Sudden Infant Deaths (SIDS) and Geomagnetic Activity: Is there a 'learned' component affected by weak physiologically patterned magnetic fields?

by

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ABSTRACT

Sudden Infant Death Syndrome (SIDS) is a primary diagnosis for the death of infants between the ages of 1 month and 1 year. This study was designed to discern the potential role of weak, naturally-patterned magnetic fields in the predisposition and precipitation of these phenomena. Several different approaches with correlational analyses indicated that the incidence of SIDS in Quebec over a 14 year period increased during periods of enhanced geomagnetic activity. Experimental studies within the laboratory involving rats demonstrated that specific temporal patterns of weak magnetic fields could affect spatial and contextual learning and memory. There was strong evidence that specific shapes of magnetic fields that compliment critical brain electrical processes might function as a conditioned stimulus to precipitate behaviours. The results support the concept that during critical periods of physiological heterostasis appropriately patterned environmental magnetic fields can affect behaviour of living systems.

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CHAPTER I

GENERAL INTRODUCTION

During development, the brain goes through periods of vulnerability to external stimuli that are ineffective later in life. It is thought that during gestation and infanthood, specific brain areas go through periods of susceptibility to these otherwise harmless stimuli which can skew natural progression. A resulting abnormal brain configuration is predisposed to later transients that can precipitate atypical responses. The investigations herein examine whether naturally occurring and synthetic magnetic fields demonstrate relationships and effects relevant to the occurrence of Sudden Infant Death in human and rat populations.

THEORY

Sudden infant death syndrome (SIDS) has been defined as the sudden death of an infant under 1 year old that is unexpected by history and unexplained after a thorough postmortem examination, including a complete autopsy, investigation of the scene of death and review of the medical history (Willinger and Catz, 1991). Although this is a relatively well agreed upon definition, it has been suggested that due to the heterogeneous nature of SIDS, that it may need revision (Krous *et al.*, 2004).

Since SIDS is a poorly understood disorder, which is difficult to study experimentally, some have relied on theoretical approaches to understanding the progression of the syndrome. These theories have later helped to direct research in investigating correlates. One such theory has been the triple-risk model proposed by

Filano and Kinney (1994). The model proposes that there are three components necessary for SIDS death to occur: a congenital or acquired vulnerability, a critical time during development of homeostatic control, and an exogenous stressor. Most studies surrounding SIDS have looked at one or more of these three components for insight into the syndrome.

Due to the space-time clustering of SIDS events and SIDS infants' dates of birth (Moreno *et al.*, 1994; Rodrigues *et al.*, 1992), some have attempted to understand the phenomenon in terms of a pervasive and unifying stimulus. Eckert (1992) has suggested that this stimulus could be disturbances in geomagnetic field. It is theorized that during congenital development, a disturbed magnetic field might interrupt the proper development of an important respiratory nucleus within the brainstem making the infant vulnerable to a stressor later in life that could precipitate SIDS. Thus, it is suggested that a disturbed geomagnetic field during a critical developmental age in a vulnerable infant could precipitate SIDS.

INCIDENCE

Though the incidence of SIDS has recently declined (Ponsonby *et al.*, 2002), it remains the number one cause of death in infants in Canada (Health Statistics Division, 2002). According to a study conducted by Health Canada, Quebec had the lowest Canadian postneonatal (28 to 364 days) death rate in the period of 1985-1989 with 0.47 deaths per 1,000 which decreased to 0.38 per 1,000 for the period of 1994-1998 (Rusen *et al.*, 2004). Areas of higher latitudes such as the Yukon and Northwest Territories had the highest SIDS rates of 2.26 and 2.18 respectively. Currently, SIDS accounts for

approximately 25% of all infant deaths between 1 month and 1 year of age (Malloy and MacDorman, 2005) with males having slightly higher risks than females (51.3% and 48.7% of cases respectively) (Rusen *et al.*, 2004). Death rates in Canada have declined over the course of the decade by approximately 50% (from 0.97 to 0.54) which has been in part due to the recent campaigns aimed at reducing some of the known risk factors associated with SIDS (Rusen *et al.*, 2004).

RISK FACTORS

Multiple risk factors correlate to the vulnerability for an infant to SIDS. These have been categorized as non-preventable and preventable risk factors (Guntheroth, 1999). Amongst the non-preventable risk factors are sex, age, and time of death. Preventable risk factors include parental cigarette smoking, prone sleeping position, and formula feeding. Identifying these risk factors and following up with preventative measures has been important in reducing the incidence of SIDS.

Although no further drop in SIDS occurred beyond the late 1990s, a significant decrease in SIDS cases occurred throughout the late 1980s and mid 1990s. This was chiefly attributed to campaigns which aimed to reduce the practice of putting infants to sleep in a prone sleeping position (Willinger *et al.*, 1998). One such effort has been the *Back to Sleep* campaign in the United States which encouraged parents to put their infants to bed in a non-prone supine sleep position rather than lying them face down which increases the risk of suffocation.

Another important risk factor for SIDS has been cigarette smoke exposure. In a study that investigated the SIDS incidence between 1989 and 1998 in the United States, it

was found that infants exposed to cigarette smoke were 1.9 times more likely to succumb to SIDS than non-smoke-exposed infants (Anderson *et al.*, 2005). Some studies have drawn the parallel between cigarette smoke constituents and those of airborne pollutants such as sulfur dioxide, nitrogen dioxide, and carbon monoxide which have also been found to increase the risk of SIDS (Dale *et al.*, 2004). Other studies found that early cigarette smoke exposure can lead to a decreased ability to arouse during sleep (Chang *et al.*, 2003) which could play a role in increasing the risk of asphyxiation. Such circumstances could be brought about by soft bedding (Kemp *et al.*, 1998), bed sharing (Scragg & Mitchell, 1998), or putting the infant to bed in a prone sleeping position.

A link with decreased arousal has also been found with bottle-feeding. In a study investigating arousal in breast-fed and formula-fed infants, it was found that 2-3 month old formula-fed infants had decreased arousal in active sleep (Horne *et al.*, 2004). Also, in a meta-analysis of SIDS studies which investigated the risk of SIDS in bottle-fed or breast-fed infants, it was found that 19 of the 23 studies favored breast-feeding as protective for SIDS with the bottle-fed infants having twice the risk (McVea *et al.*, 2000).

Age of the infant, time of day, season, and sex of the infant have also been implicated in SIDS. Of highest risk are for male infants aged between 2-4 months during the winter months (Matturri *et al.*, 2006). Some have suggested that since SIDS is sometimes correlated to obstructive sleep apnea, the highest risk occurs at night during which most of an infants' sleep occurs (White, 1995). Further, males have been found to be at higher risk of obstructive sleep apnea (Kahn *et al.*, 1992) which might explain the higher proportion of male SIDS victims (Jorgensen *et al.*, 1979).

BEHAVIOURAL CORRELATES

Although the history of the SIDS victim somehow plays an important role in assessing the risk of succumbing to the syndrome, other studies have aimed at understanding SIDS in terms of behavioural correlates. Of these, obstructive sleep apnea (OSA), arousal response, and avoidance behaviour have been the most extensively studied. It is generally thought that some relationship exists between risk factors, neuropathologies (discussed below) and the predisposition of the infant to SIDS.

Failure to arouse from a threatening asphyxiating environment during sleep has been a well researched area in SIDS research (Kahn *et al.*, 2003 for review). Although certain sleep environments such as bed sharing, soft bedding, and prone sleep position increase the risk of SIDS, most infants have the ability to arouse themselves during a potentially suffocating event. SIDS victims have been found to have abnormalities in important brainstem arousal nuclei such as delayed development of the reticular formation (Takashima and Becker, 1985) and decreased catecholamine synthesis within the locus coeruleus (Matturri *et al.*, 2006). Further, a retrospective study investigating activity levels in SIDS cases found that infants who died of SIDS had low scores on the Early Infancy Temperament Questionnaire (Kelmanson, 1996). Many studies have investigated the relationship between known risk factors and arousal in infants.

In a study conducted by Chang and colleagues (2003), infants at the age most prone to suffering from SIDS (8-12 weeks) were investigated in a binaural audiology arousal test. Groups were assessed in terms of whether their mothers smoked or not during pregnancy. The study found that infants whose mothers smoked had significantly higher arousal thresholds during non-REM but not during REM sleep. Another study

investigating hypoxic awakening threshold in infants of 2-3 months of age also found that there was a greater proportion of infants who failed to awaken if their mothers smoked during pregnancy (Lewis and Bosque, 1995).

Another area of research looking at the interactions between risk factors and arousability investigated arousal thresholds in breast- or bottle-fed infants. Similar findings to the smoking studies, it was found that infants were less arousable in quiet sleep but not active sleep (Horne *et al.*, 2004) when evaluated in a nasal air jet stimulation test. Others have found that white noise auditory thresholds were lower in infants who used a pacifier during REM sleep (Franco *et al.*, 2000). Of those who did not use a pacifier, breast-fed infants had lower arousal thresholds than those who were not breast-fed.

Sleep deprivation has also been considered a risk factor known to contribute to decreased arousal in infants. Preventing infants from sleeping on their regular schedule has been found to be associated to both increased arousal thresholds as well as obstructive sleep apnea (OSA) (Franco *et al.*, 2004; Kahn *et al.*, 1992). In large scale prospective study which extracted 30 SIDS cases from a 20,750 case sleep-study conducted in multiple laboratories, it was found that infants who eventually succumbed to SIDS had lower numbers of body movements and arousals during sleep as compared to healthy controls (Kahn, 1992). Obstructive and mixed sleep apneas were seen in 23 of 30 SIDS victims while only 9 of 60 controls exhibited these. It was also found that males were more likely to suffer from OSA than females and this was proposed as a potential explanation for the higher rates of male SIDS victims.

It is argued that since OSA is entirely a sleep-dependant disorder and that SIDS occurs mostly during sleep, the disorder could play an important role in the pathophysiology of SIDS (White, 1999). Further, the age at which most infants fall victim to SIDS is the same crucial period where breathing control is under development (Gaultier, 1999) and such cases have been found to exhibit gliosis or decreased neuronal densities in areas of the brain important for cardiorespiratory control (Matturri *et al.*, 2006; Gilson *et al.*, 1994; Hinney *et al.*, 1983). Some studies have suggested that anomalies exhibited by SIDS victims could be a result of delayed development of crucial brainstem centers involved with arousal and cardiorespiratory control or due to abnormal learning.

In a study that investigated infant escape strategies from asphyxiating microenvironments, the observers found that there could be a learning component to such avoidance (Paluszynska *et al.*, 2004). It was found that inexperienced infants had a decreased ability to escape from mild asphyxia as compared to those which had repeated exposure to such stimuli. Further evidence to support potential learning abnormalities comes from studies which have discovered evidence of increased neuronal death or apoptosis within the hippocampus in SIDS victims (Waters *et al.*, 1999; Sparks and Hunsaker, 1991).

NEUROPATHOLOGY

Many studies have been conducted to investigate the neuropathology of SIDS. Most have investigated neuronal densities, gliosis, developmental abnormalities, and/or neurotransmitter levels within important cardiorespiratory or arousal centers within the

brainstems of SIDS victims as compared to age-matched controls. Typically, congenital or developmental anomalies have been discovered and are thought to somehow predispose the infant or result from repeated asphyxiating insults in the SIDS infants.

Of the cardiorespiratory brainstem centers found to be anomalous in SIDS are the nucleus of the solitary tract (Biondo *et al.*, 2004), dorsal nucleus of the vagus (Ansari *et al.*, 2002), and the arcuate nucleus (Matturi *et al.*, 2006). Although it is difficult to decipher whether such abnormalities result from congenital development or whether they result from repeated asphyxiation, such results have been reported throughout multiple studies.

The arcuate nucleus is an important structure for respiratory control (Gozal *et al.*, 1994). Multiple studies have found decreased neuronal densities within this nucleus in approximately 50% of SIDS cases (Matturi *et al.*, 2006; Matturi *et al.*, 2002; Gilson *et al.*, 1994). Evidence suggests that such hypoplasia could be a result of cigarette exposure *in utero* as relationships between maternal smoking and decreased neuronal densities in both SIDS and stillborn infants has been found (Lavezzi *et al.*, 2005). Other anomalies within this nucleus include decreased serotonergic receptor binding (Panigrahy *et al.*, 2000). This could be important in explaining the positive relationship between tryptophan hydroxylase-positive dorsal raphe nuclei and the duration of OSAs in SIDS infants (Sawaguchi *et al.*, 2003). This study also found that such sleep apnea was related to SIDS.

Another nucleus noted to be associated with OSAs in SIDS victims has been the dorsal nucleus of the vagus. In a study investigating apoptosis through the use of glial fibrillary acidic protein, the authors found a SIDS-specific correlation between the

frequency of OSA and the degree of apoptosis in the dorsal nucleus of the vagus (Sawaguchi *et al.*, 2003). Other studies have found significantly lower volumes and total neuron numbers in this structure for SIDS victims as compared to the age-matched reference group (Ansari *et al.*, 2002). There have also been results demonstrating decreased neuronal development in this nucleus which is reflected by a larger number of dendritic spines in SIDS victims as compared to controls (Takashima and Becker, 1991; Takashima and Becker, 1985). The typical progression is for there to be an increased number of dendritic spines prenatally and a decrease postnatally (Takashima and Becker, 1986), thus the increased number of spines could reflect delayed development.

Similar developmental delay, as reflected by increased number of dendritic spines, has also been seen in the nucleus of the solitary tract and the reticular formation in SIDS victims (Takashima and Becker, 1986). The solitary nucleus has also been suggested to be damaged through repeated hypoxic-ischemic insults. This is seen by the resulting increase in astrocyte density and the percentage of necrotic cells in this nucleus in SIDS victims (Biondo *et al.*, 2004).

The reticular formation is another structure which demonstrates delayed development through an increase in dendritic spine density postnatally in SIDS victims (Quattrochi *et al.*, 1985; Takashima and Becker, 1985). This structure, thought to be involved in both respiratory control and arousal (Lydic and Baghdoyan, 2002), has also been seen to have higher levels of astrogliosis in its white matter in SIDS cases as compared to age-matched controls (Obonai and Takashima, 1998).

The last brain structure discussed in terms of anomalies in SIDS victims will be the hippocampus. This structure has been reported to exhibit significantly more apoptotic

neurons in SIDS cases as compared to age-matched controls (Waters *et al.*, 1999). Further, neurons in the hippocampus of SIDS cases show stronger evidence of oxidative stress as compared to controls. Since oxidative stress is followed by an increase in free radicals and their scavengers, Huggles and colleagues (1996) investigated the levels of glutathione peroxidase in SIDS victims. They found that there was an increase in these free radical scavengers in SIDS victims less than 6 months of age as compared to age-matched controls.

MELATONIN HYPOTHESIS

As implied by the syndrome name, SIDS has no known specific underlying mechanism. As discussed above, a large number of the heterogeneous SIDS population seems to result from cardiorespiratory or protective arousal dysfunction. Many researchers have investigated brainstems of SIDS victims for anomalies in nuclei involved in cardiac, respiratory, or autonomic function since these would escape typical autopsies. Although some animal models for SIDS have been devised, finding one that can accurately reflect the time course of fetal development and known risk factors has been difficult (Blood-Siegfried & Shelton, 2004). These animal models can contribute to our understanding of some mechanisms involved in sudden death, but they have not yet provided a proper explanation for SIDS.

One hypothesis not yet discussed is the melatonin hypothesis of SIDS which emerged from studies conducted by Sparks and Hunsaker (1988). The findings of their preliminary study revealed that SIDS cases had significantly smaller pineal glands as compared to the age-matched controls. This hypothesis was further supported by a study

that found that SIDS cases had lower concentrations of melatonin within the cerebrospinal fluid (Sturner *et al.*, 1990). The theory seems to fit with findings that SIDS tends to occur maximally in times when there is decreased or decreasing light such as during the night hours and during the winter months (Sawaguchi *et al.*, 2000). Since light exposure suppresses melatonin, concentrations of the hormone are typically highest during these time frames and would be most susceptible to influence from other factors. With such evidence, Weissbluth and Weissbluth (1994) hypothesized that SIDS was the result of dysfunction in the photoneuroendocrine system. Some have suggested that infants with such vulnerability of the pineal-melatonin system could be susceptible to factors which influence melatonin rhythms (Reiter, 1992).

Other studies that lend support to the melatonin hypothesis have shown that protective factors such as breastfeeding can stabilize sleep patterns in 3 month-old infants through the amino acid tryptophan contained in the breast milk (Cubero *et al.*, 2005) which is a melatonin precursor (Oxenkrug *et al.*, 1990). Some have suggested that melatonin could be useful in protection against SIDS (Maurizi, 1997) while others have suggested its use as a protective agent for infants born pre-term (Jan *et al.*, 2007). Since melatonin is also a free radical scavenger, it could further help to protect against the tissue damage (Reiter *et al.*, 1995) found in SIDS.

Further support for the involvement of melatonin in SIDS comes from findings that risk factors associated with the syndrome tend to decrease melatonin concentrations or disrupt melatonin rhythms. Kennaway and others (1996) have found that infants born preterm, a strong risk factor for SIDS (Blair *et al.*, 2006), have a delayed onset of the diurnal melatonin rhythm. These rhythms typically are in place at around 8-12 weeks of

age which is also the age where infants are at highest risk for SIDS. A similar cortisol rhythm appears at the same age (Mantagos *et al.*, 1998; Antonini *et al.*, 2000).

Cigarette smoke exposure has also been found to be implicated in decreased melatonin. A study investigating nursing students found that female smokers demonstrated significantly lower melatonin levels as compared to non-smoking controls (Ozguner *et al.*, 2005). Others have found that exposures to cigarette smoke can have a negative influence on exogenous but not nighttime serum melatonin levels (Ursing *et al.*, 2005). One study investigating cigarette exposure in rabbits found that melatonin was able to reverse bronchoalveolar hemorrhage which could impact proper breathing abilities (Unlu *et al.*, 2006).

Melatonin has also been implicated in OSA. Hernández and colleagues (2007) have found that there is a lacking nighttime melatonin peak in adults who suffer from apnea. It was suggested that such lack in peak could be due to a difficulty to establish a proper sleep-wake pattern. The implication of the melatonin system in the most critical risk factors for SIDS suggests that it could be an important factor. Of greatest significance is the finding that the melatonin is decreased or dysfunctional as a result of these risk factors.

As an antioxidant, melatonin plays a crucial role in reducing damage from various stressors. Some studies have found that melatonin can be protective against asphyxia-induced elevations in cortical gray matter hydroxyl radical products in sheep (Miller *et al.*, 2005). Watanabe and others (2004) have found that melatonin administration to pregnant rats can reverse ischemia-induced oxidative mitochondrial damage in the fetal brain of rats. This would suggest that low levels of melatonin during fetal development

could lead to damaged brain nuclei which could increase susceptibility to stressors later in development for SIDS cases.

Gestational melatonin levels have been implicated in proper development. Kachi and others (2006) have investigated the effects of pinealectomized mothers on their offspring. They found that rats born from pinealectomized mothers have a greater incidence of malformations. Others have found that epileptic seizures during pregnancy could impair hippocampal neurogenesis and neuronal maturation in the newborn rats when the mothers underwent pinealectomy (Turgut *et al.*, 2005). Such negative effects on the hippocampus were reversed with the administration of melatonin. Melatonin was also able to reverse high levels of cortisol found in non-human primates born from mothers exposed to constant light during pregnancy (Torres-Farfan *et al.*, 2004).

As has been demonstrated, melatonin seems to be a plausible candidate due to its involvement in the majority of risk factors and at every stage in development. This becomes especially relevant when considering the triple risk model (Filano & Kinney, 1994) since impairments in the melatonin system during fetal development could lead to changes in the brain that ultimately predispose infants to stressful stimuli later in development. Further, the onset of the melatonin rhythms at the critical age of three months suggest that further perturbation of the system could be involved in some of the SIDS cases in succumbing to the syndrome. The pineal gland is a structure that has been considered susceptible to injury (Laure-Kamionowska *et al.*, 2003). Due to the protective nature of its major compound, low concentrations of this important neurohormone could fail to combat acute stressors.

MAGNETIC FIELDS AND SIDS

In 1992, Eckert proposed that the geomagnetic field could play an important role in predisposing victims and precipitation of SIDS. It was suggested that since a magnetic field is present over entire globe, it could explain the temporal clustering of SIDS cases which has been previously reported (Moreno *et al.*, 1994; Rodrigues *et al.*, 1992). Since then, research investigating geomagnetic and synthetic magnetic fields has found supporting evidence for the hypothesis. The geomagnetic field is related to melatonin levels, and some experimental and correlative findings have suggested a relationship between magnetic fields and SIDS. Therefore, it is thought that the geomagnetic field could exert its influences on the syndrome through melatonin mechanisms.

One of the best known effects of magnetic field exposures is a resulting decrease in melatonin levels. Weydahl and colleagues (2001) investigated the relationship between a 3 hour mean value for the local geomagnetic activity index, K, and salivary melatonin concentrations. A decrease in melatonin was found to correlate with heightened geomagnetic activity only when the geomagnetic activity was above 80nT. Other studies have found that geomagnetic activity must not exceed 80nT to influence more sensitive melatonin measures. Burch and others (1999) found that overnight excretion of the melatonin metabolite 6-OHMS was reduced when average antipodal geomagnetic index (AA) exceeded 30nT.

Other experiments have simultaneously measured both corticosteroids and melatonin. Results of these studies have found that during magnetic storms, subjects demonstrated a decrease in melatonin levels but an increase in cortisol levels (Rapoport *et al.*, 1998). Some studies have suggested that due to its influence on melatonin and

other compounds such as corticosterone, the geomagnetic environment should be considered when conducting experiments and should be controlled for it in a similar manner as lighting conditions (Jozsa *et al.*, 2005).

In two separate investigations, O'Connor and Persinger have been able to lend support to the hypothesis that ambient geomagnetic conditions could play a role in SIDS. An investigation of monthly SIDS rates in Ontario between 1960 and 1961 revealed that there was a significant and strong positive correlation between the monthly number of SIDS and the geomagnetic field strength within certain specific intensity bandwidths (O'Connor & Persinger, 1997). There were increased SIDS deaths in months when there were larger numbers of days when the geomagnetic field strength was within the 11-15nT range and fewer days within the 21-30nT range. Further investigation suggested the involvement of Pc1 geomagnetic micropulsations which were within the 0.2-5Hz range (O'Connor & Persinger, 1999).

Dupont and others (2005) have devised an experimental rat model for SIDS through magnetic field exposures designed to mimic geomagnetic storms. In this study, pregnant female rats were exposed for 2 to 3 days before expected parturition to a 0.5Hz magnetic field between 5 and 10nT. Results of the study found that females exposed to the magnetic field had significantly smaller litter sizes compared to controls. Further investigation showed that brains of the surviving rat pups had significantly fewer neurons in the areas known to be compromised in SIDS such as the parasolitary nucleus at 21 days (Dupont *et al.*, 2004).

THESIS ORGANIZATION

The purpose of this thesis is to investigate the magnetic field correlates of SIDS in order to further understand the parameters that interact effectively with organisms in relation to the syndrome or its characteristics. These investigations will be conducted with the use of a SIDS database and with various rat models which are relevant to understanding some of the underlying pathologies in SIDS. Since SIDS has been found to be a syndrome with multifactorial correlates, various aspects of the disorder such as learning mechanisms and the opiate system will be investigated with the use of magnetic fields as a unifying variable. To understand the relationships between various geomagnetic variables and SIDS, Chapter II analyses a SIDS database from Quebec between 1987 and 2000. The chapter will explore how geomagnetic conditions differ during gestation and surround the times of birth and death as compared to age-matched controls. In Chapters III and IV, behavioural animal models which are thought to implicate relevant brain structures or neurotransmitter systems found to be affected in SIDS will be described. Since SIDS cases have been found to have decreased neuronal densities within the hippocampus (Waters *et al.*, 1999), an important structure for learning, the results in Chapter III will look at the relationships between magnetic fields and learning in rats. The relevance is further outlined in findings that have suggested that SIDS cases could have an underlying deficit in learned avoidance behaviours (Paluszynska *et al.*, 2004). The final chapter of the thesis is drawn from the finding that elevated opiates have been found in some post mortem analyses of SIDS cases (Storm *et al.*, 1994). In Chapter IV the influence of magnetic field parameters that have been

suggested to operate through opiate mechanisms will be described in terms of their effects on thermal nociceptive thresholds.

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CHAPTER II

THE RELATIONSHIPS BETWEEN GEOMAGNETIC VARIABLES AND THE INCIDENCE OF SUDDEN INFANT DEATH SYNDROME

ABSTRACT

It has been proposed that sudden infant death syndrome (SIDS) could have underlying relationships with changes in the earth's geomagnetic field mediated through melatonin mechanisms (O'Connor & Persinger, 1997). In the current study, a SIDS database containing 593 SIDS cases and 86 age- and gender-matched controls was used to investigate this relationship. Data representing daily, monthly, and yearly geomagnetic activity (AA index) and the number of sudden storm commencements (SSC) were loaded for days, months, and years surrounding birth, death, and monthly gestation periods. Variables were collapsed into factors which tended to load for time frames surrounding gestation, birth, or death. The analyses revealed that those who died at around 2.5 and 3.5 months of age had significantly higher geomagnetic activity on the day of death and during the month of death and that there were significantly more days where geomagnetic activity was between 60-80nT during the 30 days preceding death as compared to other age groups. When high and low frequency months (November versus May) and years (1988, 1990, 1992 versus 1996-2000) were compared, it was found that there were significantly more days where geomagnetic activity was between 20-50nT during the 30 days preceding death for high frequency years and months even when adjusting for yearly and monthly average geomagnetic activity respectively. These results support the theory that magnetic fields hold a relationship with SIDS.

INTRODUCTION

Sudden infant death syndrome (SIDS) has been described as the sudden death of an infant under 1 year of age that is unexpected by history and unexplained after a thorough postmortem examination, including a complete autopsy, investigation of the scene of death and review of the medical history (Willinger and Catz, 1991). Since the phenomenon has an unknown etiology, many studies have aimed to understand the underlying causes of the syndrome using a triple-risk model approach (Filano and Kinney 1994). This model suggests that there are three underlying components to SIDS: a congenital vulnerability, a critical time during development of homeostatic control, and an exogenous stressor. Understanding one or more of these factors has been at the root of SIDS research.

Since SIDS cases have been found to be clustered either spatially or temporally (Moreno *et al.*, 1994; Rodrigues *et al.*, 1992), some have suggested that the factor underlying SIDS could be certain specific conditions within the geomagnetic field (O'Connor & Persinger, 1997). Eckert (1992) proposed that fluctuations in the geomagnetic field could be related to a selection factor (predisposing factors) and a trigger factor (precipitating factor). It is hypothesized that exposure to certain geomagnetic perturbations interfere with the normal development of brain areas responsible for the control of breathing. Later in life, during a critical stage in development, the infant with an abnormal breathing center could be susceptible to natural fluctuations in the geomagnetic field that resonate inversely with impulses from breathing organs and result in SIDS.

