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Combined effects of complex magnetic fields and agmatine for contextual fear learning deficits in rats

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Abstract

Acute post-training exposures to weak intensity theta-burst stimulation (TBS) patterned complex magnetic fields attenuated the magnitude of conditioned fear learning for contextual stimuli. A similar learning impairment was evoked in a linear and dose-dependent manner by pre-conditioning injections of the polyamine agmatine. The present study examined the hypothesis that whole-body applications of the TBS complex magnetic field pattern when co-administered with systemic agmatine treatment may combine to evoke impairments in contextual fear learning. Within minutes of 4 mg/kg agmatine injections, male Wistar rats were fear conditioned to contextual stimuli and immediately exposed for 30 min to the TBS patterned complex magnetic field or to sham conditions. TBS patterned complex magnetic field treatment was found to linearly summate with the contextual fear learning impairment evoked by agmatine treatment alone. Furthermore, we report for sham-treated rats, but not rats exposed to the synthetic magnetic field pattern, that the magnitude of learned fear decreased and the amount of variability in learning increased, as the K-index (a measure of change in intensity of the time-varying ambient geomagnetic field) increased during the 3-hr intervals over which conditioning and testing sessions were conducted.

Keywords: Agmatine; Complex magnetic fields; Rat; Synergism; Geomagnetic indices

Introduction

Complex magnetic fields, whose temporal structures resemble the electrophysiological signatures of endogenous physiological processes, evoke behavioral impairments for inferences of learning and

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memory in a waveform-specific manner. We have previously reasoned that non-invasive whole-body applications of a theta-burst stimulation (TBS) weak intensity magnetic field stimulus might evoke learning deficits for hippocampal-dependent tasks [18]. This waveform resembles the intrinsic electrophysiological characteristics of hippocampal cells measured following learning tasks (e.g., single unit recordings from hippocampal place cells [4]), and abrogates long-term potentiation [3] when applied as electrical stimulation to hippocampal tissue.

To test the hypothesis that weak intensity complex magnetic fields might evoke learning impairments in a waveform-specific manner, we fear conditioned male Wistar rats to contextual stimuli and exposed them singly for 30 min to a TBS patterned complex magnetic field (after [22]), a burst-firing patterned complex magnetic field, and two extremely low frequency sinusoidal patterns which were intensity matched to the two complex patterns [18]. Marked attenuation of the magnitude of the learned fear, as inferred by diminished defensive freezing when returned to the conditioning chamber for retention testing 24 hr after conditioning sessions, was specific to applications of the TBS patterned complex magnetic field. When we repeated the study with conditioned fear to discrete (tone) stimuli, which from neuroanatomical studies is independent of hippocampal functioning [6], the TBS patterned complex magnetic field-evoked impairment in learning was not evident.

Cumulative evidence indicates that the effects of weak intensity magnetic fields can be augmented or diminished by specific pharmacological agents. Extremely low frequency magnetic fields with sinusoidal waveforms (e.g., those produced by 50 or 60 Hz power frequency sources, or those produced by rotating (0.5 Hz) magnets) are known to diminish opioid- or stress-evoked analgesic responses in rodents [20,10,25]. However, acute exposures to frequency-modulated (complex) magnetic fields are known to potently enhance a delta-opioidergic mediated thermal analgesic (antinociceptive) response [26,28]. One report has suggested that 50 Hz magnetic fields may act in an anti-cholinergic manner, as the behavioral effects of the magnetic fields were blocked by physostigmine [14]; other reports indicate that 60 Hz magnetic fields may block morphine-evoked analgesia [29], likely through a nitric oxide synthase mechanism [12]. An intriguing paper by Kavaliers et al. [11] reported that the non-spatial, and perhaps spatial, components of a hidden-platform water maze task were improved in female deer mice following exposures to 60 Hz magnetic fields; the effect was mimicked by the opioid antagonist naltrexone. Our own investigations have revealed that the TBS magnetic field pattern affects a variety of behavioral tasks in a manner similar to specific doses of the polyamine agmatine [19,24].

