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# MARKED RAPID ALTERATIONS IN NOCTURNAL PINEAL SEROTONIN METABOLISM IN MICE AND RATS EXPOSED TO WEAK INTERMITTENT MAGNETIC FIELDS

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Adult AMES mice and male Sprague Dawley rats were exposed to an artificial magnetic field, generated by Helmholtz coils. 3.5 hours after the onset of darkness the coils were activated for one hour resulting in an inversion of the horizontal component of the earth's magnetic field. The coils were activated and deactivated at 5 min intervals during the 1 hour exposure period. In both mice and rats, the levels of serotonin in the pineal were markedly increased by the exposure. In rats, an increase of pineal 5-hydroxyindole acetic acid and a decrease of the activity of the pineal enzyme serotonin-N-acetyltransferase also was observed. However, pineal and serum melatonin levels were not altered. The results indicate that the metabolism of serotonin in the pineal is quickly affected by the exposure of animals to a magnetic field. 

Growing public concerns about possible health risks caused by high-voltage lines are based on reports indicating a relationship between electromagnetic fields and diseases such as cancer and leukemia [1,2]. The increased risk for childhood leukemias in homes with high-current configurations has also been found [3]. Animal experiments have attempted to clarify the physiological mechanisms underlying the effects of magnetic fields (MF) on biological systems (for review see [4]). In some mammals the pineal gland responds to changes in the ambient MF [5-7]. This endocrine organ, and one of its hormones, melatonin, is involved in the regulation of annual reproductive cycles (for review see [8]). Moreover, melatonin plays an important role in the immune system [9] and suppresses the incidence of certain cancers [10]. In mammals, pineal synthesis of melatonin is controlled indirectly by the environmental light/dark cycle acting via the sympathetic innervation of the gland [11]. An important step in the synthetic chain is the activity

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Abbreviations: MF, magnetic field; NAT, serotonin N-acetyltransferase; HIOMT, hydroxyindole-O-methyltransferase; 5-HT, serotonin; 5-HIAA, 5-hydroxyindole acetic acid; 5-HTP, 5-hydroxytryptophan; MAO, monoamine oxidase; TH, tryptophan hydroxylase; AAAD, aromatic amino acid decarboxylase.

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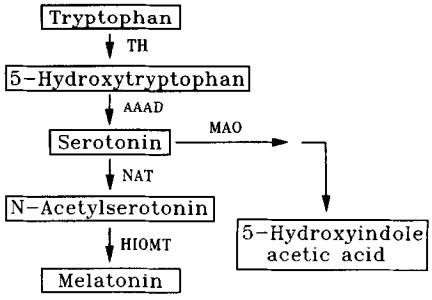


Figure 1. Diagrammatic representation of the synthesis of melatonin in the pineal gland. An important step in the chain is the activity of NAT, an enzyme which acetylates serotonin (5-HT) to N-acetylserotonin which is further O-methylated by the enzyme hydroxyindole-O-methyltransferase (HIOMT). For additional details see [12].

of the pineal enzyme serotonin-N-acetyltransferase (NAT, EC 2.3.1.5.). The activity of this enzyme as well as the melatonin levels in the pineal and in the blood show a clear diurnal pattern with highest levels occurring during the night; conversely, the substrate for NAT, serotonin (5-HT), as well as 5-HIAA show an inverse concentration pattern in the pineal with highest levels occurring during the light phase [12]. In figure 1, the synthetic pathway of melatonin is illustrated.

Exposure to a Earth strength (approx. 0.5 Gauss = 50$\mu$Tesla) static MF depresses both rat pineal NAT activity and melatonin levels when the horizontal component of the earth's MF is reversed [6,7]. Interestingly, a mere compensation of the earth's MF has no effect on either pineal NAT or melatonin [13]. However, the underlying mechanisms by which the pineal is affected by exposure to an artificial MF are poorly understood. It is not clear whether the observed effects are due to events within the pineal or caused by MF-induced alterations of the sympathetic innervation. Recent findings of Rudolph et al. [14] showed a MF-induced decrease of cAMP in the pineal, thus making the latter possibility more likely. In an attempt to clarify some of these issues, we examined the effects of MF exposure on the nocturnal metabolism of pineal 5-HT.

