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Magnetic Fields Inhibit Opioid-Induced Feeding in the Slug, *Limax maximus*

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KAVALIERS, M., K.-P. OSSENKOPP AND A. MATHERS. *Magnetic fields inhibit opioid-induced feeding in the slug,* Limax maximus. PHARMACOL BIOCHEM BEHAV **23**(5) 727–730, 1985.—Exposure to rotating and elevated magnetic fields significantly reduced over three hours the ingestive effects of the opiate agonist, morphine (10 mg/kg), in free-feeding slugs, *Limax maximus*. Magnetic field exposure also inhibited the opioid-mediated increased ingestive responses of slugs that had been food-deprived for 24 hr. These results suggest that magnetic stimuli inhibit opiate-mediated behavioral and physiological functions in invertebrates in a similar manner as observed in vertebrates.

Feeding Ingestive behaviors Magnetic fields Mollusc Morphine Opiate Opioid Slug

SUBSTANTIAL evidence exists to suggest that magnetic fields can influence biological systems. Geomagnetic cues are used in avian, fish and insect orientation [13,30]. Various types of magnetic stimuli have been shown to affect circadian rhythmicity, growth, feeding, drinking, endocrine activity and a variety of other behaviors in birds and mammals [1, 4, 9, 22–23a, 29–33, 42], including that of humans [42]. Magnetic fields have also been shown to influence a number of behaviors in a variety of species of invertebrates [1, 6–8, 13].

One of the observed effects of magnetic field exposure is a marked attenuation in the degree of analgesia and hyperactivity induced in mice by administration of the exogenous opiate, morphine [22–23b, 32]. This inhibitory effect is significantly greater at night, with fluctuating earth-strength and weak 60 Hz magnetic fields (0.5–1.5 Hz) reversibly suppressing the normal nocturnal peak in the day-night rhythm of morphine-induced analgesia [22]. In the day-time these weak magnetic stimuli are without any apparent effect on morphine-induced analgesia [23]. However, stronger magnetic fields do have significant inhibitory effects on day-time analgesia [22,32a]. The greater effects of magnetic fields on opiate actions in the night-time may in part be attributed to the inhibitory influence that magnetic stimuli are proposed to have on the vertebrate pineal gland [22]. Semm and his coworkers [37,40] indicated that exposure to time-varying fields can block the nocturnal metabolic and hormonal activity of the pineal gland. Previously, it had been shown that pinealectomy and non-invasive inhibition of the activity of the pineal reduced the nocturnal peak of morphine-induced analgesia [19,25]. However, the presence of significant effects of magnetic fields on morphine-induced responses in the day-time [23], when the hormonal activity of the pineal is at a minimum [34], suggests that magnetic stimuli may also have other more direct actions on opiate systems. It is of importance, therefore, to determine whether magnetic stimuli affect opiate mediated responses when the pineal gland is absent. Additionally, since opioid systems have been implicated in the regulation of a broad range of basic functions [17], it is also of interest to establish whether magnetic stimuli affect opiate mediated behaviors other than locomotor activity and nociceptive thresholds.

There is substantial evidence that opioid peptides have functional roles in the determination of behavioral and physiological functions in invertebrates. Opioid systems have been shown to be involved in the mediation of ingestive, locomotory, nociceptive and thermoregulatory behaviors of terrestrial molluscs [18, 20, 21]. Recent studies have revealed that opioid systems are involved in the regulation of the ingestive behaviors of the slug, *Limax maximus*, in a manner similar to that in mammals [21,23b]. Therefore, it is of interest to determine whether magnetic stimuli affect opiate-induced ingestive responses of this invertebrate. We report here that an exposure to a rotating magnetic field markedly reduces morphine- and food deprivation-induced ingestive responses in the slug, *Limax maximus*.

METHOD

Animals and Experimental Apparatus

Slugs (10–15 g) were field collected (Victoria, British Columbia) and maintained in the laboratory at 22± 1°C under a 12 hr light 12 hr dark cycle (light 125 μw/cm^2). They were communally housed in a glass aquarium (95×51×46 cm) that was provided with a soil substrate. Animals were kept moist and had continuous access to water, lettuce and other leafy vegetation.