The present study examined the possible additive effects of agmatine and TBS patterned complex magnetic field treatments for contextual fear learning, the specific behavioral paradigm we have found to respond most similarly to both complex magnetic field treatments and systemic agmatine administrations. Here we show that acute exposures to a theta-burst stimulation patterned complex magnetic field, following agmatine administration, evoked an additive impairment in the consolidation of learned fear to contextual stimuli. Because ambient time-varying geomagnetic activity has been postulated to contribute variability in studies addressing the effects of experimentally-applied weak-intensity magnetic fields, and because the proficiency of normal subjects for many behavioral tasks appears to covary with ambient (global) geomagnetic activity, the magnitude of the differences in learned fear between sham- and field-exposed groups, as well as the performances of each of these groups (means, standard deviations) were correlated with geomagnetic variables prior to and during fear learning. Here we report that the magnitude of the differences between sham-exposed and complex magnetic field-treated rats varied as a function of the changes in the intensity of the ambient geomagnetic field, specifically by modulating the performance of rats in the sham (control) groups.

Methods

A total of 52 male Wistar rats (Charles River, Quebec) approximately 12 months of age with an average mass of 750 grams were utilized in the present study. Our previous investigations have shown that rats from this age group 1) learn the association between contextual cues and footshock stimuli at levels measured in younger rats, and, 2) show the same degree of learning impairments as younger rats following exposures to specific patterns of complex magnetic fields. Rats were maintained 3/cage with food and water available ad libitum. Photophase onset was 0730h local time with a 12:12 L:D cycle. Ambient temperature was maintained at 20 ± 1 °C. All conditioning and testing sessions were conducted during the mid to late photophase (1300 to 1700 h local time).

The fear conditioning procedure and apparatus have been described previously [24]. Briefly, rats were placed in a modified operant (contextual) chamber and given a 3-min habituation period followed by 3, 2-sec, 0.5 mA footshocks delivered at 60 sec intervals by an A-615-C Master Shocker (Lafayette Instruments). The magnetic field transient generated by switching the current source on and off during shock intervals, as measured within the center of the contextual chamber, was less than 50 nT. During the habituation period rats were scored for midline chamber crossovers, defined as forward movements of the rats' limbs across the midline of the chamber. Retention testing was conducted 24-hr later when rats were returned for 8-min to the contextual conditioning chamber. During retention testing rats were scored for defensive freezing, a behavioral index of fear [2], nominally every 8-sec (for a total of 60 observations) for the presence (1) or absence (0) of defensive freezing, a characteristic and species-specific immobility posture displayed by rats 1) following aversive stimulation, and, 2) in contexts previously associated with aversive stimulation. The footshock chamber was cleaned with a dilute (0.4%) solution of aqueous acetic acid and then dried prior to conditioning or testing each animal. A removable, non-conducting, insert was placed into the chamber during all conditioning and testing sessions to facilitate the removal of the often aggressive rats following context/shock pairings.

Immediately following fear conditioning (training) sessions rats were placed for 30 min into the apparatus emitting the magnetic fields, or placed in a reference (sham) exposure cage within the treatment room in a location where the intensity of magnetic fields generated by artificial sources (both stray from the equipment and from 60 Hz sources) were below measurable limits (< 10 nT). Because practical methods for shielding fluctuations in the ambient geomagnetic field have not been developed, sham groups and synthetic magnetic field-treated groups of rats were continuously immersed in the ambient magnetic fields of geomagnetic origin.

The apparatus employed in this study has been described in detail and illustrated elsewhere [18]. Essentially this device consists of six solenoids mounted one solenoid per face on a Plexiglas cube (20-cm square faces); the circuitry to the six solenoids was arranged such that each opposing pair of solenoids were activated together, thereby generating magnetic fields in the plane of the X, Y and Z axes of the cube. This device, which was controlled by custom constructed circuits, rotated the magnetic field through each axis separately for durations of 2-sec per axis, and then generated the field through all 3 axes (all 6 solenoids) for an additional 2-sec. The rotational rate of the field through the apparatus was thus 0.5 Hz, a rotational velocity that has previously been demonstrated to be biologically effective [21]. Custom constructed Complex Software converted a series of digital values between 0 and 255 (the waveform file — e.g., the TBS pattern which consisted of 225 points) to -5 through $+5$ volts respectively, through a digital to analogue converter. The analogue equivalent was propagated to the solenoids and produced as a magnetic field. For this experiment the software had been configured to activate each digital value (pixel) within the waveform file for 1 msec; the interval between waveform (stimulus) presentations was also set at 1 msec. Each presentation of the waveform thus lasted 225 msec and was presented continuously. The design of the waveform computer file, and the temporal characteristics by which the magnetic field was emitted, generated the TBS magnetic field pattern illustrated in Fig. 1. The peak intensity of this magnetic field when measured in the center of the application chamber was approximately 1500 nT, when measured within 1-cm of any individual solenoid was approximately 2000 nT, and when measured in any of the four corners of the exposure apparatus was approximately 200 nT.

