An extremely low-frequency magnetic field can affect CREB protein conformation which may have a role in neuronal activities including memory

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Abstract
The cAMP response element-binding protein (CREB) was exposed to an extremely low-frequency magnetic field (ELF-MF) of the range (~2.4–2.4) mT intensity and at a frequency of 50 Hz. The effects of exposure were investigated in the mid-infrared region using Fourier spectroscopic analysis. The purpose of this experiment is to simulate the exposure of neuronal proteins to a low magnetic field which may naturally occur in the brain due to electrical impulse signals. The experimental results showed inconsistent fluctuations in peak positions, band shape, and intensities for several bands in the amide II, amide IV and amide VI regions. This can be due to two factors. The first suggests that hydrogen bonds can alter the frequency of stretching vibrations depending on the increase or decrease of strain on the vibrations. The second is that all these bands are caused by bending vibrations in combinations with other vibrations, which makes these vibrations susceptible to magnetic field influence. Spectra analysis showed that once the CREB protein was exposed to a magnetic field, it induces a genuine reaction changing the secondary structure and producing changes that can have a lasting effect. The resulting conformational changes in brain proteins may have an effective role in signal transduction, learning and memory formation.

1. Introduction
Recent studies have shown that exposure to an extremely low-frequency electromagnetic field increases hippocampal neurogenesis and improves memory [1]. Rats exposed to ELF-MF during cognitive training performed better in both spatial learning and long-term retention of spatial memory [2–4]. Other studies have also shown that exposure to ELF-MF can increase the levels of amino acid neurotransmitters and increase the level of dopamine in the thalamus of rat brains [5, 6]. Whereas, other investigations have demonstrated that exposure to an ELF-MF might have harmful effects on an organism’s physiology [7, 8].

ELF-MF is commonly present in our external environments, furthermore, the electrical impulse signals in neurons are capable of generating an internal magnetic field [9]. In this study, we are interested in investigating how exposure to ELF-MF might affect the protein structure of hippocampal proteins. We choose to focus our study on CREB because it is a well-conserved and ubiquitous protein that plays a critical role in the formation of long-term memory (LTM) [10, 11]. CREB is a cellular transcription factor that binds the cAMP response element (CRE), resulting in either upregulation or downregulation of its gene targets [12]. The CREB protein molecular structure is shown in figure 1, [13] it belongs to a superfamily of basic leucine zipper (bZIP) proteins, which can be divided further to smaller families based on their DNA binding and dimerization preferences.
Mutations in the gene encoding CREB protein have been linked to Rubinstein-Taybi syndrome, a human genetic disorder that impairs intellectual abilities \cite{14, 15}. Therefore, the utilization of CREB has been suggested for clinical use on cases of human memory disorders. CREB has been implicated in learning and memory and is also involved in a variety of other cellular processes, such as glucose homeostasis and growth factor-dependent cell survival \cite{16}.

Studies of Aplysia \cite{17, 18}, Drosophila \cite{19} and mouse \cite{20} suggested that cAMP-responsive transcription, mediated by the CREB family of proteins, has contributed strongly in the learning process and the formation of long term memory (LTM) by coupling neuronal activity with changes in gene expression \cite{21}. In general, these studies showed overwhelming pieces of evidence that CREB acts as a molecular switch to control the formation of LTM. CREB regulates crucial cells in the developing brain. It participates in neural plasticity, learning and memory, and has a role in adult hippocampal neurogenesis (AHN) \cite{22–24}. It has also been proposed that spontaneous neocortical activity can result from the magnetic interactions between astrocytes and neighboring neurons \cite{25}.

The nervous system works by using electrical signals. Therefore, it should not be surprising that exposure to electromagnetic fields could lead to physiological changes. Although there is a lack of consensus regarding the effects of ELF-MF on proteins \cite{26–28}, it seems that the ELF magnetic field affects proteins either by improving biological tasks or by causing dysfunction. This depends on whether the exchanged energy is appropriate for a particular task or not. It is clear from these in vivo and in vitro studies that protein exposure to (ELF-MF) has caused interference with memory mechanisms and has an effect on brain function. In a previous investigation, we exposed beta-amyloid and human serum albumin to ELF-MF and the results indicated these proteins were highly sensitive to the effect of magnetic fields \cite{29}.