## MATERIALS AND METHODS

A pair of Helmhotz coils of 1m diameter and with a clearance of 0.5 m was oriented in a North-South direction. Intensities of the magnetic fields were measured with a Gaussmeter (Model MG-7D, Walker Sci. Corp., Worcester, MA). The strength of the horizontal component of the earth's magnetic field was approximately 0.4 Gauss (40$\mu$Tesla) in the animal rooms, while the coils produced an inversion of the horizontal component when connected by a relay to a DC power supply at approximately 12 Volts and the appropiate current. A diode was connected in parallel to the coils (*p*-type terminal connected to the negative terminal of the power supply) in order to protect the power supply from high voltages when disconnected from the coils. Without this diode, the voltage reached more than 100 Volts, caused by self-induction of the coils when the field collapsed (data not shown). More important, the diode caused the field to decay within the same time frame that it took to produce it. The characteristics of the generated field

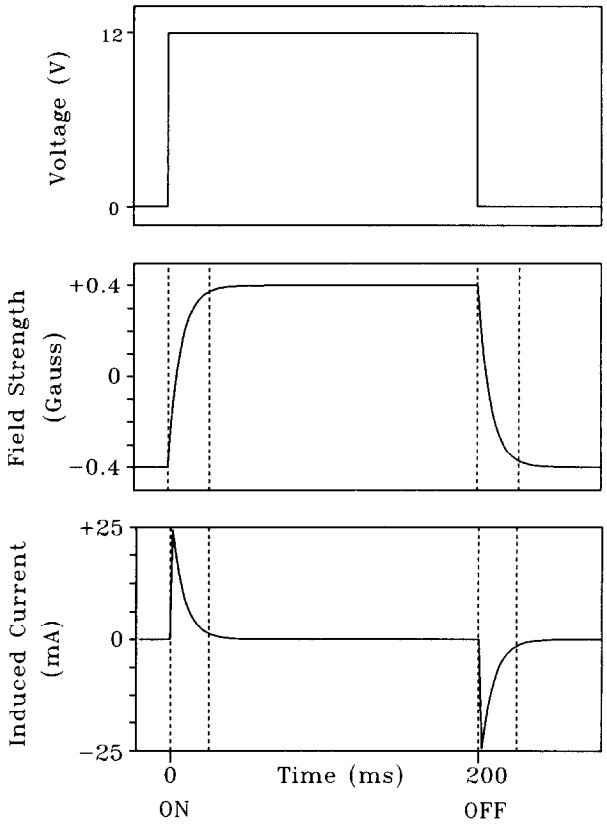


Figure 2. In order to demonstrate the characteristics of the generated MF, the coils were activated for 200msec. The following parameters are shown: Applied Voltage (upper panel), the resulting change of the MF (middle panel), and the induced current in a second coil, placed between the Helmholtz coils (lower panel). All determinations were performed by using a RM 504 oscilloscope (Tektronix) with an attached Polaroid adapter. The photographs were digitized and redrawn by means of computer programs. The dotted lines indicate the time period required for the inversion of the MF (approximately 25 msec).

are given in figure 2. As one can perceive, the reversal of the MF is accomplished within approximately 25 msec, while its compensation is reached earlier (roughly 5 msec after the coils were activated). The exponential rise and decay of the generated field are caused by the inductivity [15] of the coils (approximately 0.5 Henry; the time constant was about 7.25 msec).

Male Sprague-Dawley rats (BW 120-130g) were purchased from Harlan, (Indianapolis, IN). Adult AMES mice (BW 22-28g) were a generous gift from Dr. W. W. Morgan, this institution. The animals were adapted for one week to a 14 hours light : 10 hours dark regime at constant humidity and temperature (22°±2°C). Water and food were available ad libitum. The animals were divided into 2 groups of 7 and 8 animals each (rats), and 2 gender-balanced groups of 9 and 10 animals each (mice), respectively. Two sets of experiments were performed, each involving both groups of one species. One group was exposed to the MF for one hour, beginning 3.5 hours after the onset of darkness, while the control group was in the same room, away from the coils. The coils were activated and deactivated six times during the one hour exposure at regular intervals of 5 min each. Immediately after exposure, the animals were decapitated under dim red light in an alternating order (control and exposed) and the trunk blood was collected. The pineals were rapidly removed, frozen on dry ice and stored at -60°C until assayed.