Slugs were placed individually in 10 cm diameter petri dishes to which they were confined by 5 cm high plastic mesh slides and a plastic cover. A substrate of lettuce was placed on the bottom of the dishes. To ensure full hydration of the slugs a thin film of water was also maintained in each

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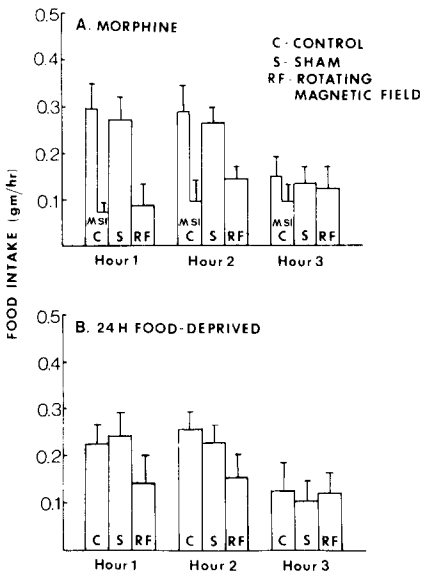

FIG. 1. Effects of rotating magnetic fields (RF) and sham rotating magnetic fields (sham) on the mean hourly food intakes of individual *Limax maximus* that were, A. Free-feeding and received intramuscular (IM) injections of morphine (10 mg/kg). B. Food-deprived for 24 hr and then presented with food. Animals were pre-exposed to the RF or sham RF conditions for 30 min before receiving either the IM injections or being presented with food. Ingestive responses were also determined of control groups (C) that received neither the RF nor the sham exposures. N=10 in all cases. Vertical lines denote two standard errors of the mean.

dish. The slugs were acclimated for 48 hr to these feeding units before any experimental manipulations were carried out.

The dishes, which were resting on 5 cm foam rubber sheets, were placed centrally between the magnets of a rotating magnetic field apparatus [22]. The apparatus consisted of two horseshoe magnets that were rotated in opposite directions along their major axes at 29 rpm. Central field intensities (with the magnets stationary and aligned along opposite poles), as measured by a Bell Incremental Gauss Meter (Model 640) ranged from 2.0–35 gauss. The spatial distribution of this field is illustrated in Kavaliers *et al.* [22]. In the sham exposure conditions the noise and vibrations of the motors were present, with the magnets replaced by lead weights of equivalent mass. In the sham exposure conditions field intensities were of constant earth strength (0.50–0.60 gauss).

Experimental Procedures

The dishes with the slugs were exposed for 30 minutes to either the rotating field (RF) or sham RF conditions. Determinations were then made of the effects of intramuscular (IM) injections of morphine sulfate (10 mg/kg/10 ml, BDH, Montreal) in saline on the amounts of pre-weighed lettuce (2.0–3.0 g) ingested by individual slugs (n=10, in all cases) each hour for three hours during the light period (900–1200 hr) while the animals were continuously exposed to either the RF or sham RF. Determinations were also made of the food intakes of control unexposed group of slugs (n=10, in both cases) that received either morphine (10 mg/kg/10 ml) or saline by itself (10 mg/kg). At the end of each hour slugs were quickly removed from the dishes and the remaining lettuce was blotted dry and weighed. Any food adhering to the slugs was removed and corrected for in the hourly determinations of food intake.

In another series of experiments slugs were deprived of food for 24 hr. They were then exposed to the RF or sham RF conditions for 30 min after which lettuce (2.0–3.0 g) was provided. Determinations were then made of the amounts of food consumed hourly over 3 hr (900–1200 hr) while the slugs (n=10, in all cases) were exposed to either the RF or sham RF conditions. Determinations were also made of the food intake of a non-exposed control group (n=10). All data was analysed by analysis of variance.

RESULTS

Administration of morphine resulted in a significant ($p<0.01$) increase in the ingestive responses and food intakes of both control and sham RF exposed free-feeding slugs (Fig. 1A). Maximal effects of morphine occurred within 1–2 hr of administration with a non-significant increase in food consumption evident in the third hour. There were no significant differences between the hourly food intakes of the morphine-treated control and sham RF exposed slugs. However, exposure to the rotating magnetic field dramatically reduced ($p<0.01$) the hourly food intakes of morphine treated animals as compared to the control or sham RF exposed individuals (Fig. 1A). The ingestive responses of the RF exposed morphine treated slugs were not significantly different from those of the saline treated control individuals. No apparent differences in activity were noted between control, sham RF and RF exposed groups of slugs.

Control and sham exposed food-deprived animals displayed significantly greater ($p<0.01$) ingestive responses than did the individuals which had food continuously present. The greatest increase in food intakes was evident during the first and second hours after food introduction, with ingestive responses returning to basal levels by the third hour. There were no significant differences in food intakes between the control and sham RF exposed groups. However, exposure to the RF significantly ($p<0.01$) reduced the hourly food intakes of the food deprived slugs. The food intakes of the RF exposed food-deprived slugs were more variable and non-significantly greater than those of the control individuals which had food present. In control procedures no significant differences were found between the basal food intakes of free-feeding control, sham RF or RF exposed groups of slugs.