O'Connor and Persinger (1997) have investigated the monthly rate of SIDS in Ontario between 1960 and 1961 with respect to certain intensity bandwidths of geomagnetic activity. It was found that SIDS deaths increased in months where fluctuations in geomagnetic intensity were between 11nT and 15nT. A linear relationship was also found between the incidence of SIDS and pc1 geomagnetic micropulsations which have frequencies within the range of the 0.7Hz breathing rate of an infant (O'Connor and Persinger, 1999).

In the current study, the influence of various geomagnetic variables on SIDS for the province of Quebec between 1987 and 2000 was investigated. The database was loaded with variables that represented daily, monthly and yearly states of the geomagnetic field as well the incidence of sudden storm commencements. In order to investigate the triple-risk model (Filano & Kinney, 1994) with respect to the geomagnetic field, various conditions of the geomagnetic field were loaded for gestation months, birth and death.

METHODS

Subjects

The SIDS database employed in this study was comprised of all documented SIDS cases in the province of Quebec between 1987 and 2000. The database contained 679 infants less than one year old that had died from unknown causes (SIDS; n=593) or known causes (age-matched controls; n=86). Dates of birth and death were included for all cases.

Geomagnetic Variables

In order to investigate the relationships between geomagnetism and SIDS, the database was loaded with various measures of geomagnetic data. All geomagnetic activity (AA) and sudden storm commencement (SSC) data were obtained from the National Oceanic & Atmospheric Administration ftp site (ftp://ftp.ngdc.noaa.gov). Data corresponding to the times for infant birth, death, or gestation period were loaded. Although the database did not include gestation lengths for the cases, it was assumed that all infants had a full term and geomagnetic data was loaded into the database to reflect activity during 9 gestation months. The geomagnetic data added to the database and the number of variables per category is described in table 2.1.

Table 2.1. Description of geomagnetic variables.

Variable	Description	# of variables
Birth		
bAA-5 to bAA+5	mean daily AA surrounding birth day (5 days before . . . birth . . . 5 days after)	11
bAm	mean monthly AA for birth month	1
bAy	yearly AA for birth year	1
bSSCs	number of days since the last SSC	1
bSSCu	number of days until the next SSC	1
Death		
dAA-5 to dAA+5	mean daily AA surrounding death day (5 days before . . . death . . . 5 days after)	11
dAm	mean monthly AA for death month	1
dAy	mean yearly AA for death year	1
d30	mean AA for the 30 days prior to death	1
dband1-11	number of days during the 30 days pre-death where AA was in certain intensity bandwidths (0-10nT, 10-20nT, 20-30nT . . . 90-100nT, >100nT)	11
dSSCs	number of days since the last SSC	1
dSSCu	number of days until the next SSC	1
Gestation		
gAA1-9	mean AA during gestation months (0-30days, 30-60days . . . 240-birth)	9
gSSC1-9	number of SSC during gestation months (0-30days, 30-60days . . . 240-birth)	9

The levels of geomagnetic disturbances or activity (AA) fluctuate over the course of years and months according to solar activity. Since some of the analyses herein aim to describe the environment during gestation, birth and death of the cases, graphs have been included for the average monthly and yearly geomagnetic activity for the years of the database (1987-2000). Figure 2.1 describes the average monthly geomagnetic activity collapsed from the 14 monthly means of 1987 to 2000 whereas Figure 2.2 describes the yearly geomagnetic activity collapsed from the 12 months per year for 1987 to 2000.

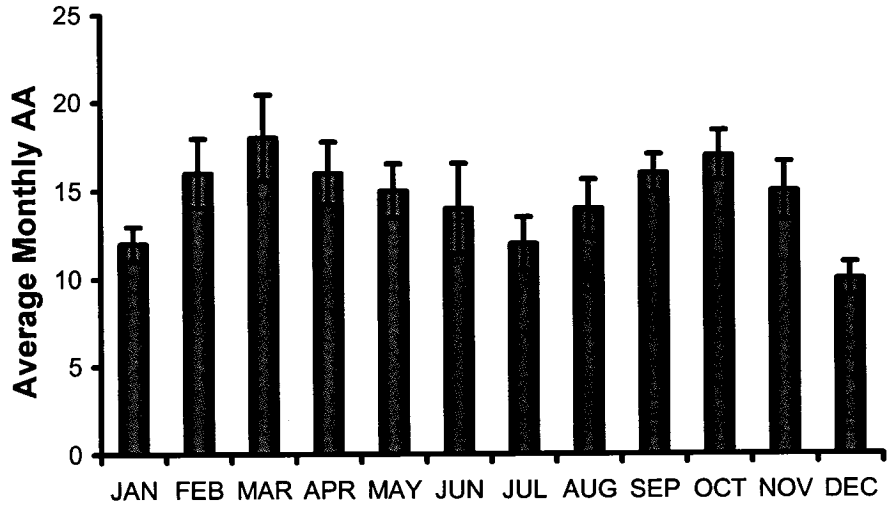


Figure 2.1. Mean (±SEM) monthly geomagnetic activity between 1987 and 2000.

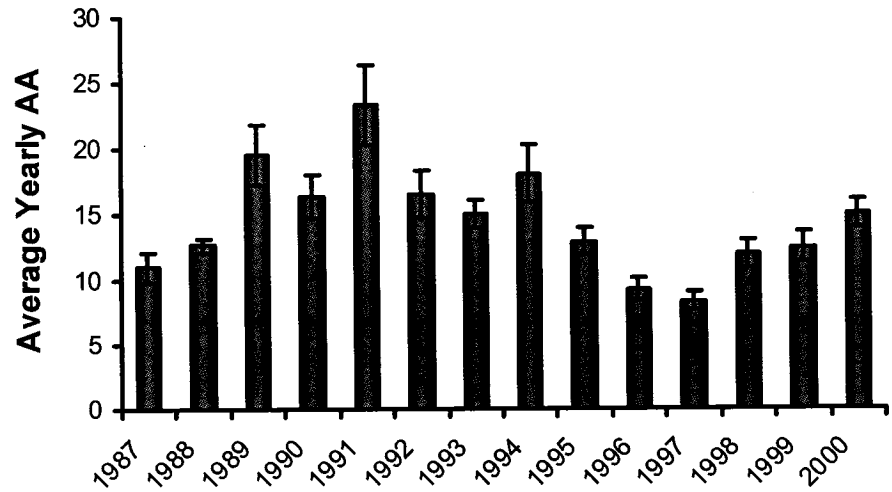


Figure 2.2. Mean (±SEM) yearly geomagnetic activity between 1987 and 2000.

Data Reduction

In order to reduce redundant variance between the variables in Table 2.1, multiple factor analyses were conducted. To increase the versatility of the resulting dataset, various factors were generated independently for each category of data as well as for some combinations of categories. The results of the factor analyses can be found in Table 2.2. The table lists the variables added to the analysis, the factors generated, the number of explained variables (Eigen Value), the variance explained by the factor (Var. %), the variables that loaded on the factor, and a short description. Only factors that had Eigen values higher than 2 were used for further analyses. Of interest is that the analyses successfully grouped variables temporally. Most of the generated factors contain variables focused around birth, death, or stages in the gestation period.

Table 2.2. Results of the factor analyses.

	Factor	Eigen Value	Var. (%)	Loaded Variables	R	Desc.
All Variables	fALL1	12.48	19.5	gAA6	0.75	Late Gestation
				gAA8	0.70	
				gAA7	0.69	
				gSSC5	0.62	
				gAA5	0.61	
				ABY	0.60	
				gSSC6	0.58	
				gSSC4	0.56	
				gSSC3	0.52	
				ADY	0.49	
				gSSC8	0.49	
				gSSC7	0.47	
				gAA4	0.44	
				gAA9	0.43	
	fALL2	4.44	6.9	gAA1	0.83	Early gestation
				gAA2	0.76	
				gAA3	0.71	
				gSSC1	0.63	
				gAA4	0.54	
	fALL3	3.55	5.5	BAA+1	0.81	Birth
				BAA+2	0.71	
				BIRTH	0.70	
				bAAm	0.42	
	fALL4	2.24	3.5	dband10	0.72	Death
				dban6	0.61	
				dband7	0.59	
				d30	0.51	
				dban8	0.43	
				dAAm	0.40	
	fALL5	2.03	3.2	dAA-4	0.86	Pre-death
				dAA-3	0.71	
				bAA+3	0.67	
				dAA-5	0.60	

Table 2.2 (cont.).

	Factor	Eigen Value	Var. (%)	Loaded Variables	R	Desc.
[redacted]	fAA1	4.17	19.0	BAA+1	0.83	
				BAA+2	0.77	Birth AA
				BIRTH	0.70	
	fAA2	2.66	12.1	DAA-1	0.86	
				DAA-2	0.82	Death AA
				DEATH	0.54	
[redacted]	f301	3.32	30.2	dband3	0.72	
				dband4	0.71	Active
				dband1	-0.62	death AA
				dband2	-0.65	
[redacted]	faaGest1	3.77	41.9	gAA8	0.82	
				gAA7	0.78	Late
				gAA9	0.72	gestation
				gAA6	0.71	AA
				gAA5	0.48	
[redacted]	fSSC1	3.68	40.9	gSSC6	0.71	
				gSSC5	0.68	
				gSSC4	0.66	
				gSSC3	0.66	
				gSSC9	0.64	All SSC
				gSSC8	0.62	
				gSSC1	0.60	
				sgSSC7	0.60	
				gSSC2	0.56	
[redacted]	fGEST1	6.33	35.2	gSSC8	0.78	
				gSSC9	0.68	
				gSSC6	0.61	Late
				gAA9	0.54	gestation
				gAA8	0.53	
				gSSC5	0.51	
	fGEST2	2.12	11.8	gAA1	0.84	
				gAA2	0.71	Early
				gSSC1	0.66	gestation
				gAA3	0.64	
				gAA4	0.59	

Age clustering

To investigate age differences, a new age variable was generated using cluster analysis. Since the average age of cases in the dataset was 3.5±2.2 months, cases more than 9 months of age (+2.5SD; n=23) were excluded from cluster analysis to restrict artifact clustering. A 4, 5, 6, and 7 group clustering method was conducted in order to investigate qualitatively the grouping method that resulted in the best distribution of cases

per cluster. The use of six age groups was decided. In order to balance the size of the age groups and to reduce the age variance, cases in the 4.5 month group and higher were collapsed into a single 4.5+ month age group (including the 9+ month olds that were excluded in the cluster analysis). The distribution of cases and mean ages (±SD) in each of the age group categories for the original cluster analysis and the new age groups can be found in Figure 2.3. All analyses conducted with age employed the four age groups.

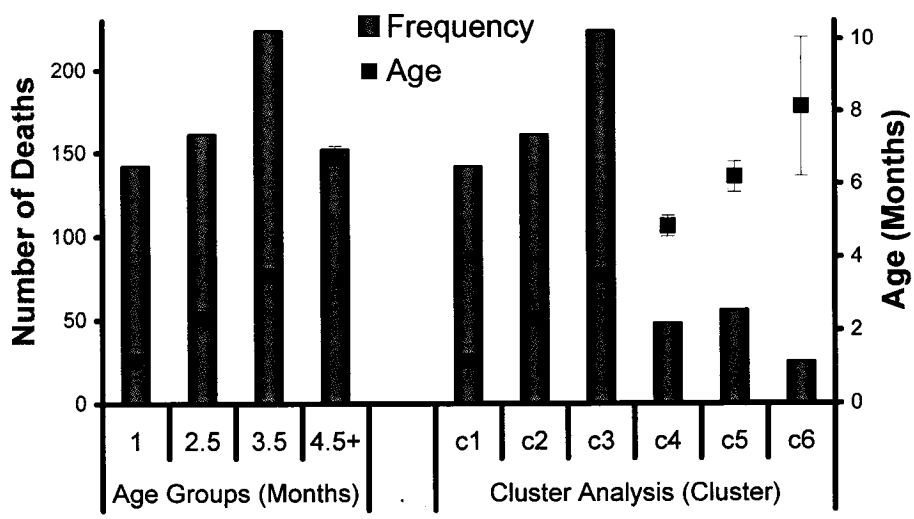


Figure 2.3. Age groups after the cluster analysis and final groups used in the analyses.

Statistical Analyses

The majority of the analyses herein employed three way analyses of variance with diagnosis (SIDS, control), gender (male, female), and age (1, 2.5, 3.5, 4.5+ months) to investigate differences in the raw variables and factors. *Post hoc* analyses were conducted with the use of Tukey's set at p=0.05. Chi square and hiloglinear analyses were employed to investigate differences in group sizes.

RESULTS

Population description

As demonstrated in previous studies (Rusen *et al.*, 2004), there were significantly more male SIDS cases (63%) than female cases (37%) [$\chi^2_{(1)}$=48.86; p<.01]. The average lifespan of cases in the database was 3.5±2.2 months. Investigating the proportion of deaths in the study population as a function of age found that there were significantly more deaths in the 3.5 month group (33%) than the 1 month (21%), 2.5 month (24%), or 4.5+ month group (22%) [$\chi^2_{(3)}$=24.18; p<.01]. Although hiloglinear analysis did not find any significant age-by-diagnosis interactions, when the SIDS and controls groups were analyzed separately, only the SIDS group was different in terms of age [1 month=20%, 2.5 month=24%, 3.5 month=35%, 4.5+ month=21%] [$\chi^2_{(3)}$=31.75; p<.01] whereas the age-dependent proportions of death in the controls did not differ [$\chi^2_{(3)}$=5.68; p>.05].

Hiloglinear analysis with the use of diagnosis, gender, and age for year of death revealed a significant year-by-diagnosis interaction [$\chi^2_{(13)}$=49.42; p<.001]. The number of deaths in the SIDS and age-matched controls populations between 1987 and 2000 can be found in Figure 2.4. *Post hoc* analyses employing a chi-square test revealed that the source of the interaction was due to a higher frequency of SIDS cases in years 1988, 1990, and 1992 (58-60 per year; 9.8-10.1%) as compared to 1996-2000 (16-29 per year; 2.7-4.9%) [$\chi^2_{(13)}$=75.93; p<.05] whereas frequencies did not differ significantly in the control group [$\chi^2_{(13)}$=21.89; p>.05]. From these analyses, an additional variable was created for high frequency years (1988, 1990, 1992) and for low frequency years (1996-2000) for the use in further analysis.

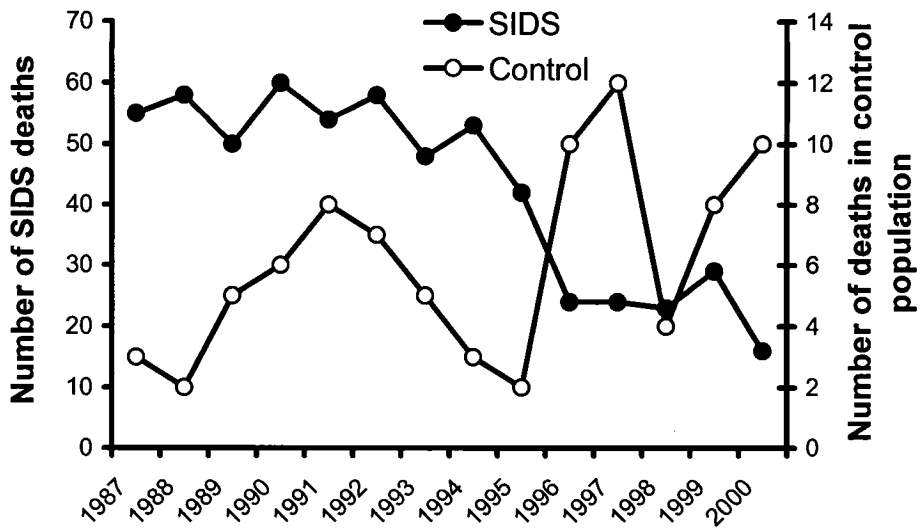


Figure 2.4. Frequency of deaths between 1987 and 2000.

Investigation of death month demonstrated that there were more deaths in the overall population during November (11.6%) and less in August (6.5%) than all other months (average 8.3%) [$\chi^2(11)=8.31$; $p>.05$]. When the SIDS group was analyzed separately, the month with the highest incidence of death was November (12.1%) and that with the lowest incidence was in May (6.4%). The overall and SIDS death frequencies over the 12 months can be found in Figure 2.5. These results were used to generate an additional variable that employed high frequency months (November) and low frequency months (May).

Geomagnetic Activity

Three way analyses of variance with gender, age, and diagnosis (SIDS or control) did not reveal any significant interactions for any of the geomagnetic variables or factors. There were no significant main effects for gender; however, the analyses did reveal some

main effects for diagnosis and age. Only individual variables demonstrated significant effects. There were no effects involving any of the factors.

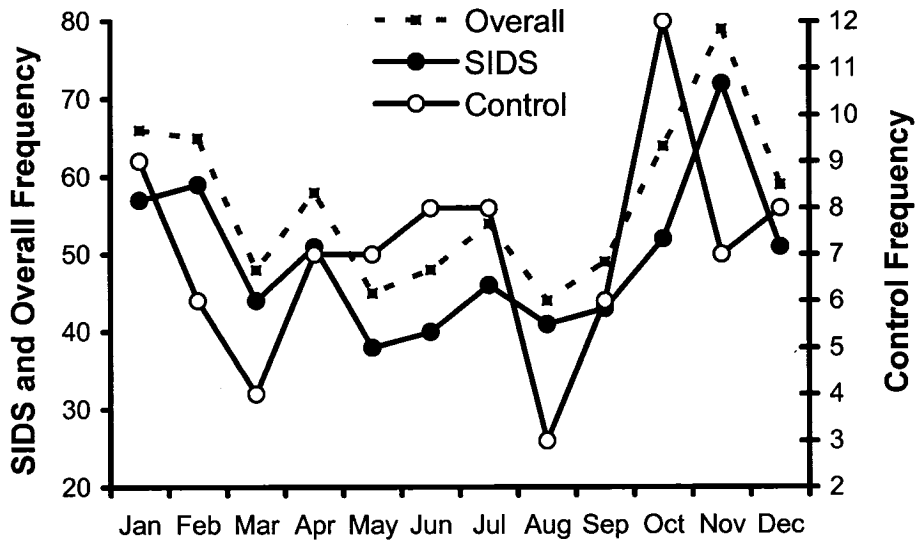


Figure 2.5. Frequency of deaths during the months of the year.

Oneway analysis of yearly geomagnetic activity as a function of diagnosis revealed that SIDS cases died in years that had significantly higher average yearly geomagnetic activity as compared to the age matched controls [$F(1,678)=5.27$; $p<.05$; $\eta^2=0.77\%$]. The means (±SEM) for average yearly geomagnetic activity as a function of diagnosis can be found in Figure 2.6.

Oneway analysis investigating the geomagnetic variables as a function of age revealed that 3.5 month old infants died in months that had significantly higher average monthly geomagnetic activity as compared to 1 month olds [$F(3,678)=3.30$; $p<.05$; $\eta^2=1.5\%$]. The means ± SEM for average monthly geomagnetic activity during the month of death for SIDS and controls (combined) can be found in Figure 2.7.

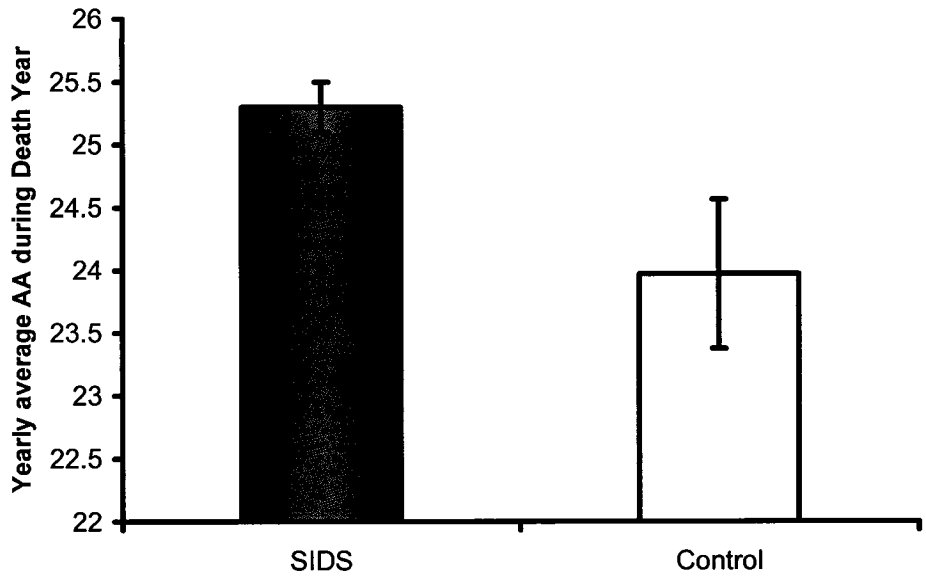


Figure 2.6. Average yearly AA for the death year across the different diagnosis conditions.

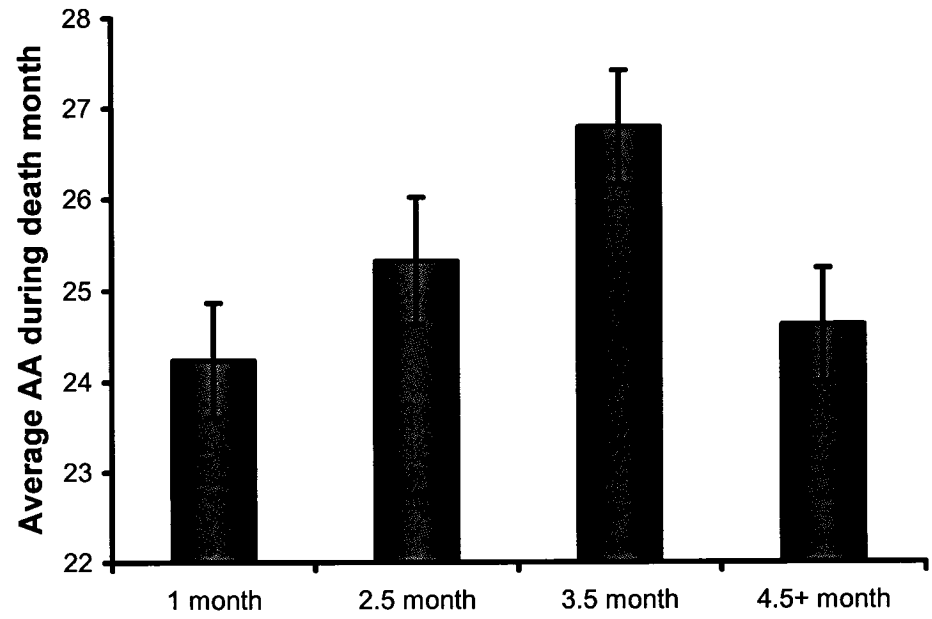


Figure 2.7. Average AA during the death month for the different age groups.

Analyses of the number of days where AA values fell within certain specific bandwidths demonstrated statistically significant differences between age groups. All of the effects involved the 2.5 or 3.5 month old groups differing from either the 1 month

and/or 4.5+ month old group. The number of days during the 30 day period before birth where geomagnetic activity fell within high intensity bandwidths (60-70nT and 70-80nT) for the different age groups can be found in Figure 2.8. The graph demonstrating low bandwidths can be found in Figure 2.9. Tukey's demonstrated that the effects were due to the 3.5 month old group having significantly less days where the AA values were between 10-20nT as compared to the 1 month and 4 month old group [$F(3,678)=5.20$; $p<.05$; $\eta^2=2.3\%$]. Also, the 3.5 month and 2.5 month old groups died following periods where there were significantly more days within the 70-80nT range as compared to the 4.5+ month old group [$F(3,678)=4.02$; $p<.01$; $\eta^2=1.8\%$]. Finally, the 2.5 month old group died following a 30 day period that had significantly more days within the 60-70nT range as compared to the 4.5 month old group [$F(3,678=3.32$; $p<.05$; $\eta^2=1.5\%$].

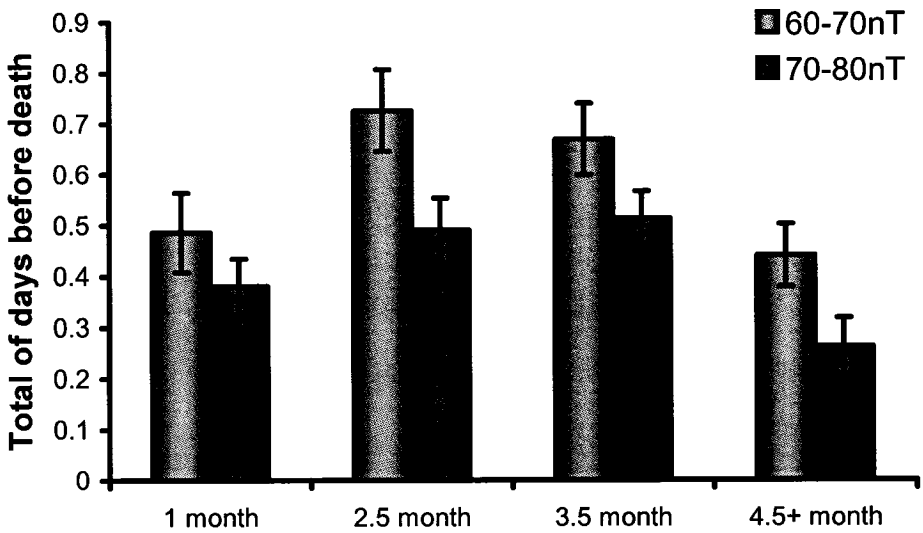


Figure 2.8. Number of days during a 30 day period before death where the geomagnetic activity was between 60-70nT and 70-80nT for the different age groups.

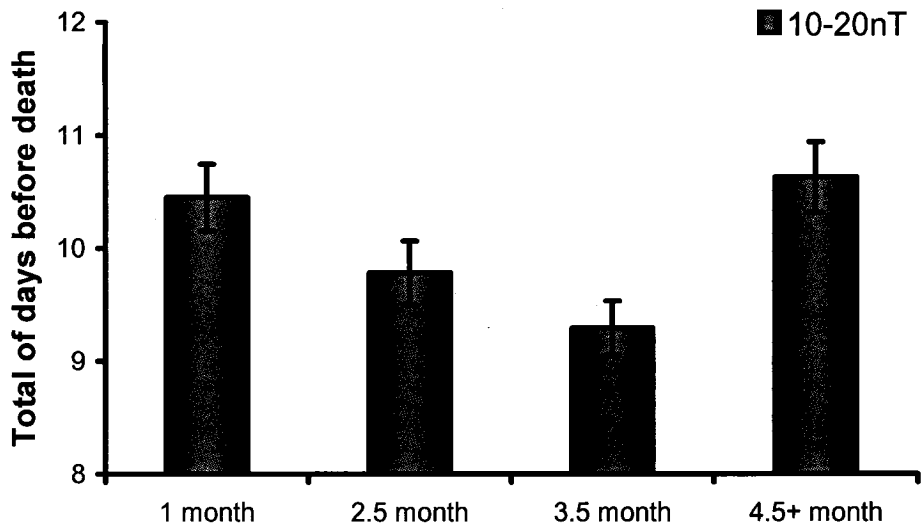


Figure 2.9. Number of days during a 30 day period before death where the geomagnetic activity was between 10-20nT for the different age groups.