The human brain has been estimated to contain close to 85 billion neurons \cite{30}, which are connected through an infinitely elaborate network of junctions to conduct neuronal signals. In this study, we suggest that when a neuronal signal is sent through this highly complex network of nerve processes, it creates an electromagnetic field that affects the structure of the surrounding proteins. The electromagnetic field can induce changes to a protein’s secondary structure by affecting both of the hydrogen bonding, and the polarization of dipole moments \cite{31, 32}. These conformational changes which result in subtle changes in the overall shape of a protein resemble the changes by the hysteresis phenomena in ferromagnetic material.

We propose that the magnetic field generated by neuronal electric signals will induce a specific conformational change to the nearby proteins. When an electrical signal comes through the neuronal network...
for the first time it could leave an imprint in terms of structural changes in the protein caused by its magnetic field. Whenever the same neuron signal is regenerated internally or externally at a later time in the neuronal network, it might revive the conformational imprint from the remnant traces of the original signal. Perhaps memory is associated with the structural changes caused by the magnetic field in the surrounding proteins. The molecular mechanism by which information is stored might be due to the hysteresis type behavior in protein conformation. The hysteresis behavior arises from the discrete structural transition induced by the magnetic field in the structure of the protein [33, 34]. The memory effect is associated with transient increases or decreases of protein vibrational frequencies induced by the field [35, 36]. Furthermore, the instantaneous acts of sensing followed by reacting and being alert most of the time for occurring events by the brain does require fast mechanisms of interaction similar to that provided by a magnetic field. Keeping an organized memory and the correlations between events fit well with the nature of magnetic field interactions.

2. Materials and methods

2.1. Preparation of stock solutions
Human recombinant CREB binding protein bromodomain (1081–1197 amino acids), was stored in a buffer of (20 μg CREB, 50 mM Tris ([hydroxymethyl] aminomethane, with the formula (HOCH2)2CNH2), pH 7.5, containing 500 mM sodium chloride, 5 mM β-mercaptoethanol, and 5% glycerol). This sample was purchased from Sigma Aldrich Chemical Company and was stored at −80 °C and did not require any further purification before use. The samples for FTIR measurement were prepared after one hour of incubating the solution at room temperature. The amount of 40 ml of the solution was placed on a silicon window plate and left to dry at room temperature before spectroscopic measurements were taken.

2.2. FTIR spectroscopic measurements
We used a Bruker IFS 66/S spectrophotometer equipped with liquid nitrogen cooled MCT detector and a KBr beam splitter to obtain the FTIR measurements.

The FTIR measurements were obtained on a Bruker IFS 66/S spectrophotometer equipped with liquid nitrogen cooled MCT detector and a KBr beam splitter. The spectrophotometer remained continuously purged with dry air during the measurements to reduce the noise signal level. The measured spectrum was the average of 60 scans for each run to increase the signal to noise ratio. The spectral resolution for all measurements was set at 4 cm−1 and the aperture setting through all the measurements was set at 8 mm since it did give the best signal to noise ratio. All needed calculations, baseline corrections, and normalization were performed by OPUS software. The peak positions were identified by obtaining the second derivative of the spectra by OPUS software and by Fourier self-deconvolution (FSD) technique which is done repeatedly through six iterations for all deconvolution processes. The technique of (FSD) decomposes the major bands of the spectrum to its original components of single peaks which are linked to the secondary structure of the protein. The FTIR spectra of CREB were obtained in the featured region of (4000–400) cm−1. The FTIR spectra were obtained through subtracting the absorption spectrum of the background from the spectrum of the protein sample. The difference spectra are used to show the net effect of the ELF magnetic field on CREB protein. For accuracy assurance, the difference spectra were calculated using the featureless region of the protein spectra (1800–2200) cm−1 where it gave a zero difference. Besides, the difference spectra for several control samples with the same protein concentration, which yielded a flat line formation as expected.