DISCUSSION

The present results show that exposure to a low fluctuating magnetic field inhibits morphine- and deprivation-induced feeding in the slug, *Limax maximus*. This extends previous demonstrations of significant attenuation of morphine-induced analgesia and hyperactivity by magnetic field stimuli [22, 23, 23a, 32, 32b] to opiate mediated ingestive responses. Evidence obtained from behavioral, biochemical, electrophysiological and pharmacological investigations of the effects of opiates, their agonists and antagonists, in molluscs, suggests the existence of regulatory opioid systems that resemble those found in mammals [18, 20, 21, 23b, 24, 38, 39]. The present results provide further evidence of this similarity, demonstrating that magnetic stimuli also inhibit opiate actions in *Limax*. The time-course and patterns of morphine and other opiate-induced ingestive responses in *Limax* are similar to those observed in mammals [28,36]. Furthermore,

administration on the opiate antagonist, naloxone, blocks the ingestive effects of morphine as well as decreasing the feeding responses of food-deprived slugs [21,23b]. These effects of naloxone are similar to those reported from mammals [5, 27, 28] and are indicative of actions at specific opiate receptors [2,8]. The similarities in the inhibitory effects of the magnetic fields and naloxone on deprivation-induced feeding also provide suggestive evidence to support the proposal that magnetic stimuli may effect endogenous opioid activity. Whether these magnetic fields have comparable effects on morphine- and deprivation-induced feeding in mammals is under investigation.

Since the animals in this experiment were well hydrated it is expected that water uptake would secondarily accompany feeding. This may be analogous to the situation in mammals where drinking is primarily considered to arise from and follow opioid-induced feeding [12,24]. There is some evidence, however, to suggest that endogenous opiates may also have independent stimulatory effects on mammalian drinking [28]. It remains to be determined whether the opioid system and magnetic stimuli have any effects on the fluid balance and water uptake of *Limax*.

These findings indicate that magnetic field stimuli have similar inhibitory effects on opiate mediated behaviors in invertebrates and vertebrates. This raises the possibility of there being an early and common phylogenetic involvement of opioid mechanisms in the mediation of the biologic effects of magnetic fields. These findings also suggest that the presence of the vertebrate pineal gland is not essential for mediating the inhibitory effects of magnetic stimuli on opiate mediated behavioral and physiological functions. This does not, however, preclude the possibility that in vertebrates the pineal organ and its hormonal products such as melatonin [34], may be involved in the mediation of day-night rhythms in sensitivity and responses to magnetic stimuli.

The inhibitory effects of the rotating magnetic fields on morphine-induced ingestive responses in *Limax* may arise from the increased levels of the magnetic fields as compared to earth strength fields and/or fluctuations in field strength. There is evidence to suggest that both of these components can influence biological systems [1], though, the mechanism(s) whereby these effects are exerted are not well understood. Evidence exists to suggest that it is the rotating or varying portions of the field rather than the static components which influence opioid activity (in preparation). Magnetic fields have been shown to influence the release of a number of neurotransmitters [1,11]. These actions are thought to involve alterations in neural calcium and in the stability of calcium binding to neuronal membranes [1–3]. Exposure to radiofrequency electromagnetic fields has been shown to increase $^{45}Ca^{++}$ efflux from isolated chicken cerebral tissue [3].

These reports fit well with the available data on the relationships between calcium, as well as other ions, and opiates [10, 14–16, 35]. In rodents intracisternal injection of calcium antagonizes morphine and opioid peptide induced analgesia [16]. The simultaneous administration of chelating agents prevents the calcium antagonism while calcium ionophores increase the antagonist effect of low doses of calcium [10]. Moreover, calcium injected into periaqueductal grey region antagonizes the analgesia produced by injections of morphine, β-endorphin or (D-Ala^2, Met^5 enkephalamide) into the same region [15]. Additionally, both the mu and delta opioid receptors on which morphine can act [28], may be coupled to voltage- and/or calcium-dependent potassium channels [41]. Thus, any alterations in calcium release and binding resulting from the magnetic field stimuli would probably have negative effects on mu and delta opioid mediated responses.

A variety of other possible modes of action of magnetic fields have also been proposed [13,26]. Further studies are needed to determine whether or not magnetic fields influence opioid mediated invertebrates and vertebrates in the same manner and on the possible causal relationships to changes in calcium and related ionic conditions.