Chemicals were purchased from Sigma, unless otherwise specified. Pineals were homogenized in 100µl of 0.05M phosphate buffer at pH 6.8. Activities of NAT and HIOMT were estimated as described previously [16,17]. Concentrations of pineal 5-HT and 5-HIAA were measured by HPLC analysis [18]. Pineal and serum concentrations of melatonin were estimated by means of a direct radioimmunoassay [19,20], with modifications: 250µl of sample, or standard (range 2pg/ml to 1000pg/ml), respectively, were combined with 100µl antiserum (initial dilution 1:9000; Batch G/S/704-8483, Stockgrand Ltd., Guildford, UK) and 100µl [$^3$H]-melatonin (TRK 798, Amersham, UK, approximately 2000 CPM/tube), all in assay buffer (0.1M tricine-buffer pH 8.0, containing 0.1% gelatine, 0.9 % NaCL, and 0.1% $NaN_3$). After incubation for 18 hours at 4°C, 500 µl of chilled dextran coated charcoal solution were added (0.5g charcoal and 0.05g dextran in 100ml assay-buffer). After vortexing and incubation for 15 min at 4°C, the tubes were centrifuged for 15 min at 4°C. 750 µl of the supernatant were added to 7.5ml of a scintillation fluid (Liquiscint; National Diagnostics, Manville, NJ) and countet for 10 min in a ß-counter. Assay sensitivity was better than 2pg/ml; intra- and inter-assay variations were 7.8% and 12.8%, respectively.

## RESULTS

As shown in figure 3, the levels of 5-HT were increased in both species due to the MF exposure. In mice, however, an unexpected sex-difference was found with higher levels in the pineals of the females. The levels of 5-HIAA were not detectable in mice while they were found to be increased in rats due to the MF-exposure. The activity of NAT in rats was decreased by the MF, while pineal HIOMT was unchanged; likewise, neither pineal nor serum melatonin levels were altered by MF exposure for 1 hour (fig.4).

## DISCUSSION

The findings that pineal NAT activity of MF-exposed rats is decreased, when compared to control animals, is consistent with former results [6,7]. The increases in

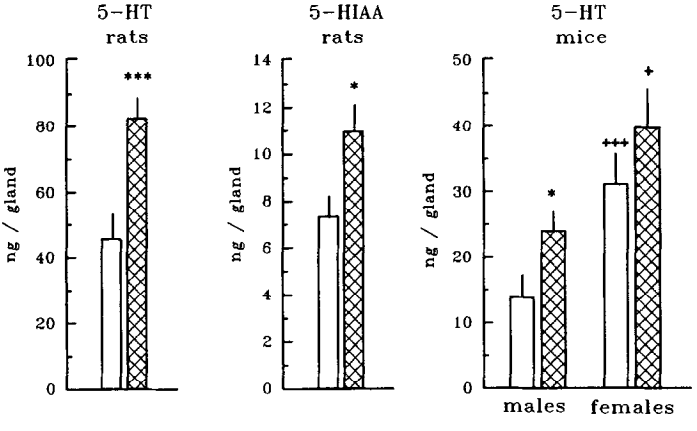


Figure 3. The effects of MF-exposure on pineal levels of 5-HT and 5-HIAA in both rats and mice. Mean values ± standard errors are shown. Open bars, control groups; cross hatched bars, exposed groups. ***, $p<0.01$ vs. control; *, $p<0.05$ vs. control; 5-HIAA was not detectable in mice. + + +, $p<0.01$ vs males; +, $p<0.05$ vs. males (ANOVA followed by Student-Newman-Keuls test).