2.3. ELF magnetic field measurements
We used an electromagnetic coil that is connected to an AC voltage regulated at 220 volts and at a frequency of 50 Hz to produce a magnetic field with a sinusoidal waveform. The sample is positioned very close to the center of the coil at a fixed holder. Therefore, keeping a uniform and perpendicular magnetic field directed at the surface of the sample. The CREB sample were exposed to varying magnetic field of the following magnitudes (0.0, 0.45, 1.4, 2.4, 1.4, 0.45, 0.0, −0.45, −1.4, −2.4) mT. The sample exposure time for each magnetic field setting was 5.0 min. The magnitude of the magnetic field was recorded for all measurements using a magnetic field axial probe PHYWE Gauss meter.

3. Results

The obtained results mainly consisted of taking the FTIR absorption spectra for the mid-infrared region (4000–400) cm−1. This range covers all the amide regions and the fingerprint region. The IR spectrum corresponds to the molecular vibration of the involved protein. The FTIR spectra in this work were obtained for the CREB sample before and after exposure to ELF magnetic fields while keeping the sample fixed in its position at all times, which allows accurate comparison of band intensities and peak positions. Any changes in CREB
spectra after exposure to a magnetic field must be due to changes in the molecular vibrations of CREB structure which can be attributed to the magnetic field effect.

Figure 2 shows the IR absorption spectrum and its second derivative of CREB protein in the range of (1800–400) cm⁻¹. The major bands are shown by the absorption spectrum while the more detailed structure of the bands is shown as individual peaks by the second derivative spectrum.

The effect of exposing CREB to a 50 Hz oscillating magnetic field at different magnitudes (0.0, 0.45, 1.4, 2.4, 1.4, 0.45, 0.0, −0.45, −1.4, −2.4) mT are shown by the changes in the FSD absorption spectrum for the different magnetic fields in figures 3 and 4. The FSD absorption spectra of CREB show the major absorption bands, including the amide bands and the fingerprint regions. The major peaks in the amide I region (1600–1700) cm⁻¹ are caused by C=O stretching vibrations coupled with the C–N stretching and C–C–N deformation mode [37].

The amide II (1480–1600) cm⁻¹ region features several peaks which are caused by out of phase combination of N–H in-plane bending and C–N stretching vibration [38]. The absorption bands in the amide III region (1220–1320) cm⁻¹ are mainly due to in-phase interference between the N–H bending and the C–N stretching with additional contributions from the C–O in-plane bending and the C–C stretching vibration [38]. The shown absorption bands in amide IV region (625–770) cm⁻¹, are mainly caused by combinations of OCN bending vibrations mixed with out of phase N–H bending [39].

The absorption bands in the amide V region (640–800), they are caused by out-of-plane NH bending, while the absorption bands in the amide VI region (537–606) are caused by out-of-plane C=O bending [38]. The FSD spectra show additional absorption bands in (900–1300) cm⁻¹ range which is assigned to C–O bending vibrations of saccharides (glucose, lactose, and glycerol) [40]. The absorption bands in the region (1360–1430) cm⁻¹ are caused by vibrations of certain amino acid chains and the absorption bands in the region (1430–1480) cm⁻¹ are caused by fatty acids, phospholipids and triglycerides [39]. All major observed peaks of the absorption bands before and after exposure to magnetic fields in the range (400–1700) cm⁻¹ are assigned in table 1 based on several previous studies [41, 42].

The spectra in figure 5 show inconsistent fluctuation in the range of (450–675) cm⁻¹, where peak positions and the shapes of bands continued to change at different magnetic fields indicating high sensitivity to the effect of the magnetic field.
Figure 3. FSD absorption spectra of CREB exposed to magnetic field at different magnitude (0.0, 0.45, 0.9, 1.35, 1.9 and 2.4) mT arranged in order from bottom to top.