Oneway analysis of variance for age group to investigate differences in the death day AA score and the average geomagnetic activity for the 30 day period before death revealed significant differences for both measures. The means (±SEM) for the geomagnetic activity on the day of death and average geomagnetic activity over the 30 days before death as a function of age can be found in Figure 2.10. Tukey's statistical test of variance revealed that there was significantly higher geomagnetic activity in the 3.5 month old group as compared to the 4.5+ month old group for the death day [$F(3,678)=3.16$; $p<.05$; $\eta^2=1.4\%$] and the 1 month old group for the average geomagnetic activity before death [$F(3,678)=4.12$; $p<.05$; $\eta^2=1.8\%$].

High and Low Frequency Years and Months

Chi-square analyses revealed that there were specific years and months within the database where SIDS frequency was higher and lower than expected if based on an even

temporal distribution. The following analyses employed isolated cases born in years and months within which there was a high (years: 1988, 1990, 1992; month: November) and low (years: 1996-2000; month: May) frequency of SIDS. All analyses employed only SIDS cases in order to investigate how geomagnetic factors differ between those born in high frequency versus low frequency time frames.

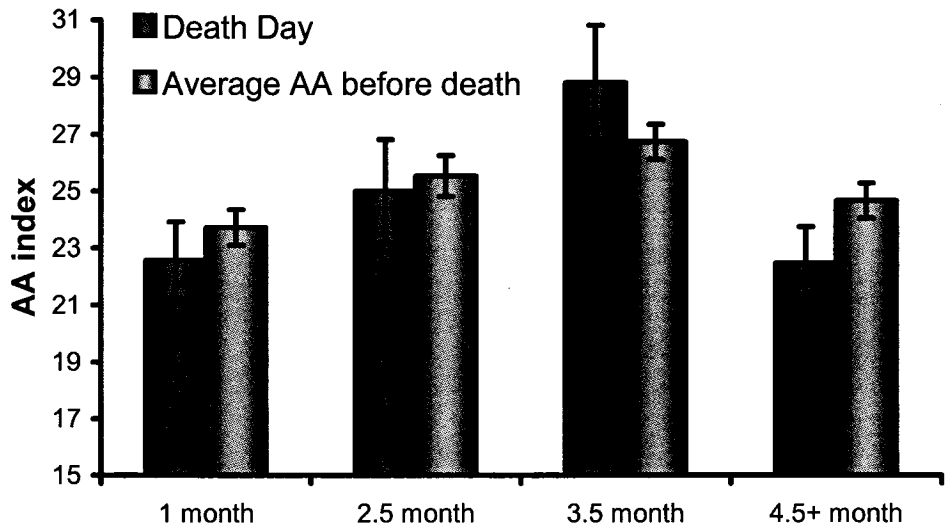


Figure 2.10. Average geomagnetic activity on the day of death and for the 30 day period preceding death for the different age groups.

When differences between years were investigated, the high frequency years demonstrated higher geomagnetic activity on almost all of the variables and factors analyzed. For this reason, only the results of the factors that were significant at $p<0.001$ are presented here. A summary of the results from oneway analyses for the factor scores as a function of the year groups can be found in Table 2.3. The means (±SEM) across the high and low frequency years for all factors generated with the use of all variables (fALL1-5) can be found in Figure 2.11. Of greatest interest is the large differences that were observed with factors representing the early and late gestation periods.

Table 2.3. Results from the analyses investigating high frequency years (1988, 1990, 1992) and low frequency years (1996-2000) for the various factors [F(1,291)].

Factor	Group	Mean	SEM	F	P	Eta^2 (%)
fALL1 Late Gestation	1996-2000	-0.56	0.045	163.04	<0.0001	36.0
	1988, 1990, 1992	0.60	0.067			
fALL2 Early Gestatoin	1996-2000	-0.53	0.050	53.43	<0.0001	15.6
	1988, 1990, 1992	0.36	0.093			
fALL3 Birth	1996-2000	-0.31	0.049	11.05	0.001	3.7
	1988, 1990, 1992	0.00	0.070			
fAA1 Birth AA	1996-2000	-0.30	0.048	10.88	0.001	3.6
	1988, 1990, 1992	0.02	0.072			
f301 Active Death AA	1996-2000	-0.60	0.080	65.49	<0.0001	18.4
	1988, 1990, 1992	0.24	0.067			
fGEST1 Late Gestation	1996-2000	-0.28	-0.283	59.61	<0.0001	17.0
	1988, 1990, 1992	0.46	0.463			
fGEST2 Early Gestation	1996-2000	-0.53	0.048	50.30	<0.0001	14.8
	1988, 1990, 1992	0.33	0.094			
Faa Gest1 Late Gestation AA	1996-2000	-0.64	0.046	117.76	<0.0001	28.9
	1988, 1990, 1992	0.40	0.072			
fSSC1 All SSC	1996-2000	-0.55	0.044	224.71	<0.0001	43.7
	1988, 1990, 1992	0.80	0.067			

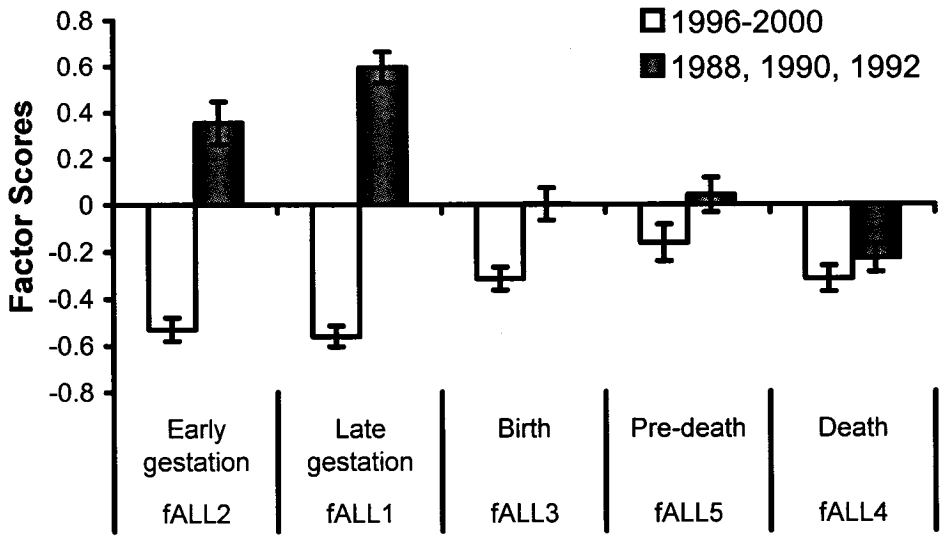


Figure 2.11. Factor scores for the various factors for SIDS cases born in low frequency (1996-2000) or high frequency years (1988, 1990, 1992).

The investigation of differences between high and low months revealed many less significant differences between month groups as compared to the year groups. The most

pertinent results came from the factor score for bandwidths 30 days prior to death (f301). A oneway analysis employing Tukey's analysis revealed that those who died during the high frequency month (mean=.363; SEM=.095) had significantly higher factor scores as compared to those who died in low frequency months (mean=-.533; SEM=.196) [F(1,109)=21.68; p<.05; η2=16.7%]. To further highlight these differences, Figure 2.12 shows that SIDS cases who died during high frequency months had significantly higher number of days before their deaths where there was medium (mid) to high levels of geomagnetic activity [F(1,109)=5.62 to 22.58; p<.05; η^2=5.0 to 17.3%] and less days where geomagnetic activity was low [F(1,109)=5.20 to 8.09; p<.05; η^2= 4.6 to 7.0%].

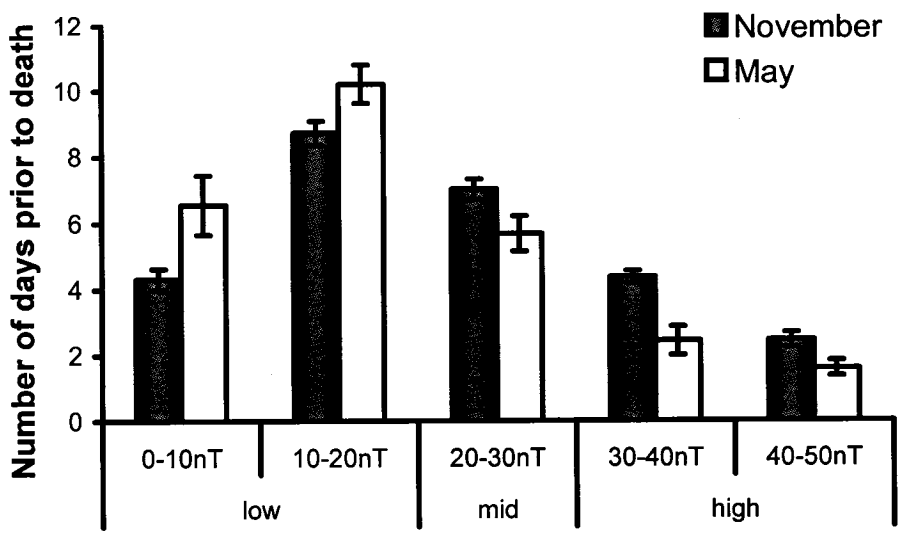


Figure 2.12. Number of days during the 30 day period prior to death where geomagnetic activity was within low (0-20nT), medium (20-30nT), and high (30-50nT) intensity bandwidths.

Since there are natural yearly and monthly fluctuations in the geomagnetic field (Figures 2.1 and 2.2), additional analyses of covariance were conducted to investigate which effects were related to events surrounding death and birth rather than seasonal or

yearly variations. For the month groups, the analyses covaried for average monthly AA during the death month and for the year groups, the analyses covaried for average yearly AA during the year of death. All variables and factors were analyzed through these methods. The only consistent effect for both year [$F(1,291)= 22.37$; $p<.0001$; $\eta^2=7.2\%$] and month [$F(1,109)=20.40$; $p<.0001$; 15.9%] frequency groups was a significantly higher residual f301 factor score for those born during high frequency time frames as compared to low frequency time frames. This factor reflects higher geomagnetic activity during the 30 days preceding the death date (Table 2.2). These residual factor scores (±SEM) for the month and year groups can be found in Figure 2.13.

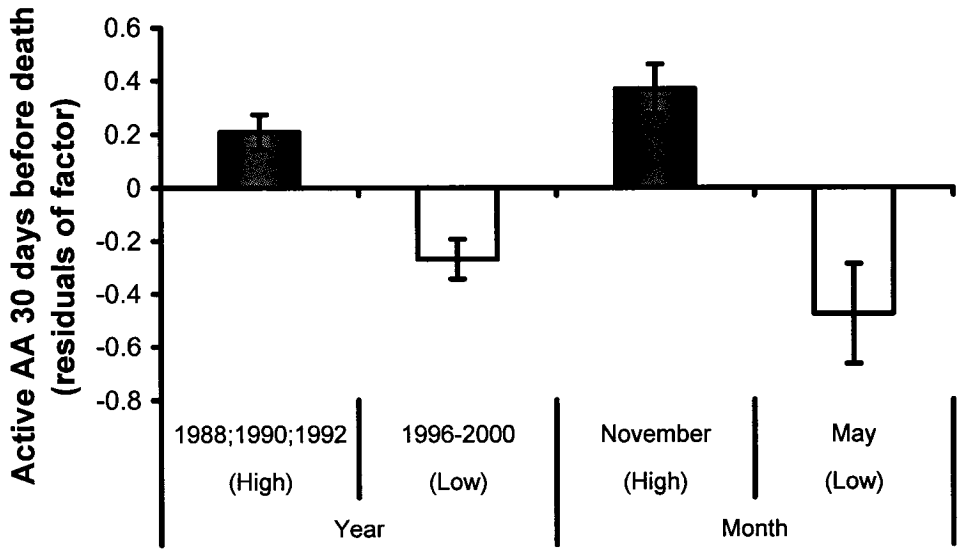


Figure 2.13. Residuals of the factor representing an active geomagnetic field during the 30 days prior to death while controlling for yearly (1988, 1990, 1992 and 1996-2000) and monthly (November and May) average geomagnetic activity.

DISCUSSION

The results herein provide some of the strongest evidence to date for an influence of the geomagnetic field upon SIDS. There were significant magnetic field effects during

the time of gestation, and at the time of death in an age-dependent manner. This is consistent with the triple risk model (Filano & Kinney, 1994) and lends strong support to the model proposed for the involvement of magnetic field disturbances in SIDS (Eckert, 1992). Further, the finding that there was only one variable (average yearly geomagnetic activity) that differed between SIDS and age-matched controls, suggests that both groups might possess a similar vulnerability to a disturbed magnetic field environment in determining the time of death independent of known pathologies. Due to the link between melatonin and geomagnetic activity (Weissbluth & Weissbluth 1994; Sparks & Hunsaker, 1988; Sturner *et al.*, 1990), the results herein suggest that research should be conducted to investigate the link between melatonin and SIDS.

The only variable that differed between SIDS and age-matched controls in this study was the average yearly geomagnetic activity in the year of death. Although the control cases were age- and gender-matched, they were not year-matched as revealed by the hiloglinear analysis showing a significant year (1987-2000) by diagnosis interaction. Since geomagnetic activity goes through 11 year cycles, year-matching becomes important in order to control for geomagnetic activity surrounding the event. Although other analyses investigating shorter time frames (months or days) can employ yearly averages as a covariate, analyses investigating yearly geomagnetic activity lack validity without proper year-matched controls. Thus the finding of differences between SIDS and controls could likely be explained due to a sampling error since not all infants that died with known causes in Quebec between 1987 and 2000 were included in this database. This suggests that the importance of temporally balancing controls and experimental groups during the investigation of any magnetic field effects. This has been suggested in

the past in studies investigating the effects of geomagnetic disturbances on tissue melatonin levels (Jozsa *et al.*, 2005).

Many studies have previously reported that the most vulnerable age for succumbing to SIDS was between 2 and 4 months of age (Mathews & MacDorman, 2007; Horne *et al.*, 2004). This happens to be within the same time frame for the development of melatonin (Kennaway *et al.*, 1996) and cortisol (Mantagos *et al.*, 1998; Antonini *et al.*, 2000) rhythm in infants. Since disturbances in the geomagnetic field can lead to decreased melatonin measures (Jozsa *et al.*, 2005; Burch *et al.*, 1999; Weydahl *et al.*, 2001; Rapoport *et al.*, 1998), these findings lend support to the theory that geomagnetic field disturbances are related to SIDS deaths through a melatonin mechanism (O'Connor & Persinger, 1997). Here we have found that the most vulnerable age groups studied (2.5 and 3.5 month olds) had higher geomagnetic activity during the death day, the death month, and during the 30 days preceding the death day as compared to the other age groups. More specific analyses of the data revealed that during the 30 days prior to death, the 2.5 and 3.5 month old groups tend to have less days with low geomagnetic activity (10-20nT) and more days with extremely high geomagnetic activity (60-70nT and 70-80nT). These higher intensities are within the same range (>80nT) previously reported to be related to decreases in salivatory melatonin levels (Weydahl *et al.*, 2001).

Of interest is that all above age-related relationships were not dependent on whether the cases in the database were within the SIDS or control group. This is of great relevance to the melatonin hypothesis. Melatonin, an important anti-oxidant and protective agent (Maurizi, 1997; Miller *et al.*, 2005; Wantanabe *et al.*, 2004) could be an

influence (decreased) in both the SIDS and controls due to a disturbance in the geomagnetic environment. Whether a known pathology is present or not, the absence of proper concentrations of this important neurohormone could be the determinant in precipitating death on a given day. This could be further exacerbated by the influence of geomagnetic field disturbances on cortisol levels (Rapoport *et al.*, 1998; Jozsa *et al.*, 2005) whose increase could lead to a decreased immune response.

To further understand the differences in the ambient geomagnetic environment surrounding SIDS, high versus low frequency years (1988, 1990, 1992 versus 1996-2000) and months (November versus May) were compared. The analysis of year differences provided compelling evidence that geomagnetic variables play an important role in contributing to SIDS. Investigation of the factors revealed that high-frequency SIDS years were characterized by higher levels of geomagnetic disturbances as compared to low frequency years. The factors that explained the largest amount of variance involved variables representing a disturbed magnetic field during late gestation (fALL1=36%, fGest1=17%), early gestation (fALL2=16%, fGest2=14%), overall gestation (faaGest1=29%) and the 30 days prior to death (f301=18%). The strongest effects were found for the factor representing the number of sudden storm commencements during gestation (fSSC1=44%). Such evidence supports the possibility that decreases in melatonin during gestation could result in developmental abnormalities (Torres-Farfan *et al.*, 2004; Turgut *et al.*, 2005; Kachi *et al.*, 2006) which could result in a vulnerability to other stressors later in development.

The differences in the geomagnetic environment between the early and late 1990s brings into question past findings that have attributed a decrease in the incidence of SIDS

during this time frame to the popular 'Back to Sleep' campaign (Mitchell *et al.*, 2007). Our data suggest that such a drop could be due to changes in the ambient magnetic field environment surrounding developing fetuses and infants. Low incidence years tended to correspond with solar minimum (1997) while high frequency years corresponded with solar maximum (1991). Since the current database has only investigated one 11 year solar cycle, it becomes difficult to elaborate on these findings. Investigation into a long-term database encompassing many solar cycles would help clarify the role of geomagnetism upon the incidence of SIDS.

Investigation of high (November) and low (May) frequency death months demonstrated that those who died during high frequency months had a larger number of days with an active geomagnetic field (20-50nT) and lower number of days with a quiet geomagnetic field (0-20nT) during the 30 day period leading to their deaths. Unlike the year differences discussed above, high and low frequency months had on average similar geomagnetic activity. Interestingly, previous research has reported that geomagnetic disturbances greater than 30nT were successful in decreasing melatonin metabolite (Burch *et al.*, 1999). Such lends further support to the melatonin hypothesis of SIDS.

The finding of an association between SIDS and geomagnetic disturbances in the 30nT range was one of the most consistent results found in the present study. Even when adjusting for average monthly and yearly geomagnetic activity for the high/low frequency months and years respectively, those who died in high frequency time frames displayed significantly higher scores for the factor (f301) representing high geomagnetic activity (20-40nT) during the 30 days preceding death. This coupled with findings that heightened geomagnetic levels were only observed in the most vulnerable age groups

strongly suggests that geomagnetic activity beyond a certain intensity level or within certain intensity bandwidths plays a key role in events leading to SIDS and possibly other known deaths through a melatonin mechanism.

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CHAPTER III

COMPLEX MAGNETIC FIELD EXPOSURES AND VARIOUS LEARNING PARADIGMS

SUMMARY

In order to investigate the effects of brief whole body exposures to a theta burst patterns of magnetic field on rat learning, this chapter investigates three different learning paradigms: conditioned place preference, contextual fear conditioning, and the six arm water maze. Since it has been found that magnetic field exposures can interfere with hippocampal function (Jenrow *et al.*, 1998, Bawin *et al.*, 1996), these experiments investigate whether impairment is specific to different learning paradigms which are acquired by different portions of the hippocampus. The consolidation of contextual stimuli required in conditioned place preference and contextual fear conditioning has been found to be dependent on the ventral hippocampus (Richmond *et al.*, 1999), whereas the dorsal hippocampus is implicated in spatial navigation required in the water maze (Pothuizen *et al.*, 2004; Richmond *et al.*, 1999). In a study investigating the effects of theta burst magnetic field patterns on fear conditioning, it was shown that a continuous 30 minute exposure might impair ventral hippocampal function since rats demonstrated decreased freezing in contextual but not discrete (tone) conditioning (McKay *et al.*, 2000). This is consistent with previous research which has found that electrolytic lesions to the ventral hippocampus impairs context-dependent freezing (Richmond *et al.*, 1999; Kim & Fanselow, 1992) but not discrete conditioning (Kim & Fanselow, 1992). Different magnetic field patterns have been employed in the present studies in order to determine whether they could selectively impair or enhance learning in the various paradigms.

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CHAPTER 3.1

MAGNETIC FIELD EFFECTS ON CONDITIONED PLACE PREFERENCE

ABSTRACT

Theta-burst magnetic field patterns (1μT) designed to mimic the electrical patterns generated *in vitro* to affect long term potentiation have been previously shown to impair the acquisition of a conditioned fear response. In the current study, we investigated whether similar magnetic field patterns could affect the consolidation of food-induced conditioned place preference. Fourteen male Wistar rats were exposed to a theta-burst magnetic field pattern (1 sec pulse of 5 trains of an LTP-evoking pattern) continuously, or with either a 5 sec or 10 sec interstimulus interval, for 15 or 30 minutes immediately following six daily conditioning trials (15 min/day) in a place preference apparatus. Testing demonstrated the durations in the food-paired chamber were significantly shorter for all of the magnetic field-exposed groups compared to the sham-exposed group (they remained for longer periods in the food-paired chamber, typical of normal rats). In addition, the group exposed continuously to the LTP-magnetic field (1 msec interstimulus duration) displayed the least time in the food-paired chamber. The treatments explained 80% of the variance in durations within the experimental setting. These results suggest that exposures to theta-burst magnetic field patterns elicit amnesic effects for contextual stimuli.

INTRODUCTION

It has previously been demonstrated that brief whole body exposures to theta burst patterned magnetic fields can impair acquisition of contextual fear conditioning (McKay *et al.*, 2000) and fear-conditioned analgesia (Stewart & Persinger, 2000). Exposure to the same magnetic field pattern in a discrete (tone) fear conditioning paradigm did not impair task acquisition (McKay *et al.*, 2000). The results of this latter study also showed that the impairments were specific to the pattern used since intensity-matched sine waves failed to elicit impairments in a contextual fear conditioning paradigm.

The current study was designed to determine whether exposure to a theta burst magnetic field pattern could impair memory consolidation in a different learning paradigm: conditioned place preference. In order to further investigate the specific parameters needed to elicit a maximal effect, variations in the interstimulus interval (ISI) between pulse presentations were employed. In addition to continuous magnetic field presentations (1ms ISI), 5 sec and 10 sec interstimulus intervals (ISI) were used as these conditions have been found to elicit long term potentiation in the entorhinal cortex when applied electrically (Yun *et al.*, 2002).

METHODS

Subjects

Fourteen (Experiment 3.1.1) and twelve (Experiment 3.1.2) individually-housed male Wistar rats aged 4 to 5 months served as subjects. Rats were food-deprived the day before the training period and only received food every second day in the conditioned place preference (CPP) apparatus. Water was available *ad libitum*.

Magnetic Field Apparatus

The magnetic field exposure system has been previously described (Martin & Persinger, 2003). Briefly, the apparatus was composed of a 25 cm x 25 cm x 25 cm Plexiglas chamber with two horizontally aligned electromagnets on opposite sides. The electromagnets were composed of 25 cm iron nails wrapped with 1050 turns of 22 gauge enameled copper wire over a length of 19cm. These were placed at the center of two sides of the chamber, 5 cm from its base (~3 cm of corn cob bedding) in an East-West geographic orientation (±5°). The electromagnets were connected to a Zenith XT personal computer through a custom made digital to analog converter that transformed a series of values between 0 and 255 to voltages ranging from -5 to +5 volts with 128 representing neutral polarity. For the waveform generated in this experiment, the values ranged from 125 (~ -0.12volts) to 250 (~4.77volts).

Magnetic Field Pattern

The magnetic field generated was similar to fields that previously employed in this laboratory and found to have amnesic effects in a contextual fear conditioning paradigm (McKay *et al.*, 2000; McKay and Persinger, 2003). The pattern was composed of an initial priming pulse followed by a 170 ms delay (theta) and four pulses at 100 Hz. This pattern was repeated 5 times and followed by an interstimulus interval (ISI). Rats were assigned randomly to either a sham condition or one of three ISI conditions: 1 ms (continuous), 5,000 ms, or 10,000 ms. Exposures in the Experiment 3.1.1 were 15 minutes in duration while those in Experiment 3.1.2 were 30 minutes in duration. The intensity of the magnetic field generated was measured to be between 0.20 μT and 1.71

μT with Metex 3800 multimeter and magnetic sensor probe (Electric Field Measurements, Stockbridge, MA).

Conditioned Place Preference

The CPP apparatus consisted of two chambers (30 cm x 40 cm x 30 cm deep) separated by a partition in which a guillotine door (10 cm x 10 cm) was installed. The chambers were made of oriented strand board (OSB) painted with alkyd paint. One chamber was painted with alternating black and white vertical lines (3cm) while the other chamber was painted with horizontal lines (3 cm). The door and the floor of both chambers were painted white.

Each day, for six days, rats were introduced into one of the two chambers in the presence or absence of food (Purina rat chow) for fifteen minutes. Approximately 50 g of food was placed in a plastic container (10 cm diameter x 3 cm deep) in the back corner furthest from the midline of the chamber. Rats were in a food-paired chamber every second day while on alternate days, they were placed in the opposite chamber without food. Rats in each field condition were counterbalanced for day of food pairing (even days or odd days) and for paired chamber (vertical or horizontal). Chambers were cleaned with 10% ethanol and allowed to dry before each trial. Following fifteen minutes in the CPP apparatus, rats were brought to a separate room where they were exposed to one of the three complex magnetic fields described above or a sham field. Food eaten during the 15 minute period was scored.

The day after the final pairing, rats were given full access to the CPP apparatus by lifting the guillotine door separating the two chambers. The rats were initially placed in

the unpaired chamber and allowed to explore both chambers for fifteen minutes while being videotaped. The videotapes were then viewed and the time spent in each chamber was recorded. A rat was considered to be in a given chamber when both front paws had crossed the midline. Midline crossings were scored. In order to assess searching and locomotor activity in each chamber, rats were scored for rearing and movement in Experiment 3.1.2. To score locomotor activity, each chamber was divided into quadrants and the rats were scored for the number of quadrants traversed.

RESULTS

Experiment 3.1.1 – 15 minutes magnetic field exposure

The means and standard errors of the means (±SEM) for length of time spent in the food-paired and unpaired chamber for the different treatment groups are shown in Figure 3.1.1. A two way analysis of variance with one level repeated (treatment) and one level not repeated (chamber) revealed a significant interaction between chamber and treatments [$F(3,12)= 10.57$; $p<.01$; $eta^2=80\%$]. *Post hoc* analyses using paired t-tests revealed that the source of this interaction could be accounted for by the magnetic field-treated groups spending equal amounts of time in both the paired and unpaired chamber whereas the sham-treated group spent significantly more time in the paired chamber than in the unpaired chamber [$t(2)= 6.16$; $p<.05$). Tukey's test of variance was used to show that animals in the 1ms ISI-treated group [$F(1,5)= 27.69$; $p<.05$], the 5,000 ms ISI-treated group [$F(1,5)= 9.36$; $p<.05$] and 10,000 ms ISI-treated group [$F(1,5)= 18.91$; $p<.05$] all spent significantly less time in the paired chamber relative to the sham-treated group.

However, the 1ms ISI-treated group spent significantly less time in the paired chamber than the 5s ISI-treated group [$F(1,5)= 7.50$; $p= .05$].