To test the hypothesis that complex magnetic fields and agmatine may interact in an additive manner to affect conditioned fear learning, an initial study requiring 32 rats (N = 8/group) was completed and was followed by a replication study employing 20 rats (N = 5/group). Immediately prior to conditioning sessions rats were injected with physiological (0.9%) saline, or with 4 mg/kg (1 cc/kg; i.p.) agmatine (Sigma, St. Louis). This dose had been selected from our previous studies with this compound where we had examined the dose-dependence of agmatine for the magnitude of attenuation of conditioned fear learning [19,24]. Following conditioning sessions half the rats in each agmatine- or saline-treated group were sham exposed or received 30 min applications of the TBS complex magnetic field pattern. All statistical analyses were completed using SPSS software on a VAX 4000 computer. Two-way analysis of variance (ANOVA) with two independent levels (sham vs. complex field; saline vs. 4 mg/kg agmatine) was the primary statistical tool. Post hoc analyses were one-way analyses of variance with Student Neuman-Keuls (SNK) set at $p < 0.05$.

The rats entered into the fear conditioning procedure were trained/tested on seven pairs of training/testing days over a period of approximately two months. Approximately 6–10 rats were conditioned during each of these seven blocks. To examine relationships between ambient geomagnetic variables and amounts of contextual fear learning, we calculated the means and standard deviations for both sham- and field-exposed groups of rats for each discrete block of training/testing (one-half the rats in each block were sham-exposed, the other half were field-exposed), as well as the F-ratios (F-statistic) representing the magnitudes of the between-group differences (sham vs. synthetic magnetic field) for each of these separate blocks (thus N = 7), and correlated these values with the ambient geomagnetic values measured at Boulder, Colorado (obtained online from http://www.dxlc.com/solar/indices.html). To obtain an indication of the day-to-day relationship between fear learning and geomagnetic variables, the planetary A-indices (in nanoTesla) for the days of

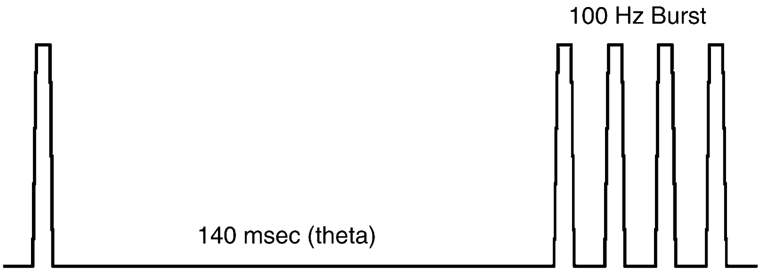

Fig. 1. The theta-burst stimulation (TBS) patterned waveform. The temporal sequence consisted of an initial priming pulse followed after 140 msec (theta rhythm) by a 100 Hz burst.

training and testing, as well as the three days preceding or following training or testing, were correlated with the means and standard deviations of fear learning for sham- and field-exposed groups, as well as the F-ratios representing the statistical contrast between these groups. For finer resolution, we correlated the K-indices (a semi-logarithmic scale reporting absolute changes in intensity of the ambient time-varying geomagnetic field over 3-hr intervals) measured during the 3-hr intervals throughout which the rats were trained or tested, as well as the two 3-hr intervals preceding, and the two 3-hr intervals following, testing, with the means, standard deviations, and F-ratios. To reduce the likelihood of outliers driving statistically significant relationships, only coefficients that were statistically significant for both Pearson r and Spearman *rho* correlations were considered (Pearson r values reported).

