colour vision in invertebrates. In *Comparative physiology and evolution of vision in invertebrates. A. Invertebrate photoreceptors* (ed. A. Atrium), pp. 534–567. New York: Springer.
- Papi, F., Luschi, P. & Limonta, P. 1992 Orientation-disturbing magnetic treatment affects the pigeon opioid system. *J. Exp. Biol.* **166**, 169–179.
- Papi, F., Ghione, S., Rosa, C., Del Seppia, C. & Luschi, P. 1995 Exposure to oscillating magnetic fields influences sensitivity to electrical stimuli: experiments on humans. *Bioelectromagnetics* **16**, 295–300.
- Phillips, J. B. & Borland, S. C. 1992 Behavioural evidence for the use of a light-dependent magnetoreception mechanism by a vertebrate. *Nature* **359**, 142–144.
- Phillips, J. B. & Sayeed, D. 1993 Wavelength-dependent effects of light on magnetic compass orientation in *Drosophila melanogaster*. *J. Comp. Physiol. A* **172**, 303–308.
- Polk, C. 1992 Dosimetry of extremely low frequency magnetic fields. *Bioelectromagnetics Suppl.* **1**, 209–235.
- Prato, F. S., Carson, J. J. L., Ossenkopp, K.-P. & Kavaliers, M. 1995 Possible mechanisms by which extremely low frequency magnetic fields affect opioid function. *FASEB J.* **9**, 807–814.
- Prato, F. S., Kavaliers, M. & Carson, J. J. L. 1996a Behavioural evidence that magnetic field effects in the land snail, *Cepaea nemoralis*, might not depend on magnetite or induced electric currents. *Bioelectromagnetics* **17**, 123–130.
- Prato, F. S., Kavaliers, M. & Carson, J. J. L. 1996b Behavioural responses to magnetic fields by land snails are dependent on both magnetic field direction and light. *Proc. R. Soc. Lond. B* **263**, 1437–1442.
- Prato, F. S., Kavaliers, M., Cullen, A. P. & Thomas, A. W. 1997 Light-dependent and -independent behavioural effects of extremely low frequency (ELF) magnetic fields in a land snail are consistent with a parametric resonance mechanism (PRM). *Bioelectromagnetics* **18**, 284–291.
- Prato, F. S., Kavaliers, M. & Thomas, A. W. 1998 Extremely low frequency magnetic fields can either increase or decrease 'analgesia' in the land snail as predicted by a parametric resonance model. *Bioelectromagnetics*. (Submitted).
- Sakharov, D., Nezlin, L., Moroz, L. & Elofsson, R. 1993 Patterns of enkephalin immunolabelling in the pulmonate snail *Cepaea nemoralis* and related molluscs. *Brain Res.* **620**, 114–121.

- Saksida, L. M., Galea, L. A. M. & Kavaliers, M. 1993 Antinociceptive effects of the enkephalinase inhibitor SCH34826 in the snail, *Cepaea nemoralis*. *Peptides* **14**, 763–768.
- Sarne, Y., Fields, A., Keren, O. & Gafni, M. 1996 Stimulatory effects of opioids on transmitter release and possible cellular mechanisms: overview and original results. *Neurochem. Res.* **21**, 1353–1361.
- Scaiano, J. C., Cozens, F. L. & McLean, J. 1994 Model for the rationalisations of magnetic field effects *in vivo*: applications of the radical pair mechanism to biological systems. *Photochem. Photobiol.* **59**, 585–589.
- Weaver, J. S. & Astumian, R. D. 1990 The response of living cells to very weak electric fields: the thermal noise limit. *Science* **247**, 459–462.
- Wiltschko, W., Munro, U., Ford, H. & Wiltschko, R. 1993 Red light disrupts magnetic orientation of migratory birds. *Nature* **364**, 525–527.
- Wiltschko, W. & Wiltschko, R. 1995 Migratory orientation of European robins is affected by the wavelength of light as well as by a magnetic pulse. *J. Comp. Physiol. A* **177**, 363–369.