To test whether the magnetic field-treated groups spend less time in the paired chamber due to deficits in memory retrieval during the testing phase as opposed to behavioural differences during the conditioning phase, an analysis of food consumption was conducted. Food consumed during the conditioning phase was analyzed through a two-way MANOVA test with one level repeated (testing day) and one level not repeated (field treatment). The analysis revealed a statistically significant main effect for field [$F(3,13)= 4.45$; $p<.05$, $\eta^2=57\%$]. Although there were no difference in rat masses across the treatment conditions [$F(3,13)=.27$; $p>.05$], the analysis investigating total food eaten during training was run once again with rat weight as a covariate. Through this analysis of covariance, the treatment effect was no longer significant [$F(3,11)=3.43$; $p>.05$].

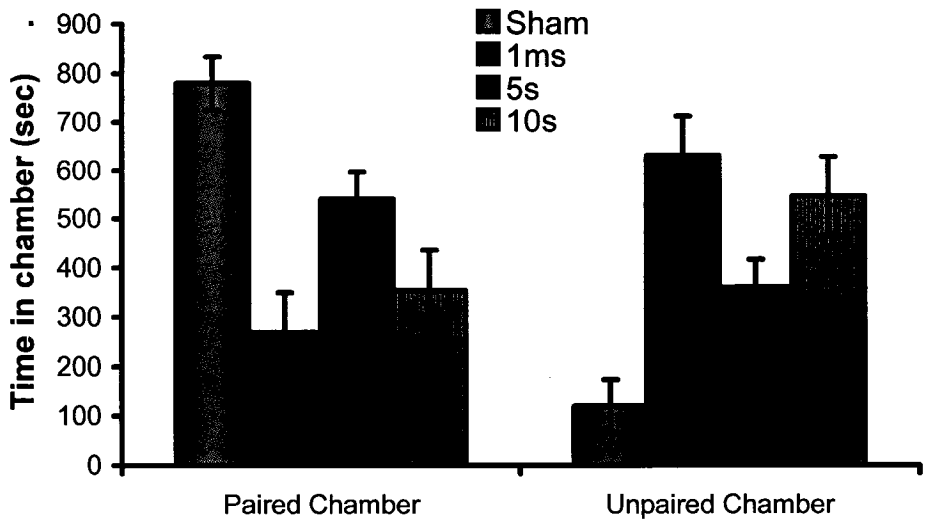


Figure 3.1.1. Time spent in the paired and unpaired chamber across the different magnetic field treatment conditions.

A second analysis to verify whether food consumption was related to time spent in the different chambers was conducted as two separate (paired chamber and unpaired chamber) oneway analyses of covariance with total food consumption employed as a covariate. The results of the analysis revealed a significant main effect for treatment group for the time spent in the food-paired chamber [$F(3,11)=8.73$; $p<.05$; $\omega^2=77\%$]. Residuals for time spent in the paired chamber (total food consumption as a covariate) were generated. The means (±SEM) for treatment group can be found in Figure 3.1.2. *Post hoc* analyses revealed that the source of the effect was due to the 1 msec ISI-treated group spending significantly less time in the food paired chamber than the 5 sec ISI-treated and sham groups which both did not differ from the 10 sec ISI-treated group.

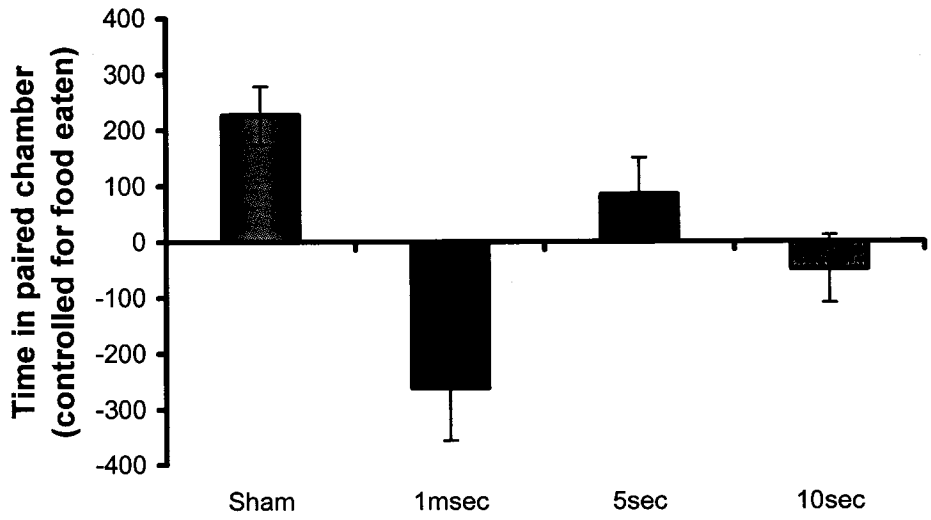


Figure 3.1.2. Time spent in the food paired chamber while controlling for total food consumption during training across the different treatment conditions.

Further tests to confirm that these differences were due to deficits in conditioning and not motor activity were conducted. A one-way analysis of variance (ANOVA) with one level not repeated (field) was conducted for the number of times that rats crossed the

midline of the CPP apparatus. A significant main effect for field was discovered [$F(3,11)= 5.406$; $p<.05$; $\eta^2=67\%$]. The means (±SEM) for midline crossings across treatment conditions can be found in Figure 3.1.3. *Post hoc* analysis using a Tukey's test revealed that the source of the effect was due a significantly higher number of crossings by the 5,000 ms ISI-treated group [$F(1,5)= 15.75$; $p<.05$] and the 10,000 ms ISI-treated group [$F(1,5)= 9.72$; $p<.05$] compared to the sham-treated animals which did not differ significantly from the 1ms ISI-treated group. There were no other significant differences between groups though animals in the 5,000 ms ISI-treated group having crossed more squares than animals in the 1 ms ISI-treated group approached significance [$F(1,5)= 5.69$; $p=.076$].

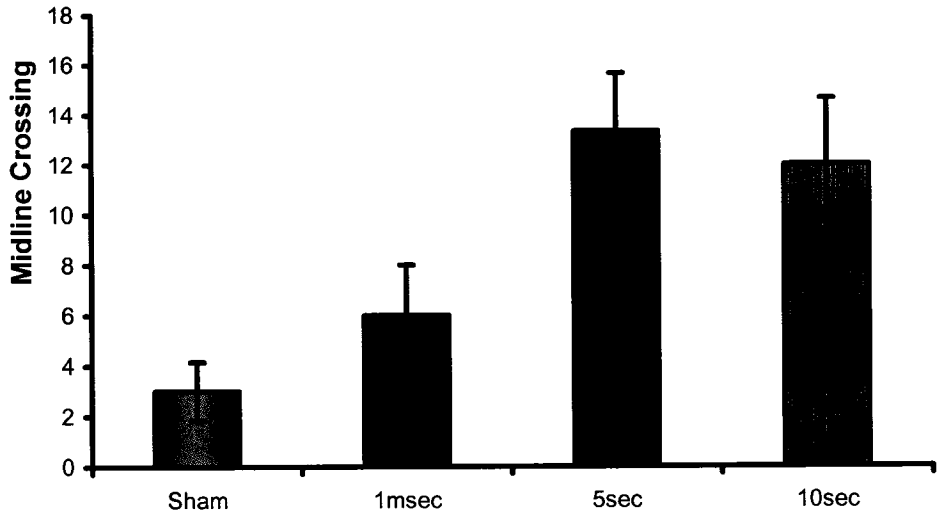


Figure 3.1.3. Total midline crossing across all treatment conditions.

A oneway analysis of covariance between treatment condition for time spent in the paired chamber with midline crossings as a covariate was significant [$F(3,11)=9.12$; $p<.05$; $\omega^2=76\%$]. The residuals for time in the food-paired chamber (control for midline crossings) for all treatment conditions can be found in Figure 3.1.4. *Post hoc* analyses

revealed that the source of the effect was due to the 1 msec ISI-treated group spending significantly less time in the paired chamber as compared to the sham-treated group which did not differ from the other treatment conditions.

Experiment 3.1.2 – 30minute magnetic field exposure

A multivariate analysis with one level repeated (chamber) and one level not repeated (field) revealed a field by chamber interaction that approached statistical significance [$F(3,11)=3.79$; $p=.059$; $\eta^2=59\%$]. The means (±SEM) for time spent in the paired and unpaired chamber for all magnetic field conditions can be found in Figure 3.1.5. *Post hoc* analysis employing a Tukey's test of significance revealed that the source of the interaction was due to the 5 sec ISI-treated animals spending significantly less time in the paired chamber than the sham [$F(1,5)=5.69$; $p=.076$] and 1ms ISI-treated animals [$F(1,5)=5.91$; $p=.072$].

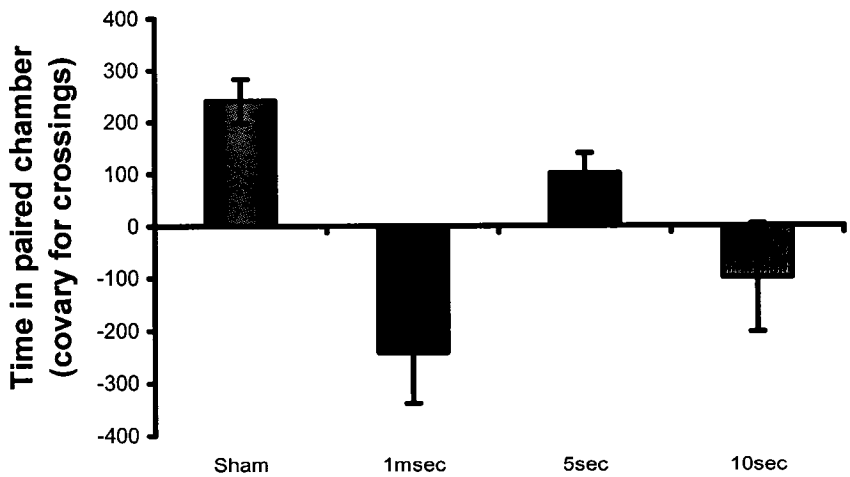


Figure 3.1.4. Time spent in the food paired chamber across the different treatment conditions while controlling for midline crossings.

To ensure that the effects were due to actual amnesic effects of the magnetic field treatments and not to locomotor activity, an analysis of covariance investigating the total time spent in the paired chamber while controlling for total movement (total quadrant crossings + total rearing) was conducted. This analysis revealed that the treatment effect was no longer significant [$F(3,11)=3.41$; $p>.05$].

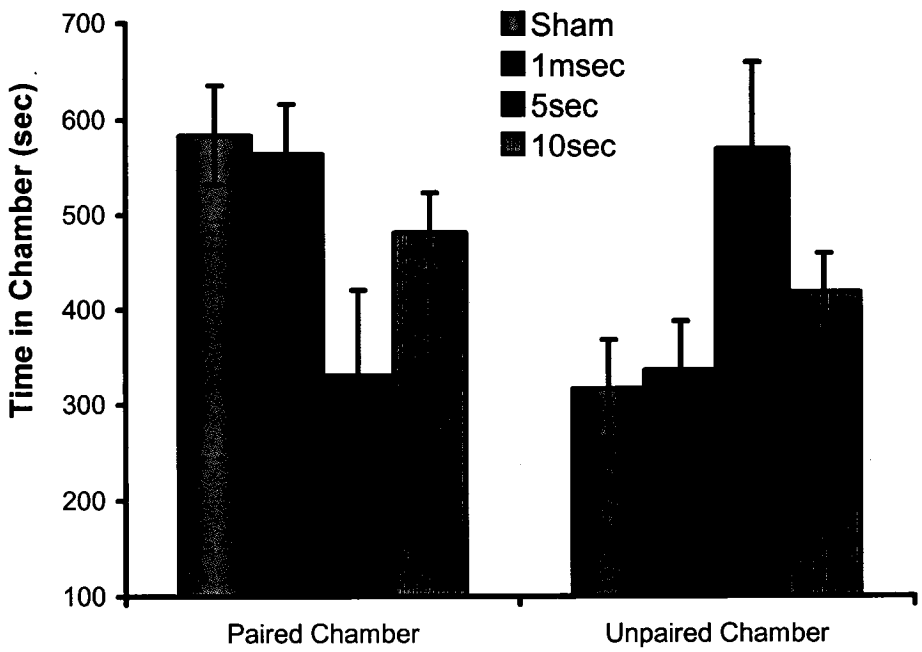


Figure 3.1.5. Time spent in the paired and unpaired chamber across the different magnetic field treatment conditions.

A two way analysis with one level repeated (chamber) and one level not repeated (field) for the amount of movement in each chamber revealed significant main effects for field [$F(3,11)=5.94$; $p<.05$; $\eta^2=60\%$] and chamber [$F(1,12)=12.02$; $\eta^2=60\%$]. The means of the two chambers were subsequently collapsed into a single mean. These means (±SEM) for movement in the whole CPP apparatus can be found in Figure 3.1.6. *Post hoc* analyses employing a Tukey's test demonstrated that the source of the interaction was

due to animals in the 5 sec ISI-treated group moving significantly less than the animals in the sham and 10 sec ISI-treated group [$F(3,11)=5.94$; $p<.05$].

The means (±SEM) for the quadrant crossings in both the paired and unpaired chambers for all rats can be found in Figure 3.1.7. *Post hoc* analyses employing paired t-tests revealed that the effect was due to rats crossing significantly more in the paired chamber as compared to the unpaired chamber [$t(11)=3.71$; $p<.05$].

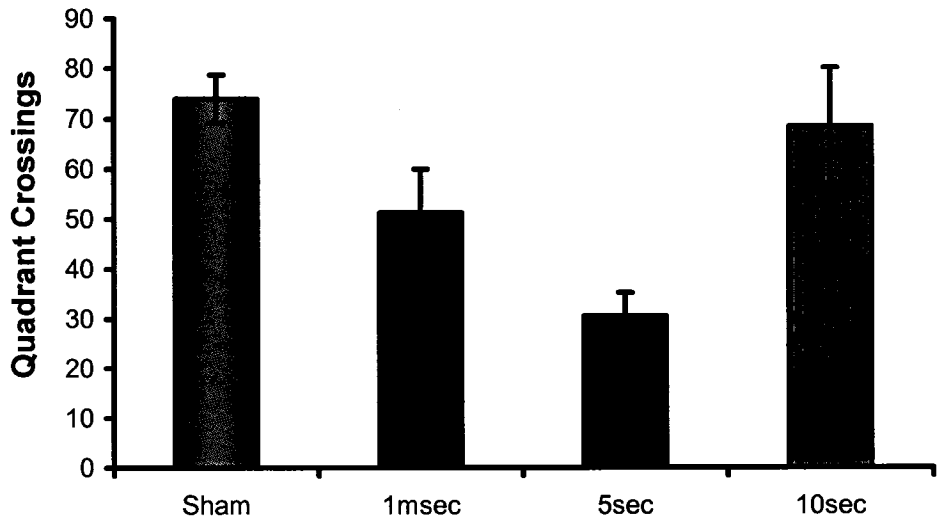


Figure 3.1.6. Total quadrant crossings in the CPP apparatus on the testing day across the different treatment conditions.

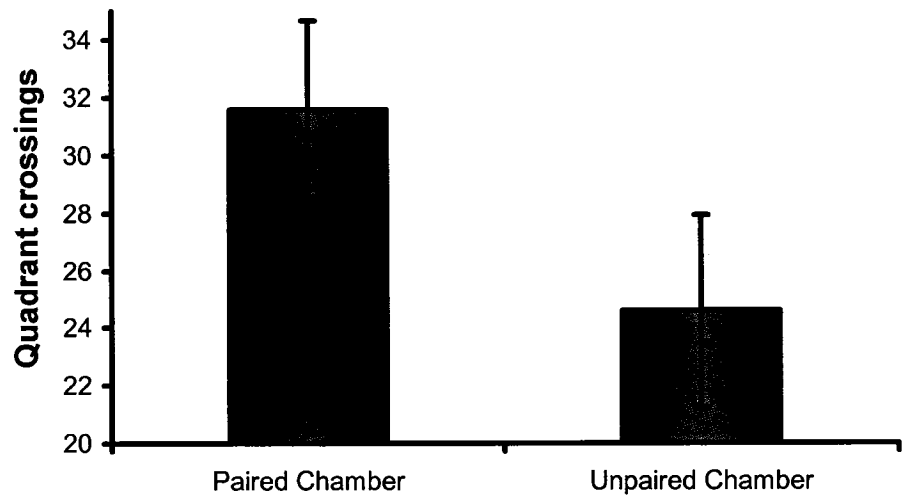


Figure 3.1.7. Quadrant crossings for all rats in the paired and unpaired chambers.

A similar multivariate analysis of searching behaviour as indicated by rearing in the paired and unpaired chambers revealed a significant field-by-chamber interaction [$F(3,11)=6.06$; $p<.05$; $\eta^2=69\%$]. The means (±SEM) for rearing in the paired and unpaired chamber across treatment conditions can be found in Figure 3.1.7. *Post hoc* analysis employing paired t-tests indicated that the source of the interaction was partially due to animals in the sham-treated group rearing significantly more in the food-paired chamber compared to the unpaired chamber [$t(2) = 7.20$; $p<.05$) while the other groups did not differ in the amount of rearing between chambers. Further, Tukey's test revealed that animals in the 5s ISI-treated group reared significantly less than those in the sham [$F(1,5)= 73.13$; $p<.05$], 1 ms ISI [$F(1,5)=12.07$; $p<.05$] and 10 sec ISI-treated group [$F(1,5)=10.57$; $p<.05$].

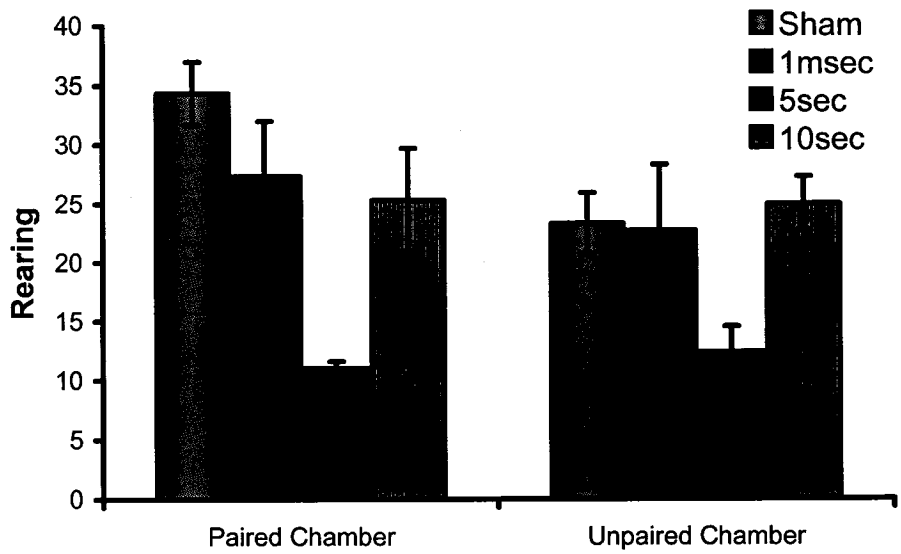


Figure 3.1.7. Rearing in the paired and unpaired chambers across the different treatment conditions.

DISCUSSION

In the current study we demonstrated that brief exposures to theta burst magnetic field patterns can impair the consolidation of conditioned place preference. Not only did

treatment of rats with all the different ISI conditions produce impairments, it was found that a shorter magnetic field exposure duration (15 min) than used in previous studies (McKay *et al.*, 2000) was sufficient in producing amnesic effects. The goal of investigating the effects of magnetic field pattern exposure on learning is to find a temporal pattern that enhances learning. However, current research seems to indicate that an impairment of natural function is more likely to result than an enhancement.

In experiment 3.1.1, all magnetic field treatment conditions demonstrated a deficit in learning as shown by their spending equal time in the paired and unpaired chamber. These effects were most pronounced for animals in the 1 msec ISI-treated group since after accounting for movement (midline crossings) and food eaten during the training phase, this group still spent less time in the food-paired chamber than sham-treated control animals. Although this pattern was the same as employed in previous studies (McKay *et al.*, 2000), this study employed a much shorter magnetic field exposure duration which might be due to the repetitive nature of the exposures in this study as compared to the single exposure.

Some studies have found that conditioned place preference can be modulated by the hormone melatonin. In an experiment investigating morphine-induced conditioned place preference, it was found that melatonin treatment caused a dose-dependentt increase in the rewarding properties of morphine (Yahyavi-Firouz-Abadi *et al.*, 2007). Disruption of the light:dark cycle by reducing the duration of the dark cycle (6 hours only) requires larger doses of morphine to induce conditioned place preference. In these studies, supplementation of melatonin abolished the need for an increased dose of morphine (Tahsili-Fahadan *et al.*, 2005). There is strong evidence that melatonin

secretion can be disrupted through exposures to complex magnetic fields (Touitou *et al.*, 2006). A magnetic field induced antagonism of the melatonin pathway could have produced effects similar to as a disruption of the light:dark cycle. Although there have not been any studies that suggest that melatonin directly modulates food-induced conditioned place preference, it is conceivable that interference with melatonin production could have decreased the rewarding properties of food and thus impaired the acquisition of the paradigm by the magnetic field-treated groups.

Of interest in this study was the finding that animals in the 5 sec and 10 sec ISI treatment conditions demonstrated hyperactivity as shown by an increase in midline crossings. Such increases in locomotor activity might be a result of the chronic exposures to the theta burst magnetic field pattern. It has been demonstrated that there is an increase in hippocampal theta activity in the rat during locomotion (Sinnamon, 2005). This rhythm has been proposed to originate in the midbrain reticular formation and reach the hippocampus through the medial septal nuclei (Vertes *et al.*, 2004). Further evidence that suggests hippocampal involvement in the resulting hyperactive effects comes from studies that have demonstrated that hyperactivity can result from hippocampal lesions (Good & Honey, 1997; McNish, *et al.*, 1997).

An alternate explanation for the increased movement could be linked to the amnesic properties of the theta burst magnetic field pattern exposure (McKay *et al.*, 2000). It has been shown that rats tend to display more movement in a novel environment compared to a familiar one (Goodwin & Yacko, 2004). Studies investigating the effects of cocaine on place preference have also demonstrated similar increases in locomotion on

test days although these are typically paired with an increase in preference for the drug-paired chamber (Zhang *et al.*, 2004) which was not seen in the current study.

The results of experiment 3.1.2, which showed that rats exposed to a theta burst magnetic field pattern for 30 minutes had the largest learning and locomotor impairments for animals in the 5 sec ISI-treated group, is contrary to the effects seen in the 15 minute exposure experiment. Unfortunately it becomes difficult to draw conclusions which are directly related to impairments in consolidation since the impairments were no longer present when accounting for the decreased activity of the 5 sec ISI-treated group. These results suggest that there might be a critical time duration window during which exposures to complex magnetic fields are most effective. Such could be analogous to dose-response curves as seen with many drugs which are most effective in specific doses and no others.

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CHAPTER 3.2

THETA BURST MAGNETIC FIELDS AND CONTEXTUAL FEAR CONDITIONING

ABSTRACT

The contextual fear conditioning paradigm has long been used to investigate learning and memory in rats. In the current experiment, 22 male Wistar rats were trained in a contextual fear conditioning paradigm following a 30 minute exposure to a theta burst magnetic field pattern which was generated in one of two compass orientations (East-West or North-South). The specific field consisted of 5 trains of 5 100 Hz pulses separated by 140 ms (theta). These trains of pulses were separated by one of three inter-stimulus intervals: 1 msec, 5 sec, or 10 sec. Rats were trained in a contextual fear conditioning chamber where they received three unsignaled foot shocks at 1 minute intervals. Following the training period, rats were tested for movement in an extinction test where they were placed back in the context where they received the shocks and observed for movement over an 8 minute period. The results of the study demonstrated that when the field was generated in the East-West orientation, rats exposed to the field with a 5 sec interstimulus interval demonstrated an increase in movement on the testing day which is indicative of impairment of consolidation.

INTRODUCTION

Contextual fear conditioning is a widely used paradigm to investigate memory and learning (Kim & Jung, 2006). This paradigm demonstrates that when rats are exposed to contextual cues paired with an aversive stimulus, they remain relatively immobile when re-exposed to the contextual cues alone. Specific brain areas, such as the hippocampus, are involved in learning the associations between stimuli and contextual cues. Lesions of the hippocampus have been found to selectively impair acquisition and consolidation of contextual fear conditioning (Phillips & LeDoux, 1992). In addition, certain drugs such as the noncompetitive NMDA receptor antagonist, MK-801, have been found to interfere with the consolidation of contextual fear conditioning but not with discrete (tone) fear conditioning (Bast *et al.*, 2003). McKay and colleagues (2000) have found similar effects resulting from brief exposures to a theta burst magnetic field pattern.

It is well known that hippocampal neurons exhibit five pulse bursts within the range of theta rhythms during the performance of spatial memory tasks (Otto *et al.*, 1991). Theta activity has also been implicated in the early stages of learning and memory (Vertes, 2005). Further, long term potentiation, a major electrophysiological correlate of learning can be induced by applying these patterns directly through electrical stimulation (Albensi, 2007). The current investigation attempts to elaborate on previous research which demonstrated learning effects as a result of theta burst magnetic field pattern exposure in the contextual fear conditioning paradigm (McKay *et al.*, 2000) while adding new parameters which have been found to be effective in eliciting long term potentiation through electrical stimulation (Yun *et al.*, 2002).

METHODS

Subjects

Twenty-two male Wistar albino rats obtained from Charles River (Quebec) served as subjects. Rats were aged 7-12 months and were housed 2-3 per cage and maintained on a 12:12 light-dark cycle with light onset at 0730 hrs local time. Food and water were available *ad libitum*. All training and testing took place between 1400 and 1800 hrs local time.

Conditioning

All testing and conditioning was conducted in a 28 cm x 20 cm x 20 cm modified operant chamber. The front, back and hinged top door of the chamber were constructed of Plexiglas, and the lateral walls were made of aluminum. The floor consisted of 18 steel rods (0.2cm diameter) spaced 1.5 cm apart, each connected to a unit designed to deliver scrambled electric shocks.

The training phase consisted of a three minute habituation period followed by three 1 sec unsignaled footshocks (0.5 mA) at 60 sec intervals. During the habituation phase, midline crossings were counted in order to receive a baseline measurement of movement. Rats were returned to their home cages immediately after the last footshock and the operant chamber was cleaned with 10% ethanol.

Twenty four hours following training, rats were returned to the operant chamber for the testing phase. Rat behaviour was scored nominally for the presence or absence of freezing through a time-sampling procedure for 1 sec every 8 sec during an 8 min extinction test. The number of freezing observations was converted to a percentage of

total observations. For the purposes of analyses, the extinction test was subdivided into 2 minute time bins. The presence of urination was also ranked on a three point scale and the numbers of fecal boluses were counted.

Magnetic Field Exposure

Prior to conditioning in the operant chamber, rats were exposed to either a sham or a complex magnetic field. The magnetic field exposure system used has been previously described in chapter 3.1 (Methods).