Results

Two-way analyses of variance indicated that midline chamber crossovers, our inference of baseline motor activity, did not differ between groups of rats as a function of dose of agmatine and did not differ a priori between would — be sham-treated or complex magnetic field-treated rats (all Fs < 2.7, ns). Grand mean midline chamber crossovers (standard deviation in parentheses) for all rats in this study were 5.8 (2.6).

As heterogeneity of variance for duration of freezing during contextual retention testing was noted between groups of rats receiving saline and sham treatments in combination vs. groups of rats receiving the TBS magnetic field pattern and agmatine in combination, parametric analyses were completed after a square-root transformation of the freezing-to-context data. Importantly, the statistically significant findings did not differ between analyses completed for transformed and untransformed data, and thus means and standard errors of the means for each group are reported based upon the raw (untransformed) data.

Combined effects of agmatine and TBS magnetic field patterns

Three-way analysis of variance (sham vs. TBS magnetic field; saline vs. agmatine; initial study vs. replication) was used to examine the combination of 4 mg/kg systemically administered agmatine and whole-body applications of a theta-burst stimulation patterned complex magnetic field for contextual fear learning. ANOVA showed a statistically significant decrease in freezing for field-exposed rats (M = 64.4, SEM = 4.2) relative to sham-exposed rats (M = 86.2, SEM = 2.1) [$F(1,29) = 30.7$, $p < 0.001$, effect size = 40%] and a statistically significant decrease in freezing for rats treated with 4 mg/kg agmatine (M = 71.4, SEM = 4.4) relative to saline-treated rats (M = 80.5, SEM = 3.0) [$F(1,29) = 11.5$, $p < 0.005$, effect size = 14%]. There were no statistically significant differences in amounts of freezing behavior between the initial study and the replication study [$F(1,29) = 0.03$, ns]. This independent level did not interact significantly between other independent levels in the analyses.

ANOVA demonstrated a statistically significant interaction between complex magnetic field treatment and agmatine treatment [$F(1,29) = 4.03$, $p < 0.05$]. Post hoc analysis (SNK, $p < 0.05$) indicated that those rats treated with 4 mg/kg agmatine and given post-conditioning exposures to the TBS patterned complex magnetic field froze significantly less during retention testing than all other combinations of groups; those rats receiving saline treatment and magnetic field treatment in combination froze significantly less than those rats receiving saline treatment and exposed to sham conditions (Fig. 2).

Correlations with ambient geomagnetic conditions

Two days prior to conditioning, the F-ratios were significantly [$r = 0.85$, $p < 0.05$] correlated with the daily low for the planetary A-index; one day prior to conditioning the F-ratios were again significantly [$r = 0.96$, $p < 0.01$] correlated with the daily low for the planetary A-index. On the day of conditioning, the means of the sham-exposed groups were negatively correlated [$r = -0.89$, $p < 0.05$] with the K-index for the 3-hr interval during which the conditionings took place; statistically significant correlations between the K-index and learned behavior were not evident during the 6-hr periods prior to or following the 3-hr conditioning interval (Fig. 3). On the conditioning day the standard deviations for the sham-exposed rats were positively correlated [$r = 0.93$, $p < 0.01$] with the 3-hr K-index value during the time of the conditionings; statistically significant correlations between the K-indices and learned fear during the intervals prior to or following conditioning sessions were not evident.

On the day of retention testing, means of the sham-exposed groups were negatively correlated [$r = -0.93$, $p < 0.01$] and the standard deviations of the sham-exposed groups were positively correlated [$r = 0.93$, $p < 0.01$] with the daily low for the planetary A-index. Additionally, on the day of retention testing the standard deviation of the sham group was positively correlated [$r = 0.83$, $p < 0.05$] with the 3-hr K-index at Boulder, and the F-ratios of between-group differences were negatively correlated [$r = -0.86$, $p < 0.05$] with the 3-hr K-index at Boulder. Both occurred during 3-hr intervals in which retention testing took place. Such relationships between ambient geomagnetic variables and learned fear