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REFERENCES

1. Adey, W. R. Tissue interactions with nonionizing electromagnetic fields. *Physiol Rev* **61:** 435–513, 1981.
2. Bawin, S. M. and W. R. Adey. Sensitivity of calcium binding in cerebral tissue to weak environmental electrical fields oscillating at low frequency. *Proc Natl Acad Sci USA* **73:** 1999–2003, 1976.
3. Bawin, S. M., W. R. Adey and I. M. Sabbot. Ionic factors in the release of $^{45}Ca^{++}$ from chicken cerebral tissue by electromagnetic fields. *Proc Natl Acad Sci USA* **75:** 6314–6318, 1978.
4. Bliss, V. L. and F. H. Heppner. Circadian activity rhythm influenced by near zero magnetic field. *Nature* **261:** 411–412, 1976.
5. Brown, D. R. and G. S. Holtzman. Suppression of deprivation-induced food intake and water intake in rats and mice by naloxone. *Pharmacol Biochem Behav* **11:** 567–573, 1979.
6. Brown, F. A., Jr. Response to pervasive geophysical factors and the biological clock problem. *Cold Spring Harbor Symp Quant Biol* **25:** 57–71, 1960.
7. Brown, F. A., Jr. Response of the planarian, *Dugesia*, to very weak horizontal electrostatic fields. *Biol Bull* **123:** 282–294, 1962.
8. Brown, F. A., Jr. and C. S. Chow. Interorganismic and environmental influences through extremely weak electromagnetic fields. *Biol Bull* **144:** 437–461, 1973.
9. Brown, F. A. and K. M. Scow. Magnetic induction of a circadian cycle in hamsters. *J Interdiscipl Cycle Res* **9:** 137–145, 1978.
10. Chapman, D. B. and E. L. Way. Modification of endorphin/enkephalin analgesia and stress induced analgesia by divalent cations, a cation chelator and an ionophore. *Br J Pharmacol* **75:** 389–396, 1981.
11. Dixey, R. and G. Rein. ^{3}H-noradrenaline release potentiated in a clonal nerve cell line by low-intensity pulsed magnetic fields. *Nature* **296:** 253–256, 1982.
12. Fitzsimons, T. J. and J. Le Magnen. Eating as a regulatory control of drinking in the rat. *J Comp Physiol Psychol* **67:** 273–283, 1969.
13. Gould, J. L. Magnetic field sensitivity in animals. *Annu Rev Physiol* **46:** 585–598, 1984.
14. Guerrero-Munoz, F., M. Guerrero, E. L. Way and C. H. Li. Effect of β-endorphin on calcium uptake in the brain. *Science* **206:** 89–91, 1979.
15. Guerrero-Munoz, F., C. Adames and E. L. Way. Calcium-opiate antagonism in the periaqueductal grey (PAG) region. *Eur J Pharmacol* **76:** 417–419, 1981.