The magnetic field pattern employed in this study was designed to mimic electrophysiological stimuli whose patterns have been known to elicit long term potentiation in hippocampal slices (Vertes, 2005). This magnetic field consisted of five pulse bursts at 100 Hz separated by 140 ms (theta) repeated 5 times and was measured to be 0.5 μT with a Metec N380 coupled to a magnetic sensor (Electric Field Measurements, Rt 183W., Stockbridge, MA 01266). Rats were assigned to one of three interstimulus interval (ISI) conditions where either 1 ms (continuous), 5 sec, or 10 sec separated each series of pulses. Also, in this experiment, rats were exposed to a magnetic field generated in one of two compass orientations (North-South or East-West)

RESULTS

Studies to investigate the movement of the rats during the testing phase were examined through a two-way multivariate analysis of variance with one level repeated (time bin) and one level not repeated (field). This analysis did not reveal any significant main effects for field [$F(3,21=0.25;\ p>.05)$ or interactions between field and time

[F(9,63)=0.65; p>.05]. The analysis did, however reveal a significant main effect for time bin [F(3,57)=22.88; p<.01; η^2=56%]. The means (+/- SEM) for movement as a function of time bin can be found in Figure 3.2.1. Post hoc analysis employing paired t-tests revealed that the source of the effect was due to rats significantly increasing their percentage movement between the second and third time bins [t(21)=-3.88; p<.01] and again between the third and fourth time bins [t(21)=-3.25; p<.01].

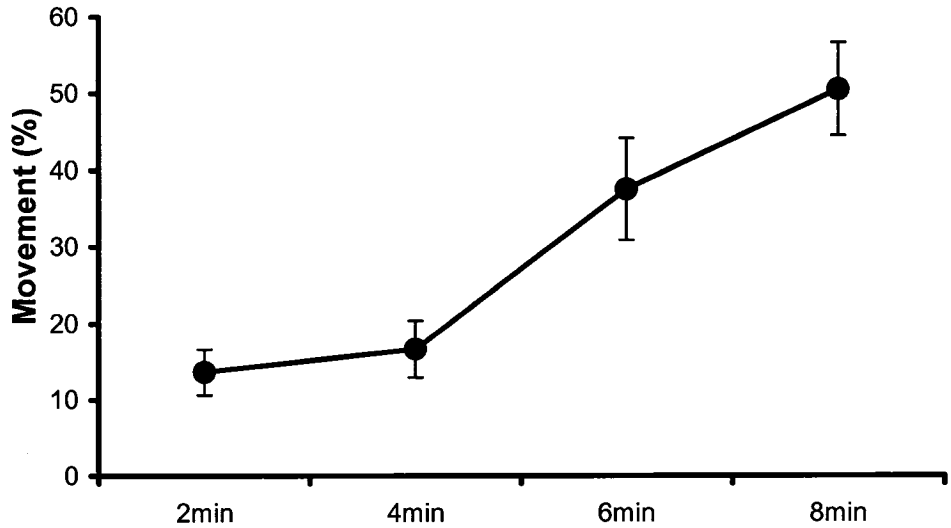


Figure 3.2.1. Movement (±SEM) for rats throughout a 8 minute extinction trial.

Oneway analyses of variance for movement, numbers of boluses, and urination did not reveal any significant main effects for magnetic field treatment (p>.05). Covarying for movement (midline crossings) during training did not change the results of these analyses.

When only the E-W field treatment was selected, investigation of freezing behaviour (movement) revealed a main effect for field during the first time bin that approached statistical significance [F(3,13 = 4.0; p=0.06]. The means (+/- SEM) can be found in Figure 3.2.2. Post hoc analysis employing Tukey's test revealed that the source

of the interaction was due to the 5 sec ISI-treated group moving significantly more than the 10 sec ISI-treated group. The other time bins for movement, number of boluses, or urination did not yield any significant effects ($p>.05$). Adjustment for movement during training did not change the results of the analyses.

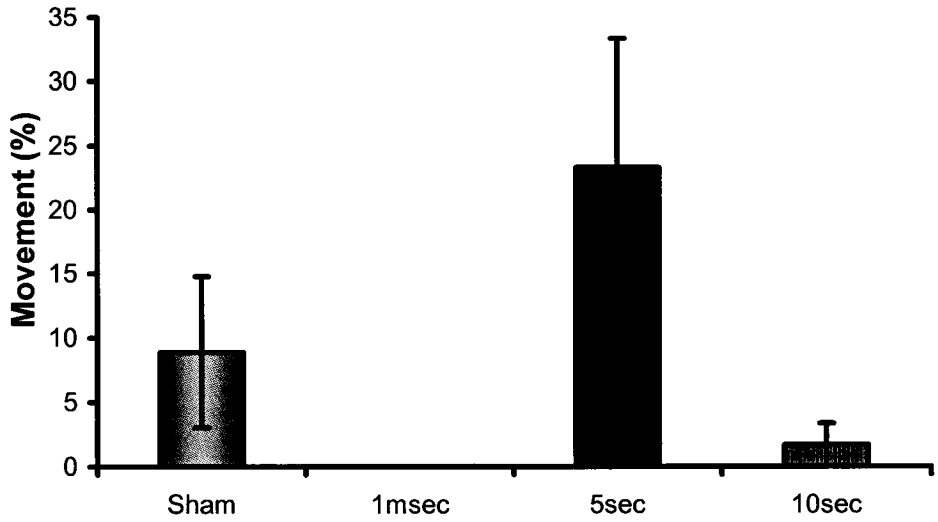


Figure 3.2.2. Rat movement during the first two minutes of the testing for the four magnetic field conditions exposed in the East-West orientation.

DISCUSSION

The results of the current study failed to replicate past experiments which employed a continuous theta burst magnetic field to impair consolidation of a contextual fear conditioning paradigm although this might have been due to the difference in the exposure apparatus (McKay *et al.*, 2000). However, as expected, rats demonstrated a typical extinction curve over the course of the testing day with percentage movement increasing as the trial approached the 8 minute mark. When rats were exposed to the field in an East-West orientation, the group treated with a 5 sec ISI pattern demonstrated increased movement in the first time bin of the experiment. This same inter-stimulus

interval has been shown to elicit long term potentiation in layer V of the entorhinal cortex when a 2 Hz frequency was used (Yun *et al.*, 2002).

That only one magnetic field orientation was successful in eliciting learning deficits in this paradigm could be related to the theory of ion resonance which states, that a magnetic field affecting a charged particle is related to the frequency of the time varying magnetic field and the background magnetic field (Blanchard & Blackman, 1994). Since the geomagnetic field has different strengths depending on the cardinal orientation, it is possible that the theta (7 Hz) magnetic field generated in the East-West orientation was able to interact with the background magnetism (d.c.) to resonate with a biologically active ion implicated in memory. Unfortunately, the background magnetic field intensity was not measured at the time of the experiment.

Looking at biologically active ions that could resonate at 7Hz, it was found that the only ion that could resonate within reasonable naturally occurring background magnetic field intensities was magnesium (Mg^{2+}). Applying the formula for parametric ion resonance (Blanchard & Blackman, 1994) to this ion, it was calculated that the background intensity would have to be 5,539 nT. Mg^{2+} makes a good candidate to be involved in learning mechanisms since it blocks N-methyl-D-aspartae (NMDA) receptor. These receptors have been those found to be most intimately implicated with the cellular correlate of learning: long term potentiation (Gruart & Delgado-García, 2007). If the 7 Hz magnetic field interfered with the Mg^{2+} ion through resonance mechanisms, there is a possibility that such could have interfered with learning too at the level of the NMDA receptor in the hippocampus or other areas.

Previous studies investigating the effects of magnetic field exposures in the hippocampus have suggested that structures which have endogenous oscillators could be most susceptible to perturbation by exogenous magnetic fields (Jenrow *et al.*, 1998, Bawin *et al.*, 1996). It has been postulated that through unknown mechanisms, theta burst magnetic field pattern exposures could disrupt these endogenous rhythms or interfere with long term potentiation (McKay & Persinger, 2003). Such disruption has been found to be partially mediated by nitric oxide. Bawin and colleagues (1996) found that hippocampal rhythmic slow activity could only be suppressed by magnetic field exposures when nitric oxide was present. Magnetic field induced hyperalgesia has also been found to have an underlying nitric oxide mechanism since a nitric oxide synthase inhibitor was found to eliminate the hyperalgesic effects (Jeong *et al.*, 2006).

Spatial learning can be disrupted through nitric oxide mechanisms. In a study investigating the effects of L-NAME, a nitric oxide synthase inhibitor, learning was found to be disrupted in a water maze paradigm (Ocakçioglu & Yavuzer, 1998). Taken together these data suggest that exposures to a theta burst magnetic field pattern could potentially disrupt learning through a nitric oxide mechanism through the disruption of naturally occurring hippocampal rhythms.

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CHAPTER 3.3

THE EFFECTS OF A THETA BURST MAGENTIC FIELD ON RAT PERFORMANCE IN A SIX ARM WATER MAZE

ABSTRACT

Spatial learning has been found to be impaired by brief whole body exposures to weak theta burst magnetic field patterns in various learning paradigms. The water maze has been widely employed as a tool to investigate the effects of various treatments on learning and memory. In this experiment, thirteen male Wistar rats were trained for 5 days in a six arm water maze where time to find a submerged platform was scored over 5 trials. Following each training day, rats were exposed to a sham or to a theta burst magnetic field pattern with one of three interstimulus intervals (ISI): 1 msec, 5 sec, and 10 sec. On the sixth day, the platform was removed and arm entries and the time spent in each arm of the maze was scored. Only animals in the 1 msec ISI-treated group showed an affinity for the goal arm in that it spent significantly more time there than did animals in any of the other treatment conditions. Animals in the 10 sec ISI-treated group displayed an impairment in learning by making significantly more arm entry errors when controlling for total arm entries. The results of this study demonstrate that various parameters of exposures to a theta burst magnetic field pattern can have varying effects on learning.

INTRODUCTION

As the previous chapters have demonstrated, learning can be disrupted by brief exposures to theta burst magnetic field patterns when applied through various parameters. A commonly employed paradigm for the investigation of learning processes in the rat is the Morris water maze (D'Hooge & De Deyn, 2001). Some studies have described an age-specific deficit in water maze performance following perinatal exposures to magnetic resonance imaging-strength magnetic field (Yang *et al.*, 2007). Whereas this study employed relatively strong static fields, few studies have been conducted investigating the effects of weak extremely low frequency magnetic field patterns on spatial learning in the Morris water maze.

Most of our learning experiments have involved the hippocampus, although learning in each of these can be modulated by different pathways and thus performance could be affected differently by the magnetic field exposure. The contextual fear conditioning paradigm has a strong amygdaloid component (Kim & Jung, 2006). The conditioned place preference paradigm implicates pathways and brain nuclei involved in reward mechanisms such as the nucleus accumbens (White & Hiroi, 1993). In the current study, we investigated whether the weak theta burst magnetic field parameters employed in previous chapters had similar learning impairment effects on performance in the water maze. The latter is less modulated by other brain areas as compared to the other learning paradigms and is more intimately involved with hippocampal function (D'Hooge & De Deyn, 2001).

METHODS

Subjects

Thirteen male Wistar albino rats obtained from Charles River (Quebec) were used in the experiments. Rats were maintained on a 12:12 light-dark cycle with light onset at 0730. Rats were between 5 and 12 months of age. All experiments were conducted between 1400 and 1900 hours local time.

Procedure

For five consecutive days, rats were trained in a six-arm water maze (training phase). One arm contained a randomly assigned submerged platform (1cm below surface) that did not change in location for the extent of the training for a specific rat. Each training day consisted of five trials where rats were randomly assigned to begin at the end of one of the five arms not containing a platform. The temporal order of the arms was counterbalanced over the five days of training. Rats were allowed to swim in the maze until they found the platform or for a maximum of 2 minutes. Once the rats found the platform, they remained there for the remainder of the trial or for at least 30 seconds if they did not find the platform in the first 1.5 minutes or more. If rats did not find the platform within the 2 minutes, they were guided to it with the use of a wooden rod. Between each trial, rats were removed from the maze and placed in a shoebox cage for 15 sec. The time taken for the rats to find the submerged platform on each of the trials was recorded.

Rats were tested on the sixth day (testing phase). For testing, the platform was removed. Rats were placed in the center of the maze facing away from the goal arm and

allowed to swim freely for 2 minutes. The time spent in each section of the maze (arms and center) as well as the total and incorrect arm entries were scored. For the purpose of analyses, each two minute trial was divided into 1 minute banks.

Water maze

The water maze was made up of a 2 m diameter children's pool containing water to a height of 15 cm. Within the pool, six Plexiglas arms (25 cm x 75 cm x 75 cm high) were placed in a star formation. A submerged platform (1 cm below the surface) was located at the end of a specific arm for the extent of the training. For the purpose of the analyses, the location of the rat was noted to be in one of five locations: goal arm, opposite arm, far arms, close arms, and center (Figure 3.3.1). The time in the close and far arms was the total time spent in these two arms divided by two.

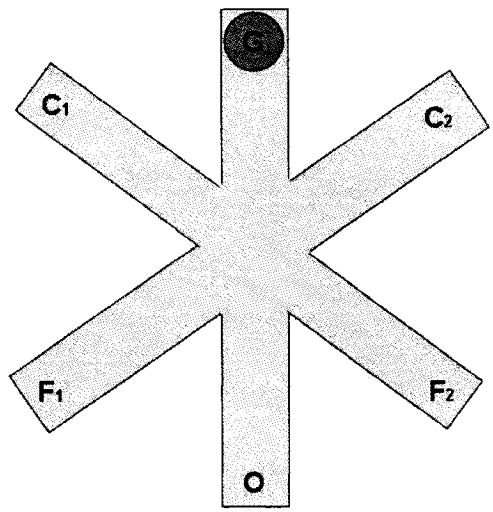


Figure 3.3.1. Six arm water maze with the goal platform (G), close arms (C_1, C_2), far arms (F_1, F_2) and opposite arms (O).

Magnetic Field Exposure

Following each training session, rats were exposed to either a sham or a complex magnetic field. The apparatus and magnetic field patterns employed in this study were the same as described in Chapter 3.1 (Methods).

Statistical Analyses

Analyses for the acquisition phase were conducted though 5X5X4 multivariate analysis of variance with two levels repeated (trial, day) and one level not repeated (field). Analyses for the testing phase were conducted through a 2X2X4 multivariate analysis of variance with two levels repeated (trial, time) and one level not repeated (field). Dependent variables for the testing phase included total arm entries, error arm entries, and total time spent in each of the arms and center of the apparatus. All between subject *post hoc* analyses were conducted with the use of Tukey's test while within subject analyses were conducted with the use of paired t-tests.

RESULTS

Training Phase

A three way mixed analysis of variance with two levels repeated (trials, days) and one level not repeated (field) for the time taken to find the platform during the acquisition phase revealed significant main effects for day [$F(4,48)=29.32$; $p<.05$; $\eta^2=79\%$] and trial [$F(4.48)=3.36$; $p<.05$; $\eta^2=31\%$]. A day-by-trial interaction [$F(16,192)=2.27$; $p<.05$; $\eta^2=22\%$] was also found. The analysis further revealed a field-by-day interaction that approached statistical significance [$F(12,48)=1.78$; $p=0.096$; $\eta^2=40\%$].

To investigate the main effect for day, the 5 trials per day were collapsed into daily means. The means (±SEM) for the time taken to find the platform can be found in Figure 3.3.2. *Post hoc* analyses employing paired t-tests revealed that the source of the effect was due to rats significantly decreasing their latencies on succeeding days except between days 4 and 5 [$t(11) \geq 2.95$; $p < .05$].

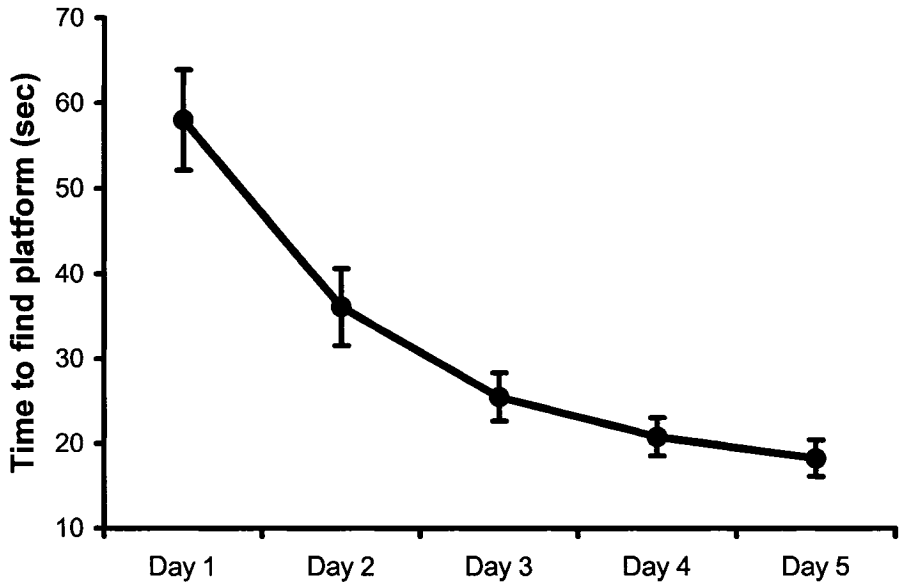


Figure 3.3.2. Time taken to find the submerged platform during the training phase over the 5 days of testing.

Post hoc analysis of the trial effect was conducted after collapsing the trial means for the 5 days of testing. The means (±SEM) for time taken to find the platform over the 5 trials (SEM) can be found in Figure 3.3.3. Post hoc analyses employing paired t-tests (all dfs=11) demonstrated that the source of the effect was a significantly lower platform latency during trial 5 as compared to trial 1 (t=3.21), trial 3 (t=2.77) and trial 4 (t=4.23).

The means (±SEM) for the time taken for rats to find the platform over the five days of testing for each of the 5 trials can be found in Figure 3.3.4. Paired t-tests revealed

that the source of the day-by-trial interaction was mainly due to differences on days 1 and 2. *Post hoc* analyses (all dfs=11) demonstrated that on day 1, rats had a significantly longer platform latency on trial 1 as compared to trials 4(t=2.51) and 5(t=3.52). On day 2, rats demonstrated significantly longer times to find the platform on trials 3 and 4 as compared to trials 2 and 5 (t≥2.25).

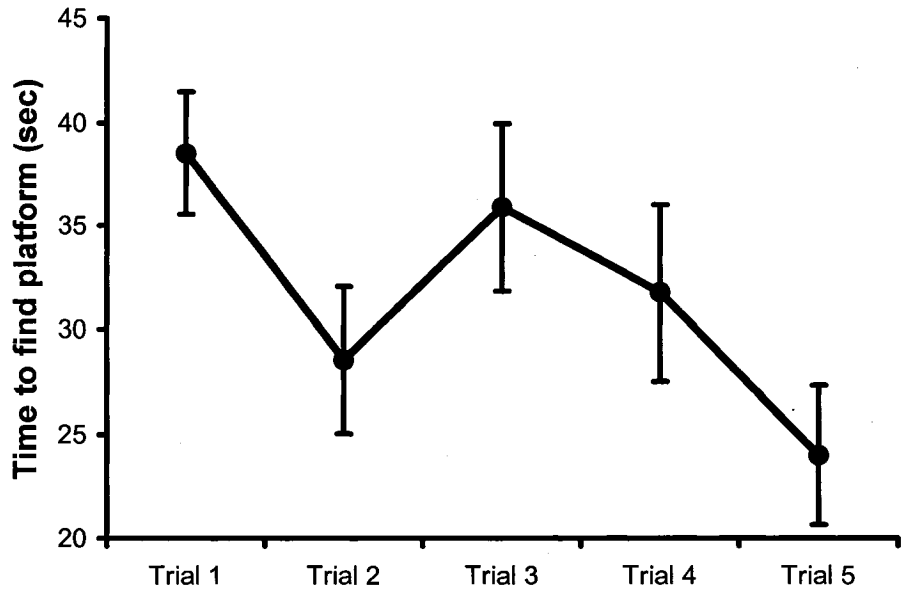


Figure 3.3.3. Mean time taken to find the submerged platform during the training phase over the 5 trials.

The near significant field-by-day interaction was analyzed subsequent to collapsing the data into daily means. These means (±SEM) for the time taken for rats treated with each condition to find the platform can be found in Figure 3.3.5. Although Tukey's test did not reveal any significant differences between animals in the different treatment conditions on any of the given days, *post hoc* analysis employing paired t-test for individual groups (all dfs=2) revealed that the source of the interaction was due to animals in the 5 sec ISI- and sham-treated group having a significantly longer latency on

day 3 than on day 5 (t=3.57) while animals in the 1 ms ISI-treated group had a significantly longer latency on day 1 as compared to all other days (t= 6.05 to 75.16).

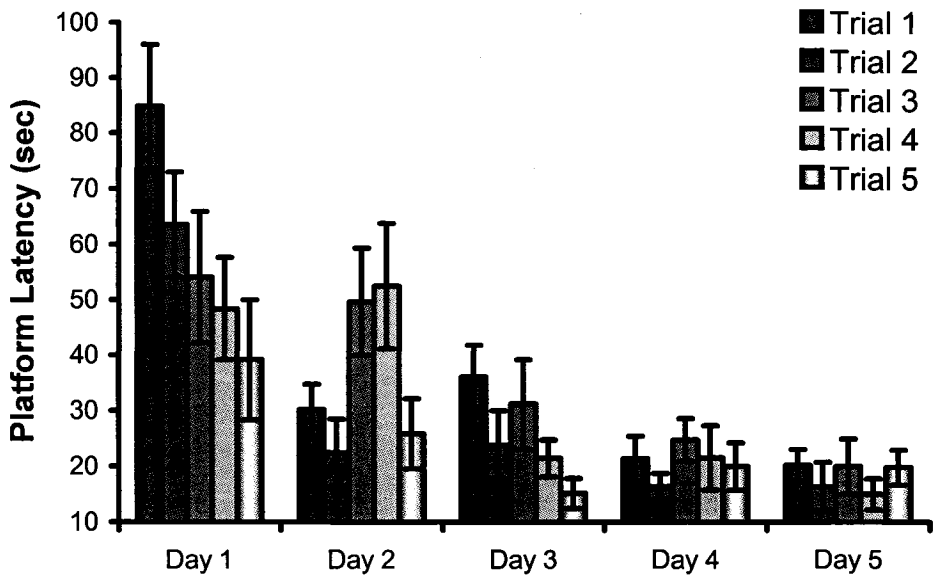


Figure 3.3.4. Time taken to find the submerged platform over the 5 trials of the 5 days of testing during the training phase.

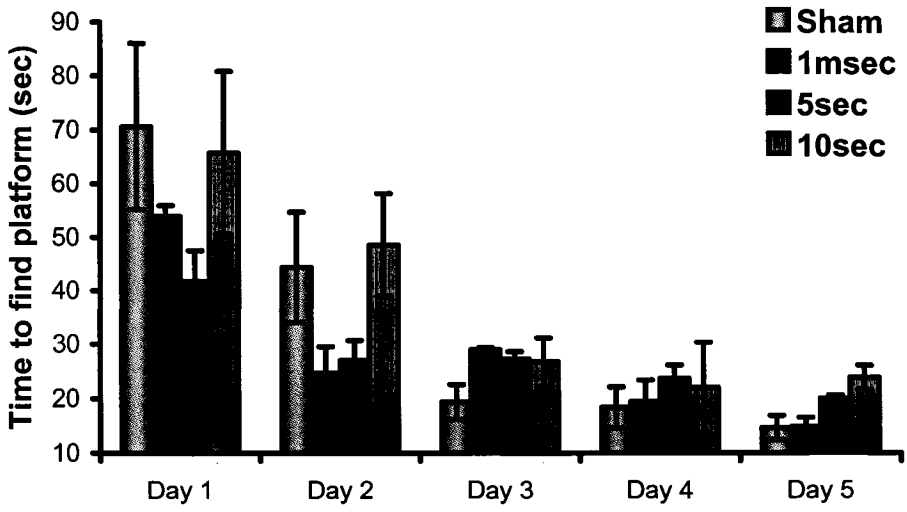


Figure 3.3.5. Time taken to find the platform during the training phase across the different treatment conditions.

Testing Phase

A three way analysis of variance with two levels repeated (trial, time) and one level not repeated (field) for the time spent in each section of the maze (arms and center) revealed a significant time-by-trial interaction for time spent in the center section [$F(1,12)=5.62$; $p<.05$; $\eta^2=41\%$], close arms [$F(1,12)=6.27$; $p<.05$; $\eta^2=44\%$], and wrong arms [$F(1,12)=15.79$; $p<.01$; $\eta^2=66.4$]. Further, a significant main effect for treatment condition for the time spent in the close arms was found [$F(3,12)=5.23$; $p<.05$; $\eta^2=66\%$].

The means (±SEM) for time spent in the center area of the maze for the two trials over the two 1 minute time banks can be found in Figure 3.3.6. *Post hoc* analyses employing paired t-tests revealed that the source of the time-by-trial interaction was due to the rats spending significantly less time in the center during the first minute of the first trial as compared to the first minute of the second trial. [$t(11)=-2.26$]. There were no other time bank differences.

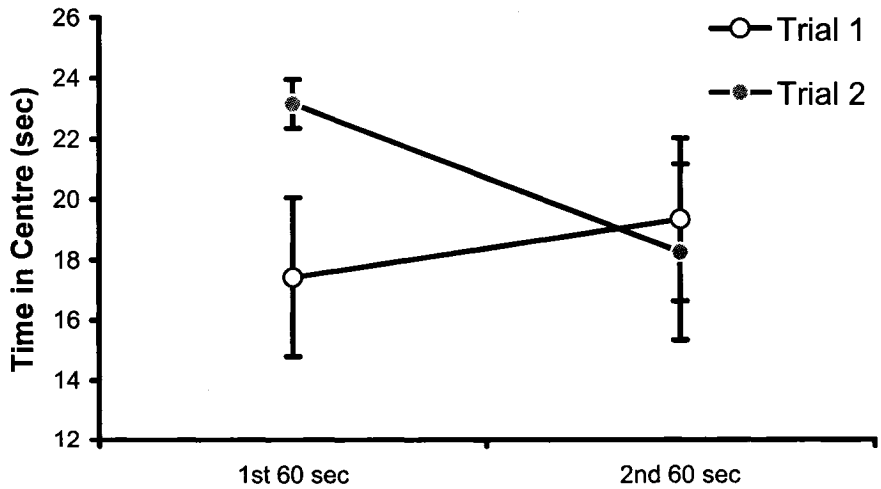


Figure 3.3.6. Time spent in the center of the water maze across the two one-minute time banks during trial 1 and trial 2.

The means (±SEM) for the time spent in the close arms for the two trials over the two one minute time bins can be found in Figure 3.3.7. *Post hoc* analyses employing paired t-tests revealed that the source of the time-by-trial interaction was due to rats spending significantly more time in the close arms during the first minute of the first trial as compared to the first minute of the second trial [t(11)=-2.26]. There were no other time bank differences.