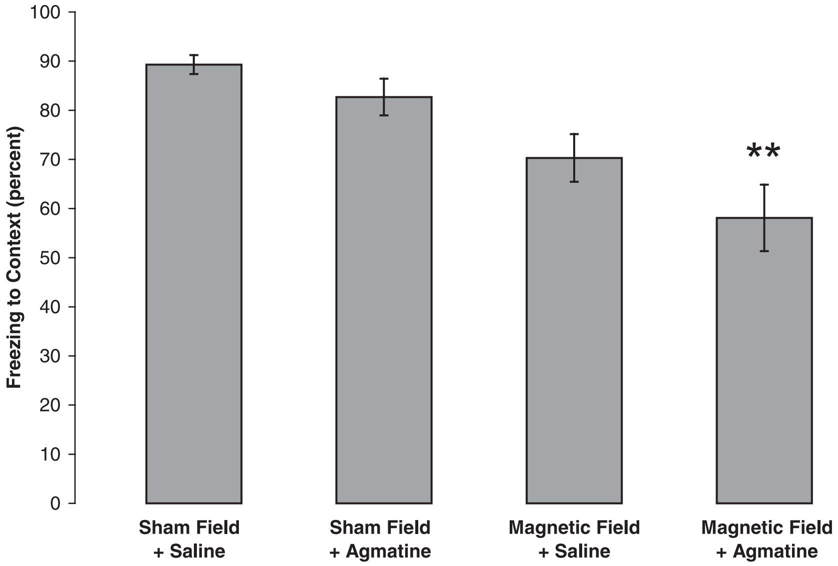

Fig. 2. Mean percent freezing to contextual stimuli during retention testing for rats given immediate pre-conditioning saline or 4 mg/kg injections and exposed for 30 min following conditioning sessions to either sham conditions or a theta-burst stimulation patterned complex magnetic field. Error bars indicate standard errors of the means. Double asterisks indicate a statistically significant difference from all other groups.

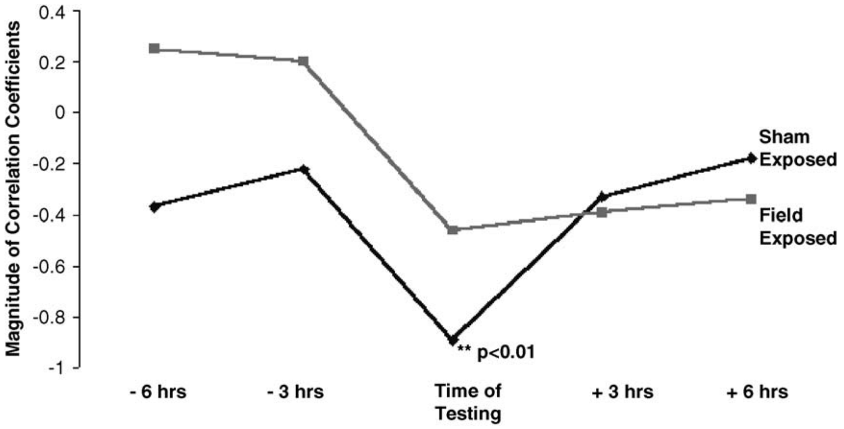

Fig. 3. Pearson correlations between the mean amount of learned fear and the intensity of the ambient geomagnetic field (K-index) throughout the 3-hr intervals during which conditioning sessions took place, or during the two 3-hr intervals prior to and two 3-hr intervals following conditioning sessions. Only the amount of learned fear for sham-exposed rats was correlated with ambient geomagnetic conditions. This relationship was only evident during the time interval of the conditioning sessions.

for these rats when divided into two groups along the saline vs. agmatine axis were not evident. These geomagnetic variables did not change the degree of statistical significance when employed as covariates in the ANOVAs described above.

Discussion

The combination of acute presentations of theta-burst patterned complex magnetic fields following fear conditioning sessions, when administered as an adjuvant with agmatine, was examined in the present study. TBS patterned complex magnetic fields augmented the fear learning impairment evoked by 4 mg/kg agmatine in a linear manner. Increased ambient geomagnetic activity during the intervals when conditioning and testing sessions were conducted was associated with decreased fear learning and increased variance (dispersion) around the mean for sham-exposed rats. Thus the magnitude of the differences between sham- and field-exposed groups was inversely related to geomagnetic intensity during fear trials. This means that as the global geomagnetic activity increased during testing the size of the treatment effect (the discrepancy between the rats exposed to the experimental magnetic field or the sham condition) decreased.