Figure 4. FSD absorption spectra of CREB exposed to magnetic field at different magnitude (0.0, 0.45, 0.9, 1.35, 1.9 and 2.4) mT arranged in order from bottom to top.
The net effect of exposing CREB to a 50 Hz oscillating magnetic field of 2.4 mT is shown in figure 6 by taking the difference spectrum: {FSD spectrum of CREB exposed to the magnetic field — the FSD spectrum of CREB}.

Figure 6 reveals the affected absorption peaks which are relevant to changes in the secondary structure of CREB protein. All major changes in the range (400–1700) cm⁻¹ are listed in table 1, where peak positions and band intensities have been affected.

The peaks of the difference spectrum in figure 6(c) above the zero line represent an increase of intensity while the inverted peaks represent a decrease in intensity. Most peaks in the range (425–675) cm⁻¹ have shown a decrease in their intensity, and in some cases were also accompanied by a little shift in positions.

Figure 7 shows the difference spectra between the original FSD spectra of CREB before being exposed to a magnetic field and to zero magnetic field exposure after a cycle of exposure to different magnetic fields: (0.0, 0.45, 1.4, 2.4, 1.4, 0.45, 0.0) mT. The difference spectrum shows unexpected fluctuations indicating a remnant effect due to previous magnetic field exposure.

It is interesting to note that amide II, amide IV and amide VI have shown sensitivity to magnetic field exposure, and all these bands are caused by bending vibrations in combinations with other bonds. One may speculate that the weak bending vibrations leave molecules more susceptible to be twisted by the magnetic field.

### Table 1. Band assignment (±0.125 cm⁻¹) before and after CREB exposure to ELF-MG.

| Region in cm⁻¹ | Band position at 0.0 mT | Band position at 2.40 mT | Changes in band intensities | Difference spectra peaks | Difference spectra inverted peaks |
|----------------|-------------------------|--------------------------|-----------------------------|--------------------------|---------------------------------|
| 427            | 427                     | decreased                |                             | 422                      |                                 |
| 447            | 450                     | increased                |                             | 438                      |                                 |
| 464            | 465                     | decreased                |                             | 463                      |                                 |
| 488            | —                       | disappeared              |                             | 484                      |                                 |
| 505            | 497                     | increased                |                             | 506                      |                                 |
| 526            | 517                     | decreased                |                             | 524                      |                                 |
| Amide IV       | 549                     | 546                      | decreased                  | 550                      |                                 |
| 586            | 587                     | decreased                |                             | 585                      |                                 |
| 599            | 600                     | decreased                |                             | 599                      |                                 |
| Amide V & VI   | 624                     | 624                      | decreased                  | 637                      | 625                             |
| 650            | 650                     | decreased                |                             | 649                      |                                 |
| 680            | 680                     | no change                |                             |                          |                                 |
| 699            | 699                     | no change                |                             |                          |                                 |
| 715            | 715                     | no change                |                             |                          |                                 |
| 759            | 759                     | no change                |                             |                          |                                 |
| 787            | 787                     | no change                |                             |                          |                                 |
| 900–1200       | no change in peak positions or intensities |
| Amide III      | 1234                    | 1234                     | no change                  |                          |                                 |
| 1283           | 1284                    | no change                |                             | 1295                     |                                 |
| 1320           | 1322                    | no change                |                             | 1317                     |                                 |
| 1338           | 1338                    | decreased                |                             | 1339                     |                                 |
| 1357           | 1356                    | no change                |                             | 1358                     |                                 |
| 1360–1430      |                         |                         |                             |                          |                                 |
| 1379           | 1380                    | no change                |                             | 1378                     |                                 |
| 1401           | 1401                    | no change                |                             | 1396                     |                                 |
| 1418           | 1418                    | no change                |                             | 1416                     |                                 |
| 1430–1480      | no change in peak positions or intensities |
| Amide II       | 1489                    | 1489                     | decreased                  | 1483                     |                                 |
| 1521           | 1521                    | no change                |                             | 1507                     |                                 |
| 1538           | 1538                    | decreased                |                             | 1539                     |                                 |
| 1563           | 1563                    | decreased                |                             | 1561                     |                                 |
| 1583           | 1583                    | decreased                |                             | 1580                     |                                 |
| Amide I        | 1600                    | 1600                     | decreased                  | 1596                     |                                 |
| 1617           | 1618                    | decreased                |                             | 1629                     |                                 |
| 1633           | 1633                    | decreased                |                             | 1650                     |                                 |
| 1650           | 1651                    | no change                |                             | 1663                     |                                 |
| 1663           | 1662                    | decreased                |                             | 1678                     |                                 |
| 1678           | 1679                    | decreased                |                             | 1696                     |                                 |
| 1696           | 1696                    | decreased                |                             | 1685                     | 1675                             |
4. Discussions