16. Harris, R. A., H. Yamamoto, H. H. Loh and E. L. Way. Discrete changes in brain calcium with morphine analgesia tolerance dependence and abstinence. *Life Sci* **20:** 501–506, 1977.
17. Henry, J. L. Circulating opioids: Possible physiological roles in central nervous function. *Neurosci Biobehav Rev* **6:** 229–256, 1982.
18. Kavalieres, M., M. Hirst and G. C. Teskey. A functional role for an opiate system in snail thermal behavior. *Science* **220:** 99–101, 1983.
19. Kavaliers, M., M. Hirst and G. C. Teskey. Ageing, opioid analgesia and the pineal gland. *Life Sci* **32:** 2279–2287, 1983.
20. Kavaliers, M. and M. Hirst. The presence of an opioid system mediating behavioral thermoregulation in the terrestrial snail, *Cepaea nemoralis*. *Neuropharmacology* **23:** 1285–1289, 1984.
21. Kavaliers, M., M. Hirst and G. C. Teskey. Opioid-induced feeding in the slug, *Limax maximus*. *Physiol Behav* **33:** 765–767, 1984.
22. Kavaliers, M., K.-P. Ossenkopp and M. Hirst. Magnetic fields abolish the enhanced nocturnal analgesic response to morphine in mice. *Physiol Behav* **32:** 261–264, 1984.
23. Kavaliers, M. and K.-P. Ossenkopp. Exposure to magnetic fields alters morphine-induced behavioral responses in two strains of mice. *Neuropharmacology* **24:** 337–340, 1985.
23a. Kavaliers, M. and K.-P. Ossenkopp. Tolerance to morphine induced analgesia in mice: Magnetic fields function as environmental specific cues and reduce tolerance development. *Life Sci* **37:** 1125–1135, 1985.
23b. Kavaliers, M., B. Rangley, G. C. Teskey and M. Hirst. Mu- and kappa-opiate agonists modulate feeding behavior in the slug, *(Limax maximus)*. *Pharmacol Biochem Behav*, in press, 1986.
24. Kissileef, H. R. Food-associated drinking in the rat. *J Comp Physiol Psychol* **67:** 284–300, 1969.
25. Lakin, M. L., C. H. Miller, M. L. Stott and W. D. Winter. Involvement of the pineal gland and melatonin in murine analgesia. *Life Sci* **29:** 2543–2551, 1981.
26. Leask, M. J. M. A physiochemical mechanism for magnetic field detection by migratory birds and homing pigeons. *Nature* **267:** 144–145, 1977.
27. Maickel, R. P., M. C. Braude and J. E. Zabik. The effects of various narcotic agonists and antagonists on deprivation-induced fluid consumption. *Neuropharmacology* **16:** 863–866, 1977.
28. Morley, J. E., A. S. Levine, G. K. Yim and M. T. Lowy. Opioid modulation of appetite. *Neurosci Biobehav Rev* **7:** 281–305, 1983.
29. Nahas, G. G., H. Boccalon, P. Berryer and B. Wagner. Effects in rodents of a 1-month exposure to magnetic fields (200–1200 gauss). *Aviat Space Environ Med* **46:** 1161–1163, 1975.
30. Ossenkopp, K.-P. and R. Barbeito. Bird orientation and the geomagnetic field: A review. *Neurosci Biobehav Rev* **2:** 255–279, 1978.
31. Ossenkopp, K.-P. and M. D. Ossenkopp. Geophysical variables and behavior. XI open-field behaviors in young rats exposed to an ELF rotating magnetic field. *Psychol Rep* **52:** 343–350, 1983.
32. Ossenkopp, K.-P., M. Kavaliers and M. Hirst. Reduced nocturnal morphine analgesia in mice following a geomagnetic disturbance. *Neurosci Lett* **40:** 321–325, 1983.
32a. Ossenkopp, K.-P., M. Kavaliers, F. R. Prato, G. C. Teskey, E. Sestini and M. Hirst. Exposure to nuclear magnetic resonance imaging procedures attenuates morphine-induced analgesia in mice. *Life Sci*, in press, 1985.
33. Persinger, M. A, G. F. Lafreniere and K.-P. Ossenkopp. Behavioral, physiological, and histological changes in rats exposed during various developmental stages to ELF magnetic fields. In: *ELF and VLF Electromagnetic Field Effects*, edited by M. S. Persinger. New York: Plenum, 1974, pp. 177–223.
34. Reiter, R. J. The mammalian pineal gland: structure and function. *Am J Anat* **162:** 287–313, 1983.
35. Sadee, W., A. Pfeiffer and A. Herz. Opiate receptor: multiple effects of metal ions. *J Neurochem* **39:** 659–667, 1982.
36. Sanger, D. J. and P. S. McCarthy. Increased food and water intake produced in rats by opiate receptor agonists. *Psychopharmacology (Berlin)* **74:** 217–220, 1981.
37. Semm, P., T. Schneider and L. Vollarth. The effects of an earth-strength magnetic field on the electrical activity of pineal cells. *Nature* **288:** 607–608, 1980.
38. Stefano, G. B., B. Hall, M. H. Markham and B. Dvorkin. Opioid inhibition of dopamine release from nervous tissue of *Mytilus edulis* and *Octopus bimaculatus*. *Science* **213:** 928–930, 1980.
39. Stefano, G. B., R. M. Kream and R. S. Zukin. Demonstration of stereospecific opiate binding in the nervous tissue of the marine mollusc *Mytilus edulis*. *Brain Res* **181:** 445–450, 1980.
40. Welker, H. A., P. Semm, R. P. Willig, J. C. Commentz, W. Wiltschko and L. Vollrath. Effects of an artificial magnetic field on serotonin N-acetyltransferase activity and melatonin content of the rat pineal gland. *Exp Brain Res* **50:** 426–432, 1983.
41. Werz, M. A. and R. L. Macdonald. Opioid peptides selective for mu- and delta-receptors reduce calcium-dependent action potential duration by increasing potassium conductance. *Neurosci Lett* **42:** 173–178, 1983.
42. Wever, R. ELF-effects on human circadian rhythms. In: *ELF and VLF Electromagnetic Field Effects*, edited by M. A. Persinger. New York: Plenum, 1974, pp. 1010–1144.