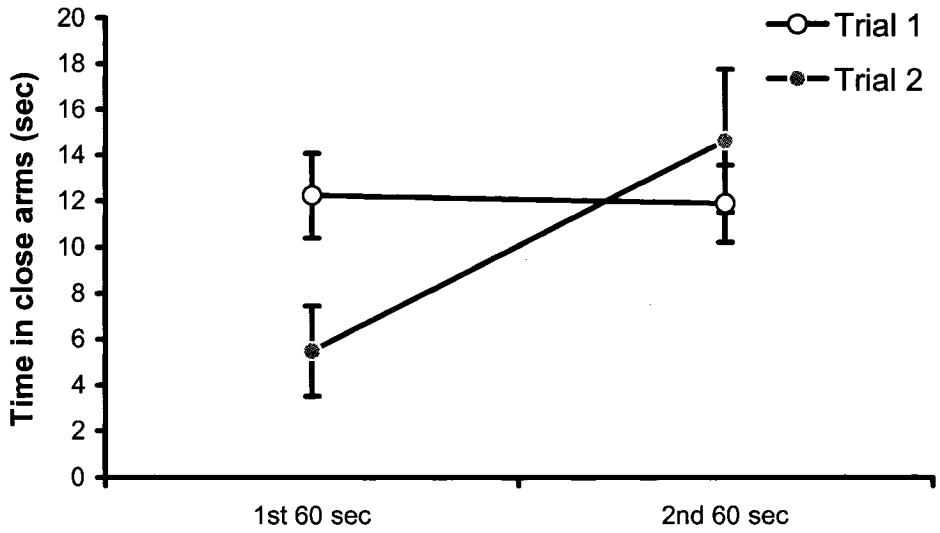


Figure 3.3.7. Time spent in the close arms across the two one-minute time banks for trial 1 and trial 2.

The means (±SEM) for the time spent in the wrong arms (anywhere but the goal arm) for the two trials over the two one minute time banks can be found in Figure 3.3.8. *Post hoc* analyses employing paired t-tests revealed that the source of the time-by-trial interaction was due to rats spending significantly less time in the wrong arms during the first minute of the second trial as compared to the second minute of the second trial [t(11)=-2.59] and the first minute of the first trial [t(11)=4.31]. There were no other time bank differences.

To analyze the significant main effect for field, for time spent in the close arms, the two one-minute time banks and 2 trials were collapsed into a single mean. These means (±SEM) for the time spent in the close arms across the different field treatment conditions can be found in Figure 3.3.9. *Post hoc* analyses employing Tukey's test revealed that the source of the effect was due to animals in the 1ms ISI-treated group spending significantly more time in the close arms as compared to those in the 10 sec ISI- and sham-treated groups which did not differ from each other or from the 5 sec ISI-treated animals.

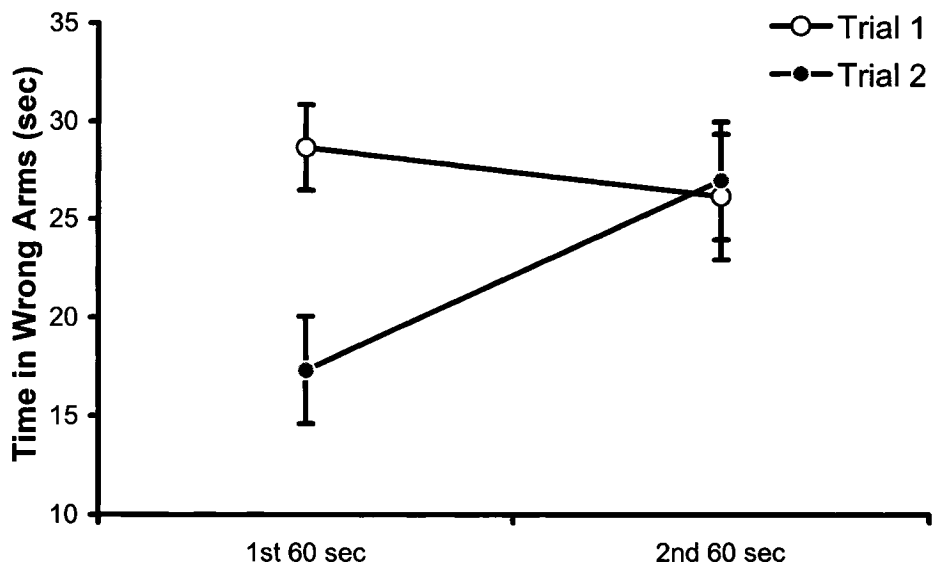


Figure 3.3.8. Time spent in the wrong arms across the two one-minute time banks for trial 1 and trial 2.

A three way analysis of variance with two levels repeated (time, trial) and one level not repeated (field) investigating total arm entries revealed a significant field-by-time-by-trial interaction [$F(3,12)=4.26$; $P<.05$; $\eta^2=61\%$]. The means (±SEM) of total arm entries for the two time banks of the two testing trials across the treatment conditions can be found in Figure 3.3.10. *Post hoc* analyses employing Tukey's test did not reveal any

significant between-subject effects (p>.05). *Post hoc* analyses employing paired t-tests (all dfs=2) revealed that the source of the interaction was due to animals in all groups except for those in the 5 sec ISI-treated group significantly decreasing their total arm entries within the two time banks for the second trial [sham (t=7.00); 1 msec (t=10.00); 10 sec (t=10.39). The animals in the 5 sec ISI-treated group had significantly decreased total arm entries within the two time banks of the first trial (t=11.00) while animals in the two other magnetic field-treated groups had decreases between the first minute of first trial and second minute of the second trial [1 msec (t=6.50); 10 sec (t=6.05)].

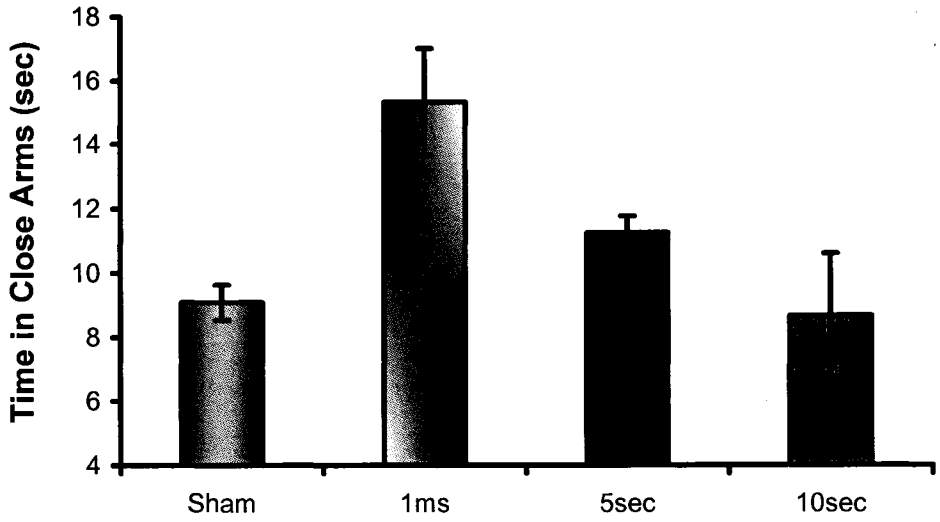


Figure 3.3.9. Time spent in the close arms across the different treatment groups.

A three way analysis of variance with two levels repeated (trial, time) and one level not repeated (field) for arm entry errors revealed a near-significant field-by-trial-by-time interaction [$F(3,11)=2.95$; $p=0.098$; $\eta^2=53\%$]. In order to control for total movement of the rats, an additional analysis of covariance was conducted to investigate arm entry errors while controlling for total arm entries. The analysis of variance revealed a

significant field-by-trial interaction [$F(3,11)=2.25$; $p<.05$; $\omega^2=82\%$]. Subsequently, residuals were generated and collapsed into trial means from the two one-minute time banks. The means (±SEM) can be found in Figure 3.3.11. *Post hoc* analyses revealed that the source of the interaction was due to animal sin the 10 sec ISI-treated group making significantly more arm entry errors than those in the 5 sec ISI- and sham-treated group during the first trial. There were no between subject differences during the second trial. There were no within subject differences.

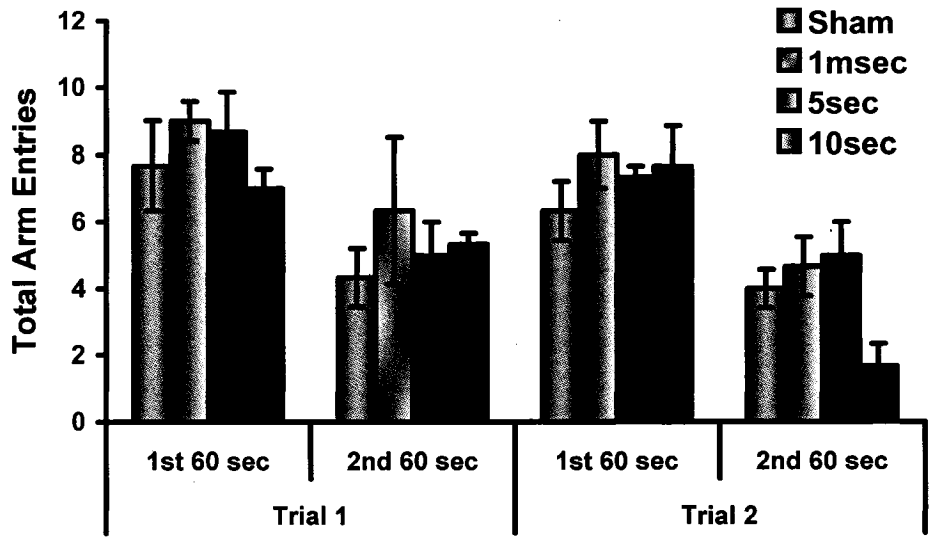


Figure 3.3.10. Total arm entries across the two time banks and two trials for the different treatment conditions.

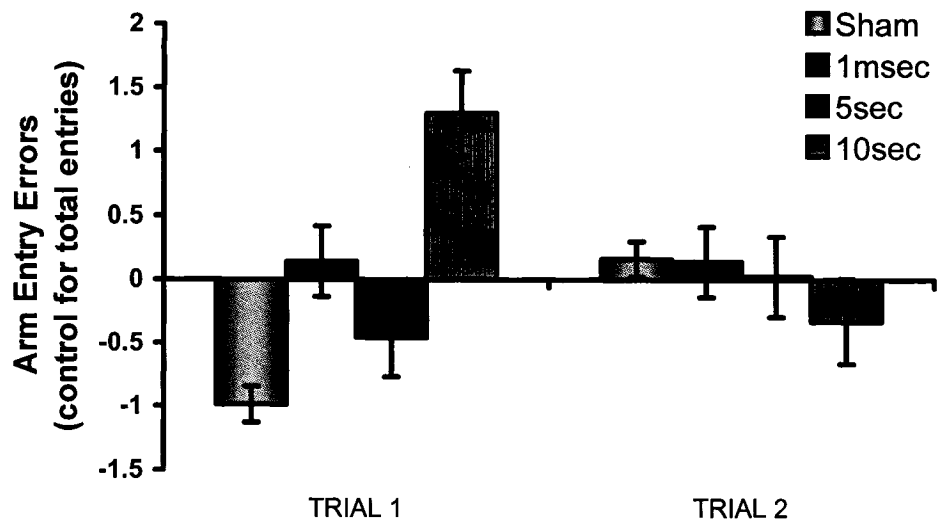


Figure 3.3.11. Arm entry errors (while controlling for total arm entries) for the different treatment conditions across the two testing trials.

DISCUSSION

The results of the current study demonstrated that five days of training in the six arm water maze was sufficient for rats to learn the paradigm as demonstrated by the decrease in time taken to find the submerged platform over days and trial. The 1 msec ISI-treated group was the only group that demonstrated a reliable decrease in latency throughout the training when the groups were analyzed separately. Although these results could be taken as sign that rats treated with these magnetic field parameters perform better in the water maze, this was likely due to small sample sizes and the chance consistency of this group within the first training day. Since subjects received their first magnetic field exposure after the training, the group consistency cannot be attributed to the treatment.

Certain patterns were observed in the testing phase of the experiment including that the rats spent more time in the center, close arms, and wrong arms during the first minute of the first trial. However, few of the analyses demonstrated clear signs that the

magnetic field treatments affected learning in any of the different conditions. One of the ambiguous results was that animals in the sham group showed significantly decreased total arm entries over the second trial whereas rats in the magnetic field-treated groups did not. This was interpreted as animals in the sham-treated group decreasing searching behaviour compared to the magnetic field-treated groups, although without a naïve control it becomes difficult to interpret these results.

The only clear difference across treatment conditions to suggest impairment in learning was that animals in the 10 sec ISI-treated group committed significantly more arm entry errors when accounting for total arm entries. A learning impairment with this group was also seen in the conditioned place preference paradigm where rats were also repeatedly exposed to the theta burst magnetic field pattern although this was also accompanied by an increase in movement.

It is not surprising that it is a different temporal parameter (interstimulus time) that elicits learning impairments in the water maze paradigm as compared to the contextual paradigms since there are different portions of the hippocampus involved in each of these paradigms. Whereas the contextual paradigms involved the use of the ventral hippocampus, (Richmond *et al.*, 1999), the dorsal hippocampus is involved with spatial navigation (Pothuizen *et al.*, 2004; Richmond *et al.*, 1999). The current series of studies suggest that different parameters of magnetic field exposures could selectively impair different areas of the hippocampus. Previous studies have found that brief exposures to a continuous theta burst magnetic field pattern was successful in impairing contextual but not discrete fear conditioning (McKay *et al.*, 2000). Together, the studies suggest that continuous theta burst magnetic field pattern can impair contextual learning

but not spatial navigation whereas a theta burst magnetic field pattern with a 10 sec ISI impairs spatial navigation while sparing contextual learning.

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CHAPTER IV

BURST FIRING MAGNETIC FIELDS AND ANALGESIA

SUMMARY

Brief whole body exposures to burst firing magnetic fields have previously been found to elicit analgesic effects in the rat (Fleming *et al.*, 1994; Martin & Persinger, 2003). Through a series of studies, Martin and Persinger (2003, 2004) have investigated the ideal parameters necessary for eliciting an increase in thermal analgesic thresholds through brief exposures to a burst firing magnetic field. The studies in the following chapter aim to further manipulate parameters in order to build upon these findings.

The first portion of this chapter contains experiments which employ temporal parameters of the burst firing magnetic field that were found to elicit analgesic responses and then build upon these observations by investigating the contributions of time of day, orientation of the generated field, rat age and rat gender. Since most of the previous studies were conducted in the morning hours, additional experiments will test if there are differences between morning and evening exposures. The effects of magnetic fields are as effective on rat pups and on older rats will be investigated. In addition, a study was conducted to show whether the cardinal orientation in which the magnetic field is generated interacts with the effects. Finally, a study that combines time of day and rat gender will investigate whether the magnetic field effects are present in both male and female rats.

The second portion of this chapter investigates long term magnetic field exposures and their effectiveness in eliciting analgesic responses. Previous finding have

shown that the analgesic effects are only present on the first day of exposure and not on the second (Martin *et al.*, 2004). Since it has been postulated that magnetic fields can elicit such responses through an opiate mechanism (Fleming, et. al, 1994, Richards *et al.*, 1993), low doses of ketamine were given to one group in order to decrease tolerance to morphine (Bell, 1999). Ketamine's activity as a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, implicated in learning mechanisms, is thought to interfere with the learning component underlying opiate dependence and tolerance.

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CHAPTER 4.1

INVESTIGATION OF RAT GENDER AND AGE IN THE ELICITATION OF ANALGESIC RESPONSES THROUGH MORNING AND EVENING EXPOSURES TO BURST FIRING MAGNETIC FIELDS

ABSTRACT

Brief whole body exposures to burst firing magnetic fields have previously been found to elicit analgesic effects in the rat. In this series of studies, various manipulations to the magnetic field experiments have been made to better define the limitations of magnetic field induced-thermal analgesia. Three separate studies were conducted to determine whether time of day (morning or evening), magnetic field orientation, and rat gender were relevant variables in eliciting analgesic responses. A fourth study was conducted to determine whether there were time of day and magnetic field generating apparatus effects in rat pups (30 days). Although these studies did not replicate past studies, in that none of the manipulations successfully elicited analgesic responses, the current studies demonstrated that there rat pups displayed significantly stronger analgesic responses in the morning as compared to the evening. Also the rat pups seemed to have quickly learned the hotplate analgesia paradigm as demonstrated by the rapid decline of hotplate latency through the six testing trials over two days. Such rapid learning was not evident in the adult rats.

INTRODUCTION

Previous experiments have demonstrated that brief (30 minutes) whole body exposures to weak (1μT) complex magnetic fields elicit elevated nociceptive thresholds (Fleming *et al.*, 1994; Martin & Persinger, 2003). Fleming and colleagues, showed that the magnetic field-induced analgesia was equivalent to a therapeutic dose of 4 mg/kg of morphine, and that the effects were also longer in duration. Many further studies have investigated the contributions of specific parameters responsible for eliciting a maximal effect using different exposure apparatuses (Martin & Persinger, 2003; Martin *et al.*, 2004).

Throughout the course of the day, fluctuations in neurotransmitter and hormone levels occur which can change the effectiveness of various drugs. It has been found that both basal and morphine-induced pain thresholds were higher at night than during the day (Karakucuk, 2006). The present studies were designed to investigate the diurnal effects of weak complex magnetic field exposures in rats of different ages. Further, the exposure orientation of the experimental magnetic field relative to the geomagnetic field was investigated. Since previous studies employed mostly male rats, both males and females were employed in some studies to investigate whether there were any sex differences in nociceptive thresholds.

METHODS

Subjects

Male and female albino Wistar rats obtained from Charles River Laboratories in Quebec were employed in the studies. All rats were aged between 6 months and 14

months of age and housed 2-3 per cage unless otherwise stated. Rats were maintained on a 12:12 light-dark cycle beginning at 0800 hr local time.

Apparatus

The exposure apparatus employed in this study has been described in previous studies (Fleming *et al.*, 1994; Martin *et al.*, 2004). The apparatus was composed of a 25 cm x 25 cm x 25 cm Plexiglas chamber with two horizontally aligned electromagnets on opposite sides. The electromagnets were composed of 25 cm iron nails wrapped 1050 turns with 22 gauge enameled copper wire over 19cm. These were placed at the center of two sides of the chamber, 5cm from its base (~3 cm of corn cob bedding) in an East-West geographic orientation (±5°). For comparisons, we will refer to this as the 'Nails' apparatus.

The electromagnets were connected to a Zenith XT personal computer through custom made digital to analog converter that transformed a series of values between 0 and 255 to voltages ranging from -5 to +5 volts with 128 representing neutral polarity. For the waveform generated in this experiment, the values ranged from 125 (~ -0.12volts) to 250 (~4.77volts). The magnetic field parameters were programmed into the computer through the Complex software. All experiments involved a burst firing magnetic field which had a 3 msec temporal pixel duration and a 4000 msec interstimulus interval since these have been shown to be optimal in eliciting analgesia (Martin *et al.*, 2004).

General Procedure

On each testing day, rats were taken from their home cages and brought to a separate room containing the hotplate analgesiometer. To assess nociceptive thresholds,

rats were placed on a hotplate analgesiometer set at 55°C and the latency for them to lick their hind paws twice in any order was noted. Following a baseline measurement, rats were exposed either to a burst firing magnetic field or a sham field for 30 minutes. Rats were assessed again immediately following the magnetic field exposure (Trial 1) and 30 minutes later (Trial 2). In some studies, rat weight was also measured after the final trial to be employed as a covariate in some analyses.

Experiment 4.1.1 – Morning and evening exposures

Twelve male Wistar rats weighing between 500 g and 600 g were tested either in the morning (0900 to 1100 hours) or evening (1700 to 1900 hours) to investigate whether there is a diurnal variation in the responding to magnetic fields. Rats were tested during two successive days. The time of day for exposure for a given rat was changed between the two days.

Experiment 4.1.2 – Magnetic field orientation

To investigate whether there is a difference caused by the orientation of the generated magnetic field relative the geomagnetic field, sixteen male Wistar rats weighing between 500 g and 600 g were exposed to the burst firing magnetic field in one of two geomagnetic orientations (North-South or East-West). All experiments were conducted between 0900 and 1200 hrs.

Experiment 4.1.3 – Morning and evening exposures of rat pups

To investigate whether younger rats could be affected by magnetic fields in a similar manner as older rats, 23 male rat pups (30 days old) were tested for two

consecutive days on the hotplate analgesiometer. Rats were tested both in the morning and evening. A group of three rats were exposed to the magnetic field in either the 'Nails' magnetic field apparatus or a Helmholtz apparatus. The Helmholtz apparatus generated more of a homogenous magnetic field (Martin & Persinger, 2003) and was made of a 41 cm x 30 cm x 31 cm Helmholtz coil (30 ohm). Rats were placed within a Plexiglas container (21 cm x 28 cm x 28 cm) within the coil. The chamber was covered with approximately 2 cm of corncob bedding. The analysis consisted of a four way mixed analysis of variance with one level repeated (day) and three levels not repeated (field, time of day, apparatus).

Experiment 4.1.4 – Morning and evening exposures of female and male rats

In order to assess whether rat gender might be a pertinent variable in magnetic field analgesia, 17 female and 15 male rats were assessed in a comprehensive study investigating gender, field, and time of day effects on nociceptive thresholds. To further ensure that effects were not masked by testing methods, both the latency to lick one paw and two paws were employed. The analysis consisted of a four way mixed analysis of variance with one level repeated (trial) and 3 levels not repeated (field, gender, time of day).

Statistical Analyses

All analyses were conducted through multiple methods in order to ensure that any possible effects were not masked by the scale of the variable. Analyses were conducted with the raw data (baseline, trial 1, trial 2), net differences in responding (Trial –

Baseline) and relative differences in responding [(Trial – Baseline)/Baseline]. Both between subject anovas and within subject multivariate analyses were conducted depending on the analysis. Although the number of between subject variables differed with each experiment, there were basically three different types of analyses conducted for every set of three trials:

1. two way multivariate analyses with one level repeated (trial: baseline, trial 1, trial 2) and one level not repeated (field: burst X, sham)
2. two way multivariate analysis with one level repeated (trial: net 1, net 2 OR relative 1, relative 2) and one level not repeated (field: burst X, sham)
3. oneway multivariate analysis of variance with field (burst X, sham) and covarying for baseline responding.

Post hoc analyses were conducted with the use of Tukey's test for between subject effects and with paired t-tests for within subject effects.

RESULTS

Experiment 4.1.1 – Morning and Evening Magnetic Field Exposure

Analysis of variance employing two way between subject analysis of variance with day (day 1 and day 2) and treatment (field and sham) for each of the morning and evening variables for baseline, trial 1 and trial 2 measurements did not reveal any significant main effects or interactions involving treatment condition. Employing net or relative scores did not change the significance of the results. Also, the use of raw scores in an analysis of covariance for baseline responses did not change the significance of the results.

Multivariate analysis of variance with two levels repeated (trial; time of day) and one level not repeated (field) for hotplate latency revealed a significant interaction between trial and time of day. The means (±SEM) for hotplate latency over the three trials during the morning and evening can be found in Figure 4.1.1. *Post hoc* analyses employing paired t-tests revealed that the source of the interaction was due to rats having a significantly higher hotplate latency in the morning on trial 2 than on all of the trials in the evening [$t(11)>2.78$; $p<.05$].

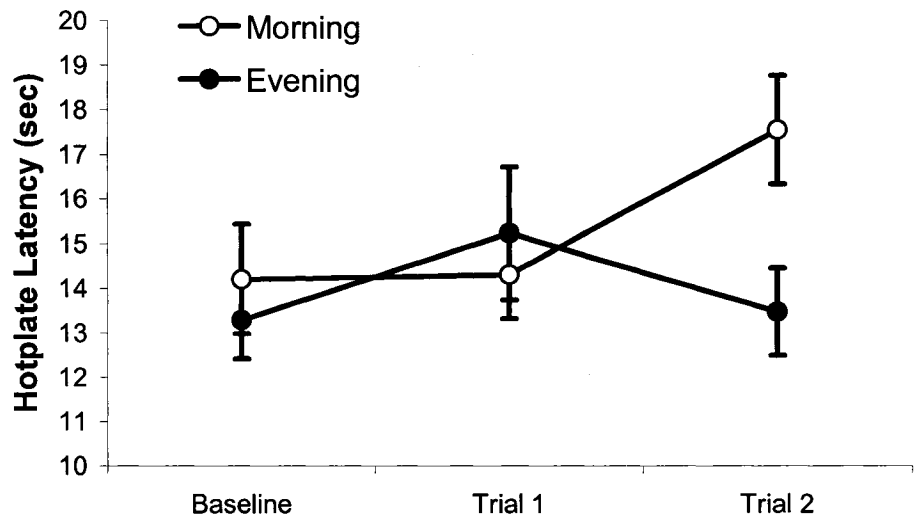


Figure 4.1.1. Hotplate latency for all trials during morning and evening testing.

Experiment 4.1.2 – Magnetic Field Orientation

Analysis of variance employing 2 X 2 X 2 between subject analysis of variance with treatment (field and sham) and magnetic field orientation (East-West and North-South) throughout the three trials did not reveal any significant main effects or interactions. Employing net or relative scores did not change the significance of the

results. Finally, the use of raw scores in an analysis of covariance for baseline responses did not change the significance of the results.

Experiment 4.1.3. Rat Pups during morning and evening

Using raw scores, a 2 X 2 X 2 X 2 X 2 mixed analysis of variance with two levels repeated (day, trial) and three levels not repeated (field, coil, time of day) revealed statistically significant main effects for time [$F(1,22)=10.62$; $p<.05$; 41%], day [$F(1,23)=32.66$; $p<.01$; 67%], and trial [$F(2,46)=4.39$; $p<.05$; 23%]. The analysis further revealed statistically significant interactions for coil-by-time [$F(1,22)=10.49$; $p<.05$; 41%], day-by-trial [$F(2,46)=4.03$; $p<.05$; 21%], and coil-by-time-by-trial [$F(2,46)=5.72$; $p<.01$; 28%]. No significant effects or interactions were found for magnetic field treatment condition with the use of raw scores ($p>.05$).

In order to analyze the effects of time of day, the six trials over the two days for each rat were collapsed into means. These means (±SEM) for the hotplate latency for morning and evening testing can be found in Figure 4.1.2. *Post hoc* analyses revealed that the source of the effect was due to rats exhibiting higher hotplate latencies during the morning hours as compared to the evening [$F(1,22)=7.37$; $p<.05$; $\omega^2=26\%$].

To further analyze the main effects for day, the three trials per day were collapsed into daily means. These means (±SEM) for the hotplate latency for day 1 and day 2 can be found in Figure 4.1.3. Post hoc analyses revealed that the source of the effect was due to rats exhibiting higher hotplate latencies on day 1 as compared to day 2 [$t(22)=6.28$; $p<.05$].