The qualitative analysis of the secondary structure for CREB protein is based on the observed changes in peak position, band intensity and bandwidth for the individual bands. It is widely accepted that vibrational spectroscopy is highly sensitive and can detect any small change (0.02%) in bond strength \[43\]. Changes in bands’ positions are caused by a shift in the frequency of the absorbed energy due to changes in the original bond vibrations of the absorption band. Some other causes can induce a shift in the frequency of vibrations. For instance, protein aggregation induces a shift to lower frequency in the secondary structure \[44\]. The intensity of an absorption band is proportional to the number of its vibrational bonds. The intensity of the vibration band can also be induced by an increase in the population of the components that give rise to that vibration. Furthermore, an increase in the intensity of a vibration band can be induced also by an increase in the dipole moment of the macromolecules due to their alignment to the applied field \[45\]. Any variation in the number of bonds is directly linked to changes in the secondary structure of the involved protein.

Changes in the FSD convoluted spectra in figures 3 and 4 indicate secondary structure changes as a result of CREB exposure to different magnetic fields. These changes are evident in most of the bands in the range of \((425–675)\) \(\text{cm}^{-1}\). The changes are characterized by inconsistent fluctuations in peak positions, bands shape, and intensities as can be seen in the following bands \((427, 447, 464, 488, 505, 526, 549, 586, 599, 625\) and \(651\) \(\text{cm}^{-1}\). The other bands in the range of \((680–1800)\) \(\text{cm}^{-1}\) have shown little or no change in peak positions with little decrease in intensity of the absorption bands in the amide II region.

The following bands have shown a shift in their peak positions due to magnetic field exposure in the range \((0.0 \rightarrow 2.4)\) mT as follows: \((505 \rightarrow 497)\) \(\text{cm}^{-1}\), \((526 \rightarrow 517)\) \(\text{cm}^{-1}\) and \((549 \rightarrow 546)\) \(\text{cm}^{-1}\). These peaks have shifted to lower energies, which indicates a decrease in the molecular vibration due to a weaker strain on the vibrating molecules. Such as been specified above, proteins aggregation induces a shift to a lower frequency in the secondary structure \[46, 47\]. This peak shift can also be supported by arguing that out of plane bending in combination with other molecular vibrations leave molecules more susceptible to be twisted by the magnetic field. Twisting of the molecular bonds can increase or decrease the stiffness of the vibration constant leading to an increase or decrease in the bond energy. Other experimental investigations suggest that a hydrogen bond to
Figure 6. Spectrum (A): obtained for CREB exposed to 2.4 mT magnetic field. Spectrum (B): obtained for CREB at 0.0 mT field. Spectrum C: shows the difference spectrum of (A-B).

Figure 7. Spectrum A: obtained for CREB before exposure to any magnetic field. Spectrum B: obtained for CREB after been exposed to magnetic field in steps and returns to 0.0 field exposure. Spectrum C shows the difference spectrum of (A-B).
the C–O group can lower the band’s frequency by 20 to 30 cm\(^{-1}\) while a hydrogen bond to the N–H group can lower it by (10–20) cm\(^{-1}\) [48]. Generally, it is easier to bend than stretch, so bending vibrations are of lower energy than stretching vibrations for the same bond. Therefore, absorptions due to bending tend to occur at lower wavenumber than that of stretches.