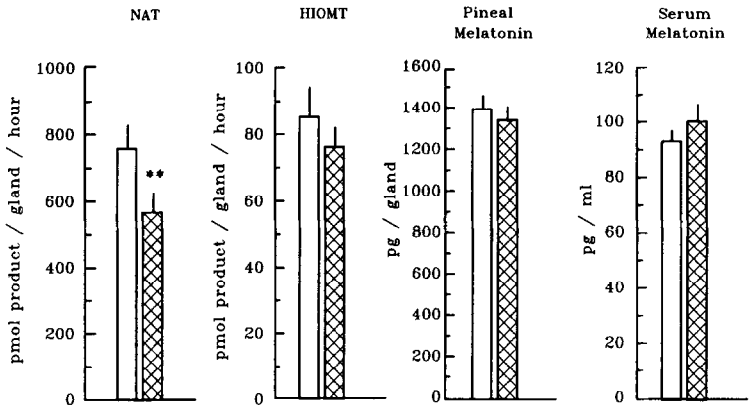


Figure 4. The effects of MF exposure on the activities of the pineal enzymes NAT and HIOMT and on pineal and serum levels of melatonin in rats. Mean values ± standard errors are shown. Open bars, control groups; cross hatched bars, exposed groups. **, $p < 0.025$ vs control (ANOVA followed by Student-Newman-Keuls test).

pineal 5-HT and 5-HIAA after MF exposure, described here for the first time, are likely a direct consequence of NAT suppression. A reduction in 5-HT acetylation would cause an accumulation of the substrate which then would be oxidatively deaminated to 5-HIAA (fig. 1). Although we assume that the suppression of NAT activity was a direct result of MF exposure, other possible explanations for the observations exist. Thus, MF exposure could have increased the synthesis of 5-HT (e.g., at the level of the trytophan hydroxylase enzyme, fig. 1) which, in turn, may suppress NAT activity via a 5-HT receptor-mediated mechanism [21].

The fact that melatonin was not detectable in the mouse pineal is not surprising since most strains of mice have lost the capability of synthesizing melatonin due to the inactivity (or absence) of the pineal enzyme HIOMT [22]. NAT activity in these mice strains are lower than in wild mice, but not completely absent [22]; MF-induced suppression of the enzyme presumably caused the increase in pineal 5-HT. The observed sex-difference in the pineal content of 5-HT is well in accordance with previous findings in C57BL mice [22].

The failure of the artificial MF to suppress pineal and serum melatonin levels in rats despite the drop in NAT activity may be a consequence of the short exposure interval, i.e., one hour, or it could relate to some aspect of the experimental procedure (e.g., the field strength). Thus, had the exposure period been prolonged, perhaps pineal and circulating melatonin levels may have dropped. Alternatively, the decrease in NAT activity may be compensated by the large increase in substrate, i.e. 5-HT, which then leads to more melatonin production at lower NAT levels.

The effects described herein are clearly a consequence of the artificial inversion of the earth's magnetic field. In the present study where the animals were moving freely, changes of the natural MF including its inversion also occurred

whenever the animals turned their heads. That these movements do not affect the normal function of the pineal is obvious [e.g., the increase in pineal melatonin synthesis during the dark phase occurs in diurnally active animals (with restricted movement during darkness) as well as in nocturnal species which are *active* during the night]. Hence, the mere inversion of the earth's MF is very likely unrelated to the reported changes in the pineal function. Rather, the *time course of change* of the MF more likely explains the observed effects. As shown in figure 2, an inversion of the MF induces a current in a second coil only during the *transients*, not *while* it is inverted. Although the additional coil may not be a good model for an animal with respect to the amount of induced current, the principle is the same: regardless the kind of matter, every change of the ambient MF induces an electrical field which then results in a current, depending on the electrical conductivity of the object [15]. The magnitude of the MF itself has no influence on the induced current; only the rate of change of the MF is significant.

The observed pineal effects of exposure to an artificially reversed MF are therefore, most likely, explained by induced currents in the nervous system caused by the on- and off-transients of the environmental MF rather than its mere inversion. However, the specific site of action in the brain remains unknown.

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