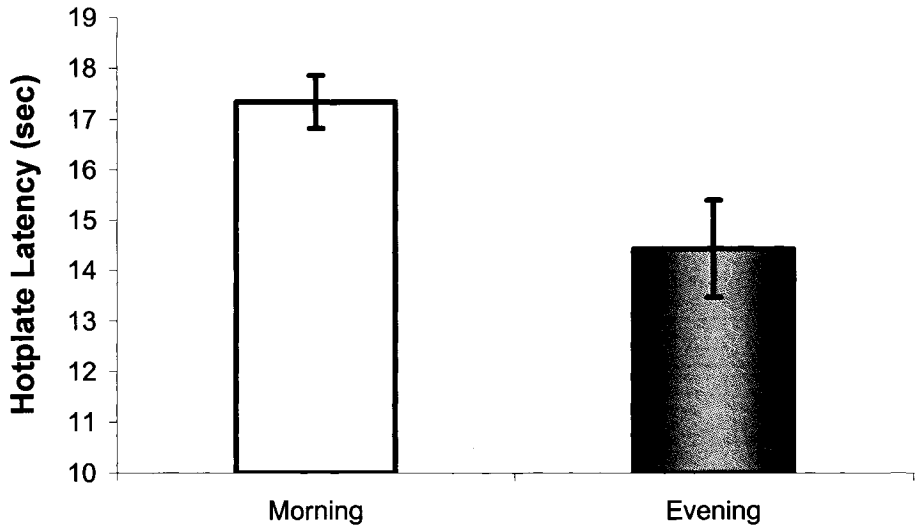


Figure 4.1.2. Mean hotplate latency during morning and evening.

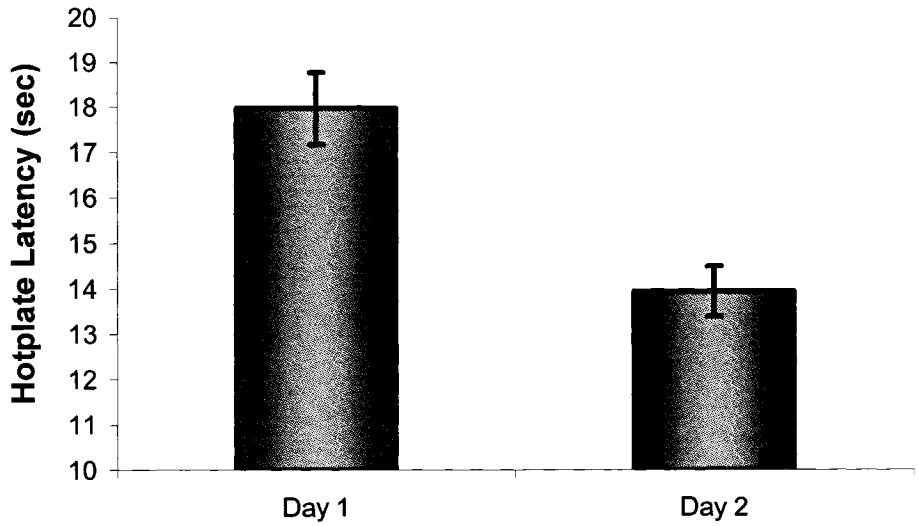


Figure 4.1.3. Mean hotplate latency during day 1 and day 2.

A significant coil-by-time interaction was analyzed with the use of the means from the 6 trials over the two days. These means (±SEM) can be found in Figure 4.1.4. *Post hoc* analyses revealed that the source of the interaction was due to rats tested in the morning exhibiting longer hotplate latencies when exposed to the Helmholtz coil as compared to the apparatus with the 'Nails' apparatus [$F(1,11)=25.01$; $p<.05$]. Further,

rats in the Helmholtz coil group demonstrated longer hotplate latencies in the morning group as compared to the evening [$F(1,11)=27.00$; $p<.05$] whereas rats in the 'Nails' group did not differ with time of day. It might be worth noting that these effects were present regardless of whether the magnetic field was present or not.

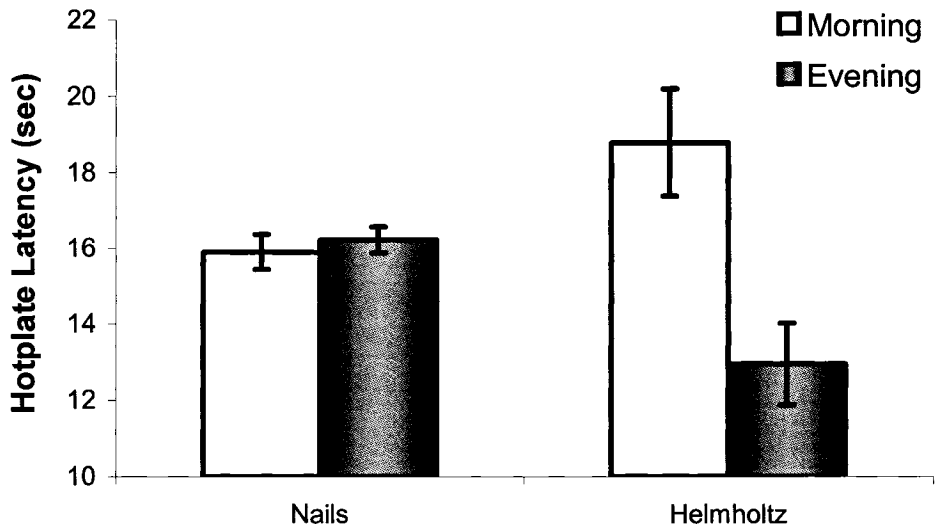


Figure 4.1.4. Mean hotplate latency during morning and evening hours for rats in the different apparatus conditions.

In order to analyze the main effect for trial, the two days of testing were collapsed into means for each trial. These means (±SEM) for hotplate latency over the three trials can be found in Figure 4.1.5. *Post hoc* analyses revealed that the rats exhibited lower latencies in trial 2 as compared to the baseline [$T(22)=2.09$].

The means (±SEM) for hotplate latency over the three trials and two days can be found in Figure 4.1.6. *Post hoc* analyses revealed that the source of the trial-by-day interaction was due to rats exhibiting a higher baseline hotplate latency on day 1 as compared to all other trials except for trial 1 on day 1 [$t(22)\geq 3.10$; $p<.05$]. Rats also exhibited higher latencies in trial 1 of day 1 as compared to all 3 trials on day 2

[t(22≥2.23; p<.05]. There were no statistically significant differences between any of the trials on day 2.

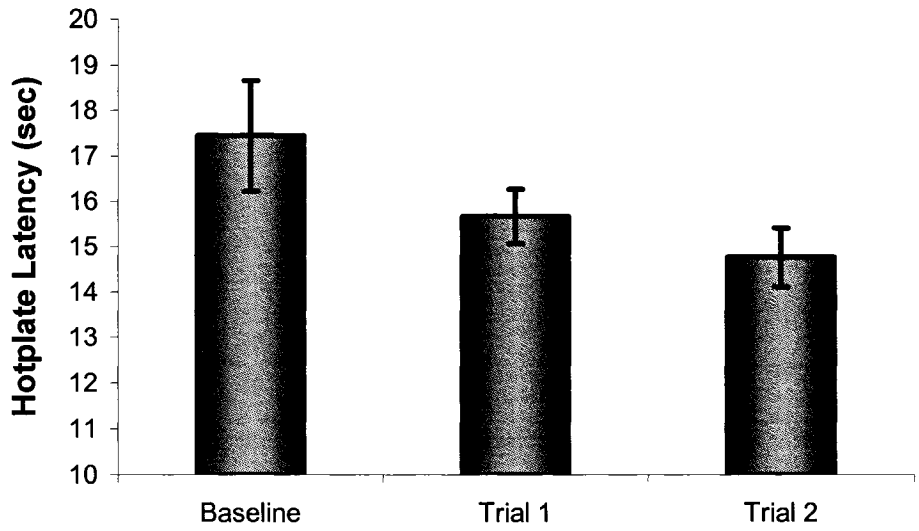


Figure 4.1.5. Mean hotplate latency over the three trials of testing.

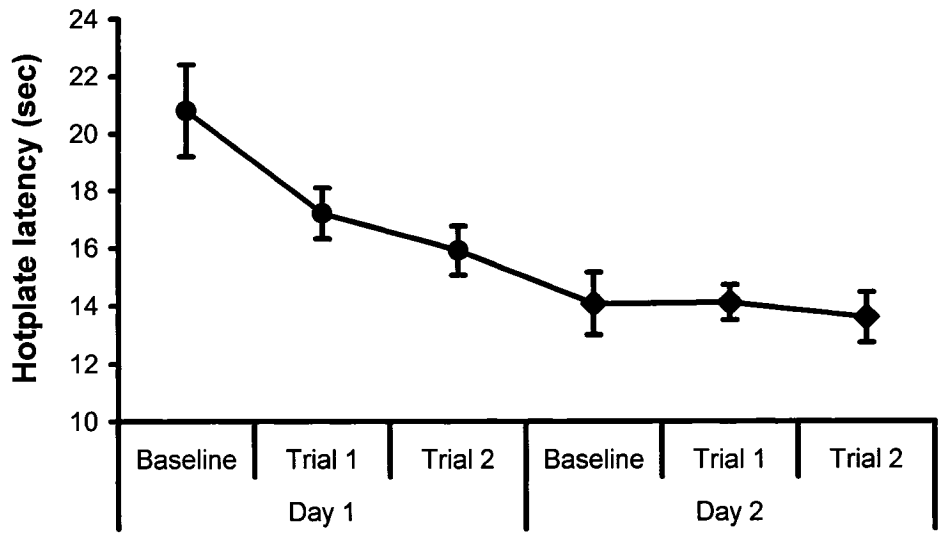


Figure 4.1.6. Hotplate latency over the three trials and two days of testing.

The analysis further revealed a three way interaction between coil, time, and trial. To investigate the source of the effect, the three collapsed trial means were employed. The means (±SEM) for hotplate latency over the three trials for morning and evening

groups for each trial can be found in Figure 4.1.7. Within subject analysis revealed that animals in the Helmoltz group exhibited near-significant lower hotplate latencies on trial 2 as compared to baseline in the morning group [t(5)=2.30; p=.07] and as compared to trial 1 in the evening group [t(5)=2.34; p=.07]. Between subject analysis investigating time of day differences demonstrated that rats in the Helmholtz group had significantly ($p<.05$) higher hotplate latencies in the morning as compared to evening for baseline [F(1,11)=24.71], trial 1 [F(1,11)=7.15], and trial 2 [F(1,11)=14.39]. Investigating coil differences, it was shown that rats in the Helmholtz group had higher baseline latency as compared to the 'Nails' apparatus group in the morning [F(1,11)=9.77] and the evening [F(1,10)=5.43].

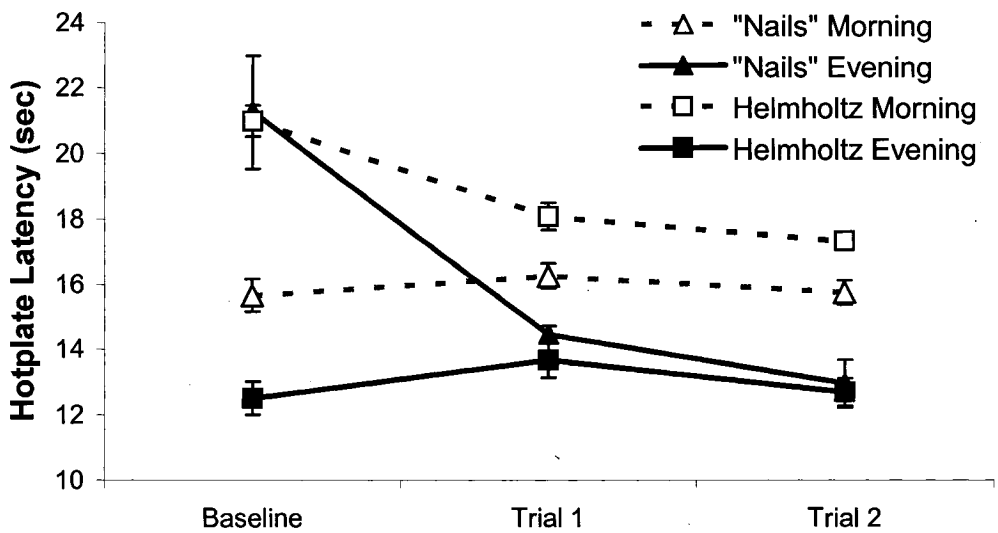


Figure 4.1.7. Hotplate latency during over the three trials of testing in the morning and evening across the different magnetic field apparatus conditions.

Although there were no magnetic field effects employing the raw data, net scores, or analysis of covariance (covarying for baseline score), the use of the relative scores

revealed coil-by-field [F(1,22)=3.72; p=.073; 20%] and field-by-trial [F(1,23)=3.35; p=.087; 18%] interactions that approached statistical significance.

The means of the two trials and two days were subsequently collapsed into single means. These means (±SEM) for the relative difference in hotplate latency for rats exposed to a magnetic field or a sham as a function of exposure apparatus can be found in Figure 4.1.8. *Post hoc* analysis revealed that the source of the interaction was due to rats in the sham-exposed group demonstrating a significantly higher relative change in hotplate latency when exposed to the 'Nails' coil as compared to the Helmholtz coil whereas rats in the field-exposed groups did not differ from each other [F(1,10)=5.23; p<.05].

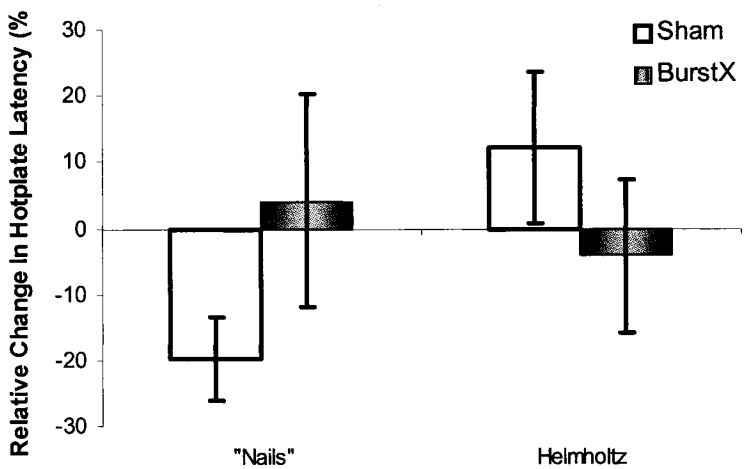


Figure 4.1.8. Relative change in hotplate latency for the two treatment conditions across the different exposure apparatuses.

In order to assess the significant field-by-trial interaction, the means of the hotplate latency over the two days were collapsed for each trial. These means (±SEM) for relative change in hotplate latency for the different field treatment conditions over the two trials can be found in Figure 4.1.9. Although *post hoc* analyses did not reveal any

significant between subject differences, within subject analysis revealed that rats in the sham-exposed group exhibited a significantly higher relative change in hotplate latency on trial 1 as compared to trial 2 [T(11)=2.52; p<.05].

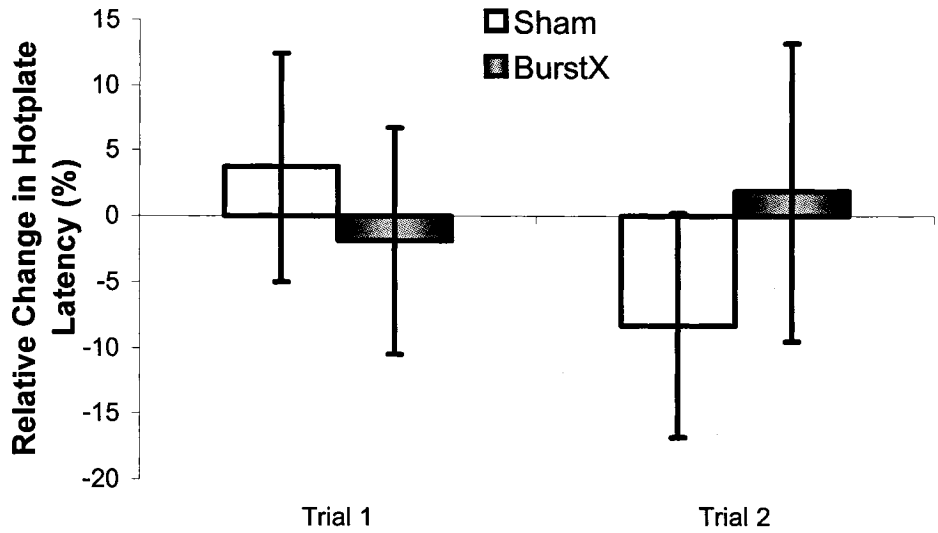


Figure 4.1.9. Relative change in hotplate latency for the two treatment conditions across the different exposure apparatuses.

Experiment 4.1.4 – Morning and evening exposure of female and male rats

The analyses for these experiments investigate both the time taken to lick one paw and two paws. Since the results of both the first lick and second lick were very similar and there was a strong correlation between the first and second lick (baseline=0.89; trial 1=0.87; trial 2=0.93; p<.01), only the results of the two licks is presented here to be consistent with the other experiments.

Although no treatment, sex, time of day, or trial effects or interactions were found with the use of the raw data or analyses of covariance for the baseline measure, the use of net and relative differences yielded near-significant results. A four way analysis of variance with one level repeated (trial) and three levels not repeated (field, sex, time of

day) yielded a near-significant field-by-trial interaction for net difference in hotplate latency [F(1,32)=3.91; p=.06; η^2=14%]. The means (±SEM) for the net change in hotplate latency across the magnetic field conditions can be found in Figure 4.1.10. *Post hoc* analyses employing Tukey's test did not reveal any between subject differences for either of the two trials. Paired t-tests revealed that the source of the interaction was due to rats in the sham-treated group having a significantly larger change in hotplate latency on trial 1 as compared to trial 2 [t(15)=2.27] whereas rats in the field-treated groups did not differ from each other.

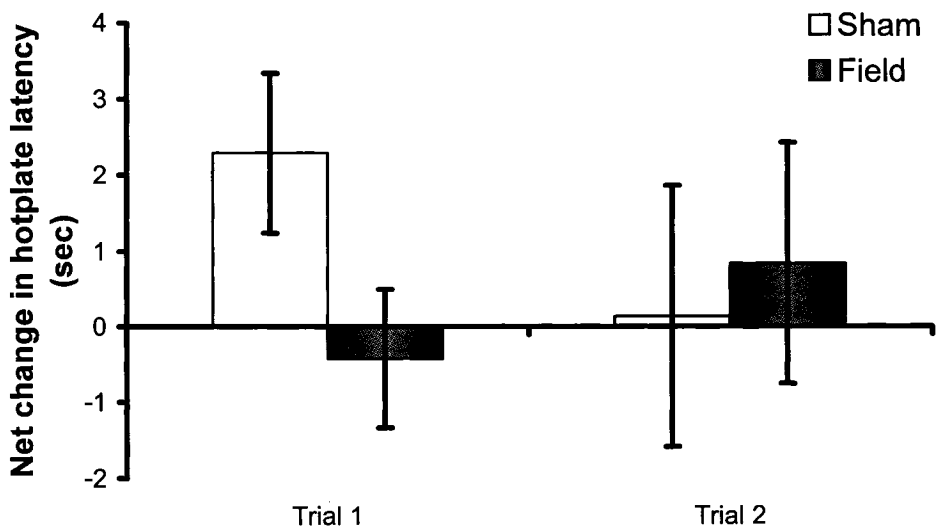


Figure 4.1.10. Net change in hotplate latency for the two treatment conditions over the two trials.

Discussion

The current set of experiments provided results that were contradictory to previous research investigating both diurnal pain sensitivity and the effects of burst firing magnetic fields on analgesia. Of further interest were the findings in the pup studies that demonstrated lower thresholds on later trials which suggested that learning could be

taking place as early as the third exposure to the hotplate analgesiometer. Curiously, these pups also demonstrated a hyperalgesic response following placement in the Helmholtz coil regardless of whether the magnetic field was turned on.

Diurnal fluctuations in thermal analgesic thresholds have been known for some time now. Frederickson and colleagues (1977) demonstrated that the thermal nociceptive thresholds in the mouse were of maximal intensity between the hours of 1530 to 0430 while the lowest thresholds were found to be between the hours of 0430 to 1130. Since these diurnal effects were blocked with the use of naloxone, a mu receptor antagonist, it was suggested that such results could be due to diurnal fluctuations in endogenous opiates. It has been found that there are differences between daytime and nighttime mu opiate receptor expression in the arcuate nucleus of the hypothalamus (Mitchell *et al.*, 1998), with the highest levels at 2000. This structure is known to be intimately involved with the endogenous antinociceptive system (Sun *et al.*, 2003). It has also been shown that melatonin is involved in increasing the secretion of beta-endorphin in mouse pituitary cell cultures (Shavali *et al.*, 2005). Melatonin shows peak binding in the suprachiasmatic nucleus at the light-dark transition (Laitinen *et al.*, 1989).

Two of the current studies were found to have conflicting results with prior findings. It was found that overall, rat pups had overall longer analgesic thresholds in the morning hours (between 0900 and 1100) and lower thresholds in the evening hours (between 1500 and 1700). In addition, adult rats had significantly higher nociceptive thresholds on the third testing trial in the morning as compared to all trials conducted in the evening. Such findings could be accounted for by other studies which have found that diurnal fluctuations in analgesic thresholds are mediated by food intake. McGivern and

Berntson (1980) were able to reverse the diurnal pattern of pain sensitivity by restricting the availability of food to the light phase. Since food was available *ad libitum* in the present study, it is unknown whether feeding patterns could have differ throughout the course of the day resulting in the observed diurnal fluctuations in nociceptive thresholds.

Some studies have found that prior experience with the hotplate apparatus diminished response time (Kayan *et al.*, 1969). Consistent with this, the experiment involving rat pups demonstrated a reliable decrease in hotplate latency over the 6 trials and over the two days (Figures 4.1.6 and 4.1.3). Although past studies investigating magnetic field effects on thermal thresholds found that they were effective on both days of testing, such studies did not report whether there were differences in baseline or overall latencies between the two days (Martin & Persinger, 2003). The findings of the current study suggest that young rats begin to learn the "paw-lick" paradigm following very few exposures.

In the experiments with the young rats, certain findings suggested that even in the absence of a magnetic field, exposures to the Helmholtz apparatus resulted in higher hotplate latencies. Care must be exercised in the interpretation of these results as differences were also seen in baseline measurements. Certain studies have suggested that proper balancing of rats among various groups should be based on baseline measurements since there is a high level of variability in rats' responding on hotplates set at 55°C (Plone *et al.*, 1996). The authors found that the responses are more stable when the hotplate is set at 50°C and that the effectiveness of drug treatments which elicit mild analgesia were also the most obvious at this temperature. The results of the current study

suggest that it is the magnetic field apparatus and not the magnetic field that is increasing nociceptive thresholds.

Analgesic effects have been found to result from magnetic field shielding in mice (Prato *et al.*, 2002). Although the field intensity within the Helmholtz coil has not been measured, the general morphology of the apparatus would be suggestive of a decreased magnetic field for the sham group. Martin and Persinger (2003) reported that there was no analgesic response which resulted from burst firing magnetic field exposures within the Helmholtz coil. If the coil itself is sufficient in attenuating the magnetic field, it is conceivable that this effect could explain the observed analgesic responses.

It remains unknown why previous studies which investigated the effects of the same burst firing magnetic field on thermal nociceptive thresholds could not be replicated (Fleming *et al.*, 1994; Martin & Persinger, 2003; Martin *et al.*, 2004). Frequency modulated magnetic fields have been shown to abolish morphine-induced analgesia (Dixon & Persinger, 2001). Results of the current series of studies suggest that there are no analgesic effects induced by the magnetic fields. In one of the experiments, it was found that while the sham-treated animals had elevated thresholds, the magnetic field-treated animals did not (Figure 4.1.10). These findings only reflected the net change in hotplate latency in trial 1 while during trial 2, no such differences were found. Further, it can be seen in Figure 4.1.9 that the sham-treated animals demonstrated a greater change in hotplate latency in trial 1 as compared to trial 2.

Stressful stimuli have been known to elevate nociceptive thresholds (Lewis *et al.*, 1980). Although much research has focused on stressors such as exercise (Blustein *et al.*, 2006) and electric shocks (Lewis *et al.*, 1980), few have investigated whether brief

exposure to thermal stimuli can result in elevated nociceptive thresholds. The elevation in thermal threshold from the sham-treated animals 30 minutes later but not 60 minutes later suggests that a nociceptive thermal stimulus might be enough to elicit stress-induced analgesia in the current experiments. If such analgesic responses were due to the endogenous opiate system, it is possible that exposures to magnetic fields blocked the responses to the stress-induced analgesia in a similar manner as previous research that has demonstrated a block in morphine-induced analgesia (Dixon & Persinger, 2001).

If magnetic field therapy is to be employed as an alternative to conventional analgesics, it is important that a consistent model can be found to evaluate potential and safety. Although previous research has investigated some of the temporal parameters required in order to elicit a maximal analgesic response (Martin *et al.*, 2004), it is apparent through the current set of experiments that certain other factors can still interact with the field exposures. Measuring analgesic thresholds with a thermal analgesiometer set at 55°C may yield inconsistent results and future research should consider counterbalancing for baseline threshold effects (Plone *et al.*, 1996). Finally, since the current research compared current results to other studies done in different periods of the solar cycle, it may be important to consider recent fluctuations in the geomagnetic field as a potential confound. Such fluctuations have been shown to increase the variability in the response of rats to nociceptive thermal stimuli (see Chapter 4.2) which could skew the results of studies which employ small sample sizes.

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CHAPTER 4.2

THE EFFECTS OF LOW DOSE KETAMINE AND MAGNETIC FIELDS ON REPEATED EXPOSURES TO A THERMAL NOCICEPTION APPARATUS

ABSTRACT

The decrease in effectiveness of opiate drugs over time (tolerance) has been well documented. It has been suggested that the analgesic properties of complex magnetic field exposures could have an underlying opiate mechanism. The current study was conducted to investigate whether a drug known to decrease the development of tolerance to opiates (ketamine) could prolong the analgesic properties of magnetic field exposure. Following a fourteen day study to determine a suitable duration for repeated testing on the hotplate, six days were chosen as the appropriate length of the study. This study also demonstrated that group variability in responding over the fourteen days of testing was positively correlated with geomagnetic activity (AA index) two days prior to testing. In the main study, on each day of the study, following a baseline hotplate latency measure, twelve male albino Wistar rats received either an injection of saline or ketamine (1 mg/kg). Rats were then exposed to either a burst firing magnetic field or a sham field. The results of this study showed that over the course of the six days of testing, only the first day demonstrated significant difference between treatment conditions with the saline group having a significantly lower hotplate latency when exposed to a magnetic field as compared to the sham field. These results further

demonstrate the inconsistency of studies employing magnetic fields in eliciting analgesic effects and suggest that factors, such as geomagnetic activity, which increase group variability should be considered during studies investigating behavioural responses in rats.

INTRODUCTION

The effects of magnetic fields in producing analgesic responses are beginning to accumulate in the literature. Magnetic fields have been shown to elicit effects that ranged from producing analgesia (Fleming et. al., 1994; Martin & Persinger, 2003; Martin et. al., 2004; Ryczko & Persinger, 2003), blocking the effects of analgesic drugs (Tysdale, 1991; Dixon & Persinger, 2001) or to having hyperalgesic properties of their own (Del Seppia et. al., 2003). This is not surprising considering that these studies have employed different patterns, or temporal morphology, of magnetic fields to induce these effects. It has been suggested that the effect of the magnetic fields on biological systems is dependent on the temporal morphology of the field in a similar manner as the effects of pharmacological treatments vary with different drug concentrations (Persinger, 1997).