Our measurements showed a large loss of intensity to most of the bands in the amide IV, amide VI regions, in addition to a small decrease of intensity to some bands in the amide II region. The loss of intensity implies that exposure to the magnetic field induces a damping effect on the amplitude of oscillation which alters molecular vibrations at the bonding sites. In general, the exposure of a protein to a magnetic field may induce forces that can influence the vibration of charged particles. For example Lorentz force \((F = qv \times B)\), and the magnetic gradient force \((F = (pm \nabla) B)\), where \(q\) and \(v\) are the charges and the velocity of the particle, \(B\) is the magnetic induction, and \(pm\) is the dipole magnetic moment [49]. The quenching effect on the spectra of amide IV and amide VI was stronger than the quenching effect on the other regions. One may postulate that the magnetic field has a larger damping effect on out of plane bending vibrations than that of other vibrations.

The spectra for the range (1000–1800) cm\(^{-1}\) shows no variations for the whole range of different magnetic fields as shown in figure 4. While, distinct variations in the spectra are shown in figures 3 and 5 in the range (450–675) cm\(^{-1}\), where some peaks disappeared and others showed a change in their intensity. Even repeated exposures to the same magnetic fields yielded a change in the spectra as shown in figure 5 for (0.0, 0.45, and 1.4) mT cases. The changes in the spectra for this range showed hysteresis behavior in CREB structure when exposed to a magnetic field. The spectra kept on changing while the magnetic field is changed through a complete cycle. It means CREB response depends not only on the current state but also upon its past exposure history. Hysteresis behavior in different proteins has been reported earlier by different researchers [33, 34, and 47]. Hysteresis in the complex system of CREB may arise from a bifurcation in the energy landscape due to functional regions on the protein’s surface, or actual three-dimensional domains which add complexity to folding landscapes [50]. The applied magnetic field can impose twisting on molecular vibrations in the protein. Furthermore, if the field is turned off the vibrational bonds do not return to their original state. Hysteresis is most likely to be a consequence of the untwisting of the bonds, where the protein shows less elasticity. These changes in the spectra reveal that the magnetic field causes changes in the three-dimensional shapes of protein structure.

On the other hand, the magnetic field has little or no interference with the in-phase combinations of N–H bending and C–N stretching in the amide III region leaving this region with minor changes in its bands’ intensities.

Figure 6 shows a minor position shift for the listed peaks in table 1: (427 \(\rightarrow\) 422, 447 \(\rightarrow\) 438, 464 \(\rightarrow\) 463, 488 \(\rightarrow\) 484, 505 \(\rightarrow\) 506, 526 \(\rightarrow\) 524, 1563 \(\rightarrow\) 1561, 1583 \(\rightarrow\) 1580, 1598 \(\rightarrow\) 1596, 1633 \(\rightarrow\) 1629, 1678 \(\rightarrow\) 1675) cm\(^{-1}\). In addition to a strong magnetic field effect in decreasing bands’ intensities occurring at (585, 599, 625 and 649) cm\(^{-1}\) and a small increase in band intensities at the following bands (1507, 1550 and 1569) cm\(^{-1}\).

The peaks in figure 6(c), represent the gain of intensities due to the magnetic field effect, which is caused by constructive interference with the absorbed frequency at the peaks’ positions. While the inverted peaks represent a loss of intensity due to the magnetic field caused by a destructive interference with the absorbed frequency at their positions. If the difference spectrum is a straight line, it indicates no contribution from the magnetic field implying no interference or energy exchange took place.

Exposing vibrating molecules to the magnetic field forces the vibrating molecules to be aligned in the direction of the applied magnetic field. The resulting torque provokes a twisting effect on the vibrating molecules which leads to a change in the vibration of the involved bonds. The change of the vibration yields a shift in the absorption frequency of the involved bands.

The difference spectrum in figure 7(c) shows the net changes in CREB protein due to increasing the magnetic field in increments from 0.0 mT to 2.4 mT and then reducing it back to 0.0 mT. Small fluctuations are observed all over the spectrum with all peaks and inverted peaks are listed in table 2. The largest inverted peak which indicates a major drop of intensity is located at 564 cm\(^{-1}\) in the amide VI region. The difference spectrum in figure 7(c) shows that once the CREB protein was exposed to a magnetic field, it induces a genuine interaction changing its secondary structure which also yields momentarily changes in the tertiary structure of the protein. This is also supported by the inconsistent changes in the spectra of figures 3–5 due to the successive changes in the magnetic field.