Agmatine is known to evoke a voltage- and concentration-dependent block of the NMDA ionophore [31]; other pharmacological effects include inhibition of nitric oxide synthesis [8], and under specific experimental conditions antagonism of alpha-2 adrenergic and imidazoline receptors [15]. However, we have previously shown through extensive behavioral work [19,24] that the most likely pharmacological bases for the effects of agmatine in the fear conditioning paradigm are those related to its effects on the NMDA receptor. Interestingly, the decreased fear learning following applications of TBS patterned complex magnetic fields noted in this study and in a past study [18] is reminiscent of the adverse effects of NMDA antagonism for contextual fear memory [5].

Weak intensity magnetic fields destabilize rhythmic slow activity in hippocampal slices [1,9]. Although it remains to be verified by direct electrophysiological measurement (optimally after focally ejecting agmatine into synaptic sites implicated in the fear conditioning circuit), theta-burst stimulation patterned complex magnetic field may have disrupted some aspect of hippocampal physiology, perhaps by destabilizing rhythmic slow activity as previously reported, or perhaps by occluding the development of long-term potentiation. Because direct application of NMDA antagonists to activated synapses occludes the generation of LTP [16], the TBS magnetic field pattern may have complemented the agmatine-mediated antagonism of glutamatergic receptor sites [31] implicated in the generation of LTP. Stated alternatively, it may be that the TBS patterned magnetic field has NMDA-like properties, acting through an as yet undiscerned transduction mechanism.

Investigations into the pharmacological bases of applied weak intensity and complex magnetic fields have pointed to augmentation of endogenous opioid activity [7,23,26–28]. Interestingly, agmatine at very low doses has been found to potentiate the effects of morphine by 5- to 9-fold, depending upon the route of morphine administration [13]. Moreover, agmatine has been shown to block the acquired tolerance to the delta-opioid receptor ligand [D-Pen2,D-Pen5]-enkephalin [13]. This specific opioid receptor subtype has been implicated as a putative transduction step for complex magnetic fields interacting with biological systems [28]. Although our earlier results [18], which employed a magnetic field with known opioid-augmenting effects [7], had no discernable effect on the magnitude of learned fear to contextual stimuli, we cannot at this time rule out the possibility that some augmentation of magnetic field-evoked opioid activity was at least in part responsible for the present effects. Microinjection of the non-specific opioid agonist morphine into the amygdala is known to potently impair contextual fear conditioning [30].

We have shown that both the magnitude of learned fear to contextual stimuli, and the variability in the amount of contextual fear learning, for sham-exposed rats were systematically related to changes in intensity of the ambient geomagnetic field at the time of training sessions. Converging evidence from other paradigms indicates that the magnitude of between-group differences (field-exposed vs. sham-treated) may vary with ambient geomagnetic conditions as well. For example, the size of the group differences (F-ratios) between magnetic field-treated groups (synthetic treatment and exposed to ambient conditions) and control groups (exposed only to ambient conditions) for numbers of working errors during a spatial radial maze learning task were negatively correlated [$rho = -0.70$] with the K-index (measured at Boulder, Colorado) during the daily 3-hr periods wherein testing was conducted [17]). We have also noted that applications of (synthetic) sudden storm commencement-modeled magnetic fields during the 24 hr interval following pilocarpine-induced limbic epilepsy markedly increased the mortality of the exposed rats over this interval. The mortality of sham-exposed rats within reference cages, however, was systematically related to increases in ambient geomagnetic activity (unpublished observations). Furthermore, we have reported in the present study that the magnitude of differences between sham- and magnetic field-treated rats was correlated with changes in ambient geomagnetic activity during the 2 d period prior to conditioning sessions.

Because of the relationship between geomagnetic variables prior to and during the time of behavioral testing, as demonstrated for sham-exposed (e.g., control) rats for several behavioral endpoints, the geomagnetic field might optimally be considered a potential source of variability in many animal studies. Importantly, the observed statistical relationship between F-ratios (which are used to calculate statistically significant results between groups) and ambient geomagnetic conditions raises the possibility that inabilities to replicate magnetic field effects across experiments and between laboratories may be related to ambient geomagnetic variables. They are either not measured or even considered by many experimenters.

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