Of greatest interest are the effects of magnetic fields in the production of analgesia, which could be of therapeutic value. Martin and colleagues (2004a) have described temporal parameters of the magnetic field generated that elicit a maximal analgesic effect. These parameters have been found to elicit an analgesic response that is in the order of that produced by 4 mg/kg of morphine in rats (Fleming *et al.*, 1994). It has been postulated that there could be a role for the endogenous opiate system in the

generation of analgesia in response to the exposure to this complex magnetic fields (Fleming, et. al, 1994, Richards *et al.*, 1993).

What remains uninvestigated is the effect of repeated exposures of magnetic fields in obtaining analgesic responses. In some studies that have been conducted over two days, and involved the repeated exposures of the rats to magnetic fields, it has been found that there is often a decrease or an abolishment in responding on day 2 (Martin *et al.*, 2004a). It is suggested that these effects could be of similar nature as the development of tolerance to the repeated administration of opiates.

The first study measured the duration of time required for a rat to reduce hotplate latency over repeated days of testing. In order to ensure that the decrease in hotplate latency over days was not due to the rat learning the paradigm and to determine the proper time frame for the second study, fourteen days of testing were employed. The second experiment assessed the effects of low doses of ketamine on repeated exposures to magnetic fields. Some reports have indicated that ketamine's role as a non-competitive NMDA receptor antagonist (O'Shaughnessy & Lodge, 1988) could have a role in decreasing tolerance developed to opiates, such as morphine, over long-term use (Trujillo, Akil, 1994; Shimoyama et. al., 1996). Low doses of ketamine were used in this study as these have been known to reduce tolerance to opiates (Bell, 1999).

METHODS

Subjects

Twelve male albino Wistar rats obtained from Charles River Laboratories in Quebec were employed in the main study. Unless otherwise stated, all rats were aged

between 6 months and 14 months and housed 2-3 per cage. Rats were maintained on a 12:12 light-dark cycle beginning at 0800 hr local time.

Experiment 4.2.1. Hotplate Learning

The first experiment took place in order to determine whether rats could learn the hotplate analgesia paradigm. Three male Wistar rats aged between 120 and 150 days were used in the experiment. For fourteen consecutive days, rats were tested three times on a hotplate analgesiometer at 30 minute intervals. Between trials, the rats were returned to their home cages. Thermal thresholds were assessed by placing a rat on a hotplate set at 55°C until it licked its two hind paws, in any order, at which time it was removed from the apparatus. Hotplate latency throughout the three trials and fourteen days was analyzed with the use of a two way within subject analysis of variance. Pearson and Spearman correlations were also conducted for daily hotplate latency and geomagnetic activity (AA) for the day of testing and geomagnetic activity for up to 5 days prior to testing. Correlations investigated relationships between hotplate latency and the group mean and standard error of the mean (SEM) for hotplate latency.

Experiment 4.2.2. Magnetic Field Analgesia and Ketamine

Apparatus

The magnetic field exposure system used has been previously described (Fleming, et. al., 1994; Martin & Persinger, 2003). The apparatus was composed of a 25 cm x 25 cm x 25 cm plastic chamber that had two horizontally aligned solenoids on opposite sides. The solenoids were composed of 25 cm long iron nails (1 cm diameter) wrapped

with 1050 turns of 20 gauge, insulated wire. These were placed as to be centered on the horizontal plane of the chamber and approximately 5 cm from its base (2 cm of corn cob bedding) in order to be at the same level as the rats' heads. The circuitry of the system was such that the field generated would be between the two solenoids, which acted as north and south poles. The solenoids were connected to a Zenith XT personal computer through a differential (d.c. coupled) amplifier. The intensity of the magnetic field generated was between 0.15 μT and 2.4μT (Martin & Persinger, 2003). The presence of the field was verified at the beginning and end of the magnetic stimulation by the use of a Metex 3800 multimeter and magnetic sensor probe (Electric Field Measurements, Stockbridge, MA).

The complex magnetic field pattern generated was based on a series of 230 points that were fed into the computer and output as a voltage between 5 volts and -5 volts. The duration of these points was set at 3 msec and a 4 sec interstimulus interval was set as the time between the repetitions of the BurstX pattern. All exposures were 30 minutes in duration (365 cycles) since this was the optimal exposure time found in previous studies (Martin *et al.*, 2004).

Nociceptive thresholds were assessed by the use of an Omitech hotplate surrounded by a plastic chamber (26 cm x 26 cm x 26 cm). The temperature of the plate was maintained at 55°C. According to the findings of the hot plate learning analysis and the goals of this study, six days were chosen as the appropriate duration (see results section). There were no significant differences in hotplate latency between day 6 and day 1 (baseline) in the hotplate learning study (see results below).

A total of 12 rats served as subjects in this study. Every day of the study, each rat was first taken from its home cage and brought to a different room were its baseline nociceptive threshold was measured. This was done by placing the rat on a hotplate and noting the time taken for it to lick its two hind paws in any combination. Following this, the rats' mass was measured and it received an injection either of ketamine (1 mg/kg, s.c.; N = 6) or of saline (1 cc/kg, s.c.; N = 6). The rat was then brought to a separate room where it was placed in a chamber for 30 minutes and was either exposed to a magnetic field or a sham (software not initiated). Following exposure, another hotplate measurement was taken (Trial 1). The rat was then returned to its home cage and a third measurement of hot plate latency was taken 30 minutes later (Trial 2).

For the purpose of analyses, net (trial – baseline) and relative [(trial – baseline)*100/baseline) differences were calculated for both trials following magnetic field treatments. All trials were analyzed independently using the raw data, net differences, relative differences, and their daily means [(trial 1 + trial 2)/2]. The trials were analyzed using a three-way multivariate analysis of variance with one level repeated (day) and two levels not repeated (field; drug). Post hoc analyses involved a Tukey's test set at $p < .05$ for between subject effects and paired t-tests for within subject effects.

RESULTS

Experiment 4.2.1. Hotplate Learning

A 2 x 2 multivariate analysis of variance with two levels repeated (days and trials) revealed a significant main effect for day [$F(13,39)=7.56$; $p<.05$; $\eta 2=79\%$]. There was no statistically significant interaction between trial and day ($p>.05$). The three trials per day

were subsequently collapsed into daily means and paired t-tests were conducted to find the source of the main effect. The means (±SEM) for hotplate latency over the 14 days of testing can be found in Figure 4.2.1. *Post hoc* analyses employing paired t-tests revealed that the source of the day effect was that the rats had significantly longer hotplate latencies on days 1 through 6 (except day 3) as compared to days 9 through 13. Responding on days 1 through 8 did not significantly differ from each other nor did days 8 through 14. These data are taken to signify that rats learn the hotplate paradigm after 6 to 8 days of testing.

To assess the relationships between the rats responding over time, both parametric (Pearson r) and non-parametric (Spearman ρ) correlations were computed to view the relationship of the group's mean responding over days. There were no significant ($p>.05$) correlations for the first seven days when using the parametric test. On the eighth day, a significant correlation emerged ($r = -.83$; $p<.05$). The correlation continued to strengthen until day 14 ($r = -.91$; $p < .01$). The Spearman correlation demonstrated slightly different results. For this test, a significant correlation appeared as early as day 6 ($\rho=-.83$; $p<.05$). After 8 days ($\rho=-.88$; $p<.01$) and 14 days ($\rho=-.91$; $p<0.001$) of the study, there were similar correlation coefficients to those demonstrated by the Pearson correlational analysis.

The relationships between group means and SEM over the 14 days exposure to geomagnetic activity (AA index) demonstrated a significant correlation between SEM and geomagnetic activity 2 days before the testing day ($r=0.56$, $p<.05$; $\rho=0.40$, $p=0.08$). There was also a significant Spearman correlation between SEM and the AA index on the

day of testing (ρ=-0.52). The SEMs and daily geomagnetic AA index plotted over the 14 of testing can be seen in Figure 4.2.2.

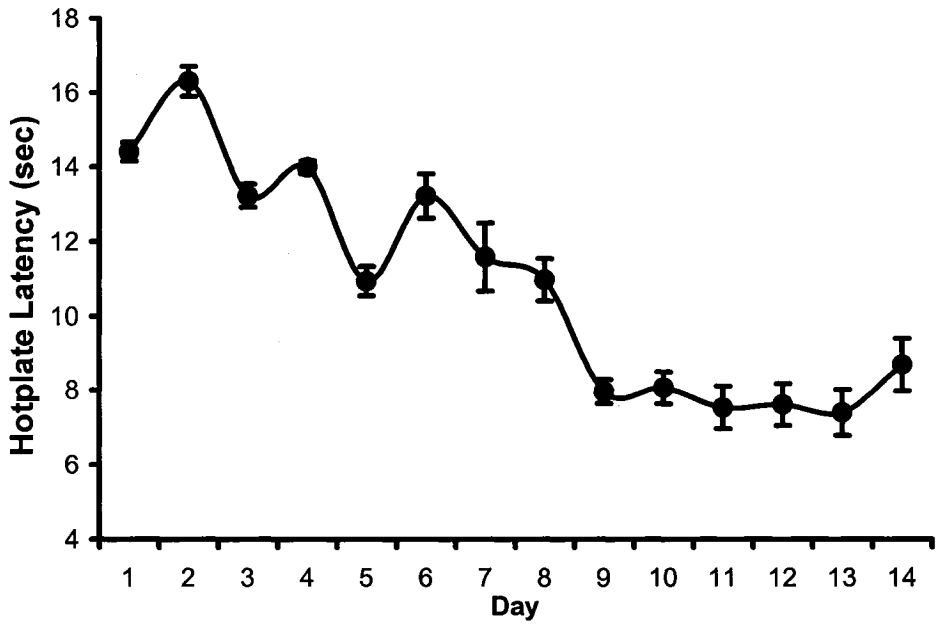


Figure 4.2.1. Average daily hotplate latency over the 14 days of testing.

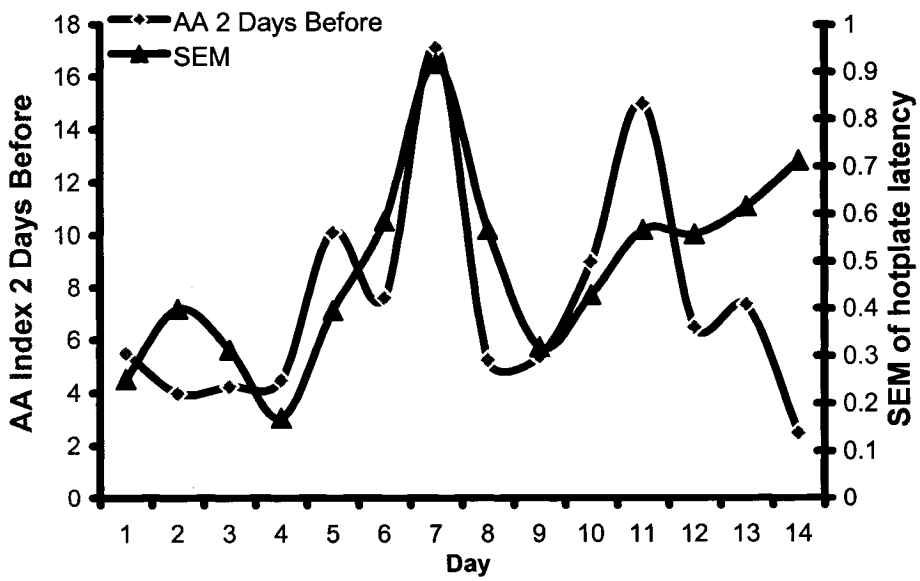


Figure 4.2.2. Correlation between the standard error of the mean of hotplate latency and daily geomagnetic activity 2 days prior.

In order to investigate whether any correlations were masked by the learning effect, partial correlations were conducted controlling for day. It was shown that, when controlling for day of testing, there was a significant correlation between hotplate latency and AA index on the day of testing (r=-0.64). Also, the correlation between SEM and geomagnetic activity 2 days before testing remained significant (r=0.58).

Experiment 4.2.2. Magnetic field analgesia and ketamine

Since the use of different variables (raw, net, relative) all yielded similar results and the use of net differences demonstrated the most pronounced effects, these will be the only results reported. A three way analysis of variance with one level repeated (day) and two levels not repeated (field, drug) demonstrated a significant field-by-drug interaction for the net difference in hotplate responding on trial 2 [$F(1,8)]=8.36$; $p<.05$; $\eta^2=51\%$]. The six days of testing were subsequently collapsed in to a single mean. These means (±SEM) for net change in hotplate latency over the magnetic field and drug conditions can be found in Figure 4.2.3. *Post hoc* analyses employing Tukey's test demonstrated that the source of the interaction was due to rats in the saline-treated group having a significantly lower hotplate latency when exposed to the magnetic field than when exposed to a sham field while rats in the ketamine-treated group did not differ between magnetic field treatment. No other main effects or interactions were found with the use of any of the variables measured during the experiment.

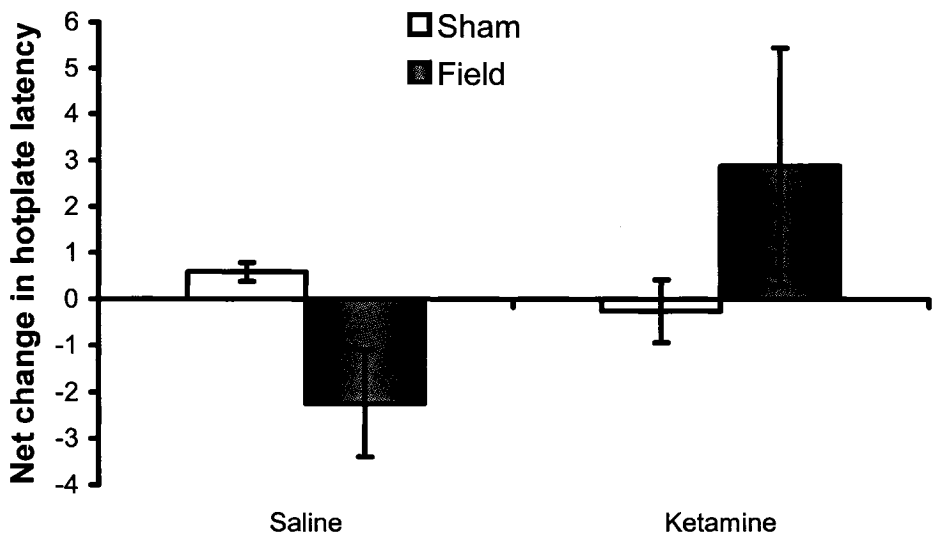


Figure 4.2.3. Six day average for net change in hotplate latency on trial 2 for the different magnetic field and drug treatment (ketamine = 1mg/kg).

When individual days were analyzed through two way between subject analyses with field and drug, only the first day of testing yielded any significant effects for change in hotplate latency. Analysis of the averaged net change in hotplate latency for the two trials revealed a significant field-by-drug interaction [$F(1,11)=7.68$; $p<.05$; $\eta^2=21\%$]. The means (±SEM) for the average (two trials) hotplate responding on day 1 can be found in Figure 4.2.4. *Post hoc* analyses employing Tukey's test demonstrated that the source of the interaction was due to rats in the saline-treated group having a significant decrease in hotplate latency when exposed to the magnetic field as compared to rats in the sham field-treated group [$F(1,5)=10.06$; $p<.05$]. There were no other significant differences between treatment conditions.

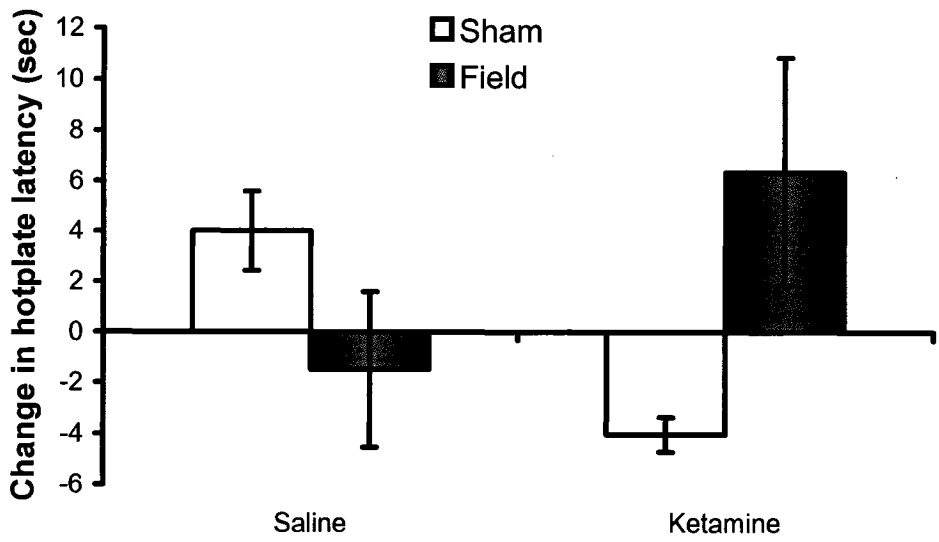


Figure 4.2.4. Average change in hotplate latency of the two trials across magnetic field and drug treatment conditions.

DISCUSSION

The results of the current study have demonstrated rats have the ability to learn the hotplate paradigm. As can be seen in Figure 4.2.1, there was a fairly steady decrease in overall hotplate latency over the 14 days of testing. It was shown that the group variability was positively correlated with geomagnetic activity two days prior to testing. This relationship was still significant when controlling for the learning effect. These results are consistent with previous research which found that geomagnetic activity during the day of testing was correlated with the variance of responding in a contextual fear conditioning paradigm (McKay & Persinger, 2003).

These results suggest that certain rats might be vulnerable to the effects of geomagnetic fluctuations. There could be some predisposition to responding to synthetic magnetic fields as generated in these experiments. Further, these results suggest that fluctuations in the ubiquitous geomagnetic field are important to consider and control in

behavioural (and perhaps other types of) testing. While more salient environmental variables such as temperature and lighting are painstakingly controlled, geomagnetism has been neglected for decades in experiments investigating rat behaviour. Much research has already demonstrated that there are significant interactions between drugs and synthetic magnetic fields (Dixon & Persinger, 2001; McKay & Persiner, 2003; Martin *et al.*, 2004b). It remains to be investigated whether drug responses might be differentially effective with dynamic changes in the geomagnetic field.

Although it was not expected that rats treated with a subclinical dose of ketamine would respond differently than rats in the saline-treated group, it was thought that the rats would demonstrate a different pattern of responses to the magnetic field exposures over the course of the six day experiment. However, the current study failed to demonstrate an analgesic effect resulting from a magnetic field exposure even on the first day of testing as expected from previous studies. Such results could be due to the use of such small sample sizes since Figure 4.2.4 is suggestive that magnetic field-treated rats had higher hotplate latencies following the treatment as compared to sham field-treated rats. Past studies have used much larger sample sizes to demonstrate the effects (Martin & Persinger, 2003; Martin *et al.*, 2004a, 2004b).

Results from this study again suggest that magnetic field exposure could block the effects of stress-induced analgesia as discussed in Chapter 4.1. Differences between the sham- and magnetic field-treated group were only seen on day 1 in this study which is indicative that rats could be habituating to the mild stress of a brief nociceptive thermal stimulus. It remains unknown why these studies investigating magnetic field effects are yielding conflicting results. The large variability seen in the current study coupled with

such small sample sizes makes it difficult to yield conclusive evidence. Future research should consider controlling for baseline hotplate responses (Plone *et al.*, 1996) as well as current and peri-experimental geomagnetic activity (McKay & Persinger, 2003) in order to minimize group variance. This would enable researchers to maintain the small sample sizes necessary to approve contemporary research in many institutions.

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CHAPTER V

GENERAL DISCUSSION

The results described in this thesis have provided strong support for the idea that weak, complex magnetic fields can exert effects upon biological systems in a way that is relevant to the Sudden Infant Death Syndrome (SIDS). Analysis of the SIDS database demonstrated a correlation between geomagnetism and SIDS at different points during gestation, birth and surrounding death. Of further interest was that effects were most pronounced for the age groups most susceptible to SIDS. Results of the studies investigating the effects of complex pulsed magnetic fields in different learning paradigms further supported previous findings that showed that learning can indeed be disrupted through the acute exposure to magnetic fields (McKay *et al.*, 2000; Stewart & Persinger, 2000).

These effects may be relevant to SIDS research because previous findings have revealed abnormalities within the hippocampus in SIDS victims (Waters *et al.*, 1999) as well as dysfunction in the learned avoidance behaviour of infants prone to SIDS (Bollen *et al.*, 2007; Paluszynska *et al.*, 2004). The investigation of magnetic field effects on thermal nociceptive thresholds failed to elicit analgesic responses as previously reported (Fleming *et al.*, 1994; Martin & Persinger, 2003; Martin & Persinger, 2004). This suggests the opiate mechanism proposed in previous magnetic field studies (Fleming, et. al, 1994, Richards *et al.*, 1993) might not underlie these effects. In comparison to studies showing a role for melatonin and learning mechanisms for SIDS, those which have found disruption of the opiate system are few (Storm *et al.*, 1994).

In chapter II many relationships between SIDS and geomagnetic activity at various points in development including gestation, birth, and death were shown. All effects found pointed to an enhanced geomagnetic index during the time frames investigated and for the age groups most prone to SIDS. One of the most frequently observed effects of geomagnetism on biological effects has been a decrease in levels of melatonin (Jozsa *et al.*, 2005; Burch *et al.*, 1999; Weydahl *et al.*, 2001; Rapoport *et al.*, 1998). This compound has been known to exude important antioxidant properties. A decrease in this compound during development has been observed to lead to macroscopic (Turgut *et al.*, 2005) and microscopic (Kachi *et al.*, 2006) anatomical abnormalities which could be an important factor in predisposing (gestation) and precipitating (death) SIDS.

The most vulnerable age for SIDS victims has been shown to be between 2 and 4 months of age. In these studies we have found that cases within these age ranges are those which have demonstrated the highest geomagnetic activity during the days preceding death. Melatonin rhythms begin to appear around 8-12 weeks but have also been shown to be delayed in preterm infants where the cycle is delayed by a couple weeks (Kennaway *et al.*, 1996). Since heightened geomagnetic activity at the time of death was found in this age group, the relationships between geomagnetic disturbances and decreased melatonin levels suggest that perturbation of the geomagnetic field during a critical age could play a role in the precipitation of SIDS. Further, since the study did not find any significant differences between SIDS cases and controls, it is proposed that the melatonin mechanism involved in precipitating deaths for infants with no known

pathologies could be the same as that which determines the fate of infants with previously detected pathologies.

It has been proposed that SIDS results from repeated hypoxic events which lead to a slow and eventual critical deterioration of important breathing centers (Sawaguchi *et al.*, 2003). A decrease in circulating melatonin, whether caused by well known risk factors such as cigarette smoking (Ozguner *et al.*, 2005) or less known risk factors such as geomagnetic storms, could fail to exert the proper protective effects upon vulnerable centers. Previous research has found that melatonin can prevent hypoxic damage to infants during pregnancy (Watanabe *et al.*, 2004) as well as during other hypoxic insults (Miller *et al.*, 2005). These results suggest that the multifactorial model for SIDS could involve a balance between melatonin and hypoxic mechanisms and that decreases in the compound by various risk factors could lead to the eventual precipitation of the death.

In Chapter III, the effects of theta burst magnetic field upon learning was investigated. Various experimental models were used to show that learning can be impaired through different magnetic field parameters. Although the theta burst magnetic field pattern is not a naturally occurring frequency within the environment, other studies have found that certain environmental frequencies such as the 50Hz power line frequencies can also impair learning (Sienkiewicz *et al.*, 1998). External magnetic fields could also interfere with the physiologically patterned magnetic fields within the brain.

Magnetic field effects on learning become important in understanding SIDS since previous researchers have found that victims prone to succumbing to SIDS have difficulty with learned avoidance to noxious stimuli (Paluszynska *et al.*, 2004). Some studies have found that associative learning may shape the defense response to hypoxia in

newborns (Bollen *et al.*, 2007; Paluszynska *et al.*, 2004). If magnetic fields can exert effects which impair learning, avoidance behaviour could be partially disrupted through this mechanism, implicating these fields further with the syndrome. Findings that certain specific magnetic fields can impair learning indicate that days of heightened magnetic activity could be involved in disrupted learned avoidance behaviour as observed in SIDS. A decreased neuronal density has been reported in the hippocampus of SIDS victims (Waters *et al.*, 1999). Because the hippocampus is considered the gateway to memory, it is possible that repeated hypoxic insults that result from such impaired avoidance behaviour could become significant. Unfortunately, it is not possible to ascertain whether the damage precedes the behaviour or vice versa.

Eckert (1994) has suggested that perturbations in the Earth's magnetic field during gestation can predispose infants to succumb to infant deaths. The data within Chapter 2 suggest that there is indeed a disrupted magnetic field during the gestational months of infants who have died in high-frequency years as compared to those who have died in low-frequency years. Because exposures to magnetic fields have been implicated in memory processes, the question arises as to whether such an active geomagnetic field conditions can function as conditioned stimuli for some individuals and hence contribute to the recreation of an *in utero* environment. Considering that magnetic field exposures have been found to have similar effects as various drugs it is plausible that an optimal configuration of the magnetic field could have a strong conditioning potential. In some instances such as conditioned taste aversion, it has been demonstrated that a single pairing of a stimulus can have long-lasting effects. If an optimal magnetic field configuration was present at one point in gestation, later exposure to this stimulus could

provide a strong environmental cue to recreate the uterine environment. Other findings that there is a similar active magnetic field present surrounding the infant's death provide further evidence of this conditioned stimulus' ability to precipitate the death. If one continues with the idea that there is a recreation of the fetal environment in which an infant does not have the capability of breathing, this active magnetic field could have the potential to precipitate such fetal behaviours.

In chapter IV we investigated the effects of magnetic field patterns on the ability to elicit analgesic responses through putative opiate mechanisms (Fleming, et. al, 1994, Richards *et al.*, 1993). Although the experiments failed to replicate past findings (Fleming *et al.*, 1994; Martin & Persinger, 2003; Martin & Persinger, 2004), one explanation could involve the variability in responses due to background magnetic fields as found in the hotplate learning experiment. This increased variability suggests a certain predisposition to the effects of magnetic fields by some rats. The effect may also be relevant to the understanding of SIDS in light of the triple risk model (Filano & Kinney, 1994). It suggests that there must be an underlying predisposition to the disorder, mediated through genetic or congenital variables. Such a predisposition to the effects of magnetic fields could provide an additional variable in understanding the multifactorial substrates to SIDS.

This thesis has found strong evidence for the relationships between SIDS and geomagnetic activity. The findings that there were significant effects during the time of gestation, and at the time of death in an age-dependent manner support the triple risk model (Filano & Kinney, 1994) and lends strong support to the model proposed for the involvement of magnetic field disturbances in SIDS (Eckert, 1992). Congruent with the

previously established melatonin hypothesis of SIDS (Weissbluth & Weissbluth 1994), other researchers have suggested that magnetic fields can exert their effects via a melatonin mechanism (O'Connor & Persinger, 1997). The results of the analyses presented here are consistent with these claims and outline an important new risk factor for parents to consider during the critical early developmental stages of the infant.

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