The exposure of CREB protein to the ELF-magnetic field showed internal energy changes in the molecular structure of the protein. A magnetic field can cause charge motion and induces molecule polarization within the exposed protein. According to Lenz’s law, charge motion can generate a new magnetic field to counter the effect of the original magnetic field. The primary magnetic fields and their induced opposing magnetic fields perpetuate energy oscillation within the protein causing time delay for a full recovery and the return to the original state.

The results of this study lead to the following suggestive hypothesis, but it needs to be confirmed by biochemical studies. The effects of the electromagnetic field on brain proteins are accumulative and evolutionary which allows for the uniqueness of each brain. Each time a neuron signal is emitted due to the observation of a certain object, it may be a little different from the previous signal emitted in observing the same object. This requires repeated modification and updating of memory imprints with the new changes which keep
the whole process relatively dynamic. Each one of these signals produces its magnetic field which in turn induces a unique three-dimensional structural change in the molecular structure of the protein. Keeping in mind, that each set of these changes corresponds to a specific interaction by the involved brain.

The instantaneous acts of sensing followed by reacting require fast mechanisms of interaction similar to that provided by a magnetic field. Staying alert and maintaining an organized dynamic memory demands a state of continuous interaction with an existing field.

Further investigations incorporating different experimental techniques in protein dynamics and ELF-magnetic effects on other important brain proteins are needed to help us better understand brain activity and function.

5. Conclusions

The experimental results showed that CREB is highly sensitive to ELF magnetic field exposure and the following changes have been observed: (1) Peak positions’ shifts and intensity changes were highly noticeable for the bands of amide IV, amide VI and amide II (2). The loss of intensity implies that CREB exposure to magnetic field introduces a damping factor on the amplitude of oscillation which alters molecular vibrations at the bonding sites (3). No major changes in the amide III and amide I region have been observed (4). The conformational changes of the protein are formed by a spontaneous chain of interactions with the magnetic field, which leads to internal energy changes in the protein (5). Hysteresis like behavior in CREB protein suggests a possible role in the learning and memory processes in the brain.

Table 2. Peak positions (±0.125 cm⁻¹) of the difference spectrum between CREB before exposure (0.0 mT) and after an ascending and descending cycle of exposure to the following magnetic fields (0.0, 0.45, 1.4, 2.4, 1.4, 0.45, and 0.0 mT).

| Region cm⁻¹ | Peak positions before exposure (0.0 mT) | Peak positions after exposure (0.0 mT) | Difference spectra peaks | Difference spectra inverted peaks |
|-------------|----------------------------------------|--------------------------------------|--------------------------|---------------------------------|
| 427         | 427                                    | 420                                  |                          | 431                             |
| 447         | 450                                    |                                      |                          |                                 |
| 464         | 464                                    | 442                                  |                          |                                 |
| 488         |                                        | 461                                  |                          |                                 |
| 503         | 497                                    | 482                                  |                          |                                 |
| 526         | 517                                    | 525                                  |                          |                                 |
| 549         | 547                                    | 545                                  |                          |                                 |
| 586         | 587                                    |                                      |                          | 564                             |
| 599         | 600                                    | 685                                  | 603                      | 623                             |
| Amide IV 537–606 | 625 | 624 | 637 | | |
| 650         | 651                                    | 647                                  |                          |                                 |
| 679         | 680                                    |                                      |                          |                                 |
| 698         | 699                                    |                                      |                          |                                 |
| 715         | 715                                    |                                      |                          |                                 |
| 786         | 786                                    |                                      |                          |                                 |
| 800–1220    | No change in peak position              | 1018                                 |                          |                                 |
| Amide III 1220–1320 | No change in peak position | | 1317 | |
| 1330–1480   | No change in peak position              |                                      |                          |                                 |
| Amide I 1480–1600 & II 1600–1700 | No Change in peak position | | 1452 | |

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