UNIVERSITY OF ILLINOIS

MAY 1989

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED EFFECTS OF THE PULSED RADIO FREQUENCY USED IN MAGNETIC RESONANCE IMAGING ON THE CORNEAL ENDOTHELIUM OF HUMANS

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF BACHELOR OF SCIENCE IN CHEMISTRY

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0-1364

Effects of the Pulsed Radio Frequency Used in Magnetic Resonance Imaging on the Corneal Endothelium of Humans

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Thesis

for the

Degree of Bachelor of Science

in

Chemistry

College of Liberal Arts and Sciences

University of Illinois

Urbana, Illinois

1989

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Chapter One: Magnetic Resonance Imaging (MRI)

A) Medical Uses Of MRI

Magnetic Resonance Imaging (MRI) is a non-invasive medical diagnostic technique capable of producing spatial images of various tissues and monitoring dynamic processes occurring within them. Clear images of many tissues, including the brain, spinal cord, liver, and inner ear have been obtained using MRI. The primary use of MRI is the detection of malignant tumors which give MRI signals different from those by benign tumors or normal tissues.

MRI can also be used to detect bone fractures, and it is safer than using x-rays. To obtain x-ray images, high-energy x-ray radiation is used. Like ultraviolet radiation which has been linked to skin cancer, x-ray radiation is capable of producing harmful mutations by altering chromosomes. In contrast to the ionizing ability of x-rays, MRI uses only magnetic field gradients and pulsed radiofrequency (RF) energy to produce an image, so no chromosomes are damaged by MRI. In fact, there are no known deleterous effects of any kind associated with MRI.

The harmful, mutagenic effect of x-rays has led, in part, to the replacement of other x-ray techniques, such as computed tomography (CT) and angiography, by MRI. In CT, the x-ray attenuation by each tissue voxel is used

to generate an image. Although CT is still a common technique used to image the brain, MRI often produces brain images superior to those generated by CT. In addition, MRI is much more versatile than CT. CT can only measures x-ray absorption by tissue, whereas MRI can be used to measure several properties of tissue, including the concentration of some elements, characteristic relaxation times T_1 and T_2, and blood flow parameters.

The ability of MRI to monitor dynamic processes, such as blood flow, provides a safe, non-invasive method to assess heart damage after a heart attack or myocardial infarction. Since blood does not flow to dead tissue, monitoring the blood flow in the heart can be used to determine the size and location of damaged tissue. Angiography, a conventional method of monitoring blood flow in the heart, requires the injection of a contrast agent into blood vessels in the heart which are then observed with x-rays. This procedure is both invasive and potentially mutagenic, so the use of MRI to monitor blood flow is much safer.

B) THE PHYSICS OF MRI

Although Magnetic Resonance Imaging and Nuclear Magnetic Resonance (NMR) imaging are equivalent terms, physicians prefer to use the shorter term, MRI, because many people have a negative impression of the word nuclear. They associate it with nuclear weapons, wherein nuclear refers to the fission or the fusion of nuclei to release large amounts of energy. However, as it is used in NMR, nuclear has a completely different meaning. It

is used to stress that NMR is based on the phenomenon of "spinning nuclei." Some nuclei, such as ^{1}H, ^{13}C, ^{19}F, and ^{31}P possess a property, called nuclear spin, which causes them to behave as if they were spinning. Since the hydrogen nucleus (^{1}H) or proton (H^{+}) is used most frequently in MRI, the discussion of MRI will mainly be focused on the magnetic properties of the proton.

Although MRI is a complex technique, it can be viewed as consisting of the following four main steps:

1) Apply a magnetic field (B_o) to magnetize the tissue in the direction of the field. This magnetization allows the tissue to act as a radiofrequency (RF) receiver.

2) Apply a RF signal to rotate the magnetization away from B_o. This step excites the protons and allows them to emit a NMR signal.

3) Measure the NMR signals (data acquisition).

4) Image reconstruction using a Two-dimensional Fourier Transform.

1. Magnetize Area of Interest

Because the proton is a charged particle, its spin gives rise to a magnetic field (or moment) identical to that of a tiny bar magnet. The vector sum of the magnetic fields produced by the protons in the sample is called the net magnetization (M). In the absence of an external magnetic field, the protons have random orientations, so the net magnetization is zero. According to quantum mechanics, however, a proton in a magnetic field can be in only one

of two possible nuclear spin states (orientations). In the more stable (lower energy) nuclear spin state the proton's magnet field is "parallel" to the field, and in the less favorable (higher energy) nuclear spin state its magnetic field is "antiparallel" to the field.

Instead of aligning parallel or antiparallel to the magnetic field, the proton's angular momentum causes it to precess about the field. The frequency of precession is called the Larmor frequency, and it is equal to $\gamma B_0/2\pi$, where γ is the magnetogyric ratio for the proton (magnetic moment / angular momentum), B_0 is the magnetic field strength at the proton, and π is equal to pi. Each spinning nucleus has a characteristic γ value; the proton's magnetogyric ratio equals $2\pi(42.576)$MHz/ tesla. The significance of the Larmor frequency is that it is equal to the energy difference (in frequency units) between the two nuclear spin states predicted by quantum mechanics. Therefore, in a typical MRI field strength of 0.5 tesla, the energy difference between the two possible proton orientations is equal to 21.29 MHZ, and each proton precesses about the field at 21.29 MHz.

Substituting this radiofrequency (RF) energy difference of 21.29 MHz into the Boltzmann Distribution Law shows that the number of protons in an orientation "parallel" to the field exceeds the number of protons "antiparallel" to the field by only a few parts per million. Nevertheless, this slight excess of protons oriented "parallel" to the field is necessary for the NMR process to occur, and on a macroscopic scale this uneven distribution of proton spins produces a net magnetization parallel to the field, called the longitudinal magnetization (M_Z). Although all the protons in a homogeneous magnetic

field precess at the same frequency, their phasing is random, so the net magnetization perpendicular to B_O (the transverse magnetization, M_X) is zero.

2. *Application of RF pulses to Rotate Magnetization*

After a magnetic field has longitudinally magnetized the region of interest, the correct amount of energy must be resonantly added before a NMR signal can be produced. If an oscillating magnetic field (B_1) applied perpendicular to the main field (B_O) is oscillating at the Larmor frequency, then it can be resonantly absorbed. This absorption of RF energy excites some of the protons by causing them to flip from a parallel to an antiparallel configuration. The small magnetic field (B_1) produces a torque on the protons which causes them to precess about it, while simultaneously precessing about the main field.

At the macroscopic level, the effect of the RF pulse is to rotate the magnetization (M) away from B_O. The angle through which a RF pulse rotates M is given by the product $\gamma(B_1)t$, where $B_1$is the magnetic field strength of the RF pulse, t is the duration of the pulse, and g is the magnetogyric ratio for the proton. Two commonly used RF pulses are the 90^o pulse and the 180^o pulse, which rotate M by 90^o and 180^o, respectively. A 90^o pulse is useful because it produces a transverse magnetization, whose precessional motion may be used to induce a voltage (the NMR signal) in a pick-up coil.

Immediately following a 90^o RF pulse, the transverse magnetization is at a maximum and the longitudinal magnetization is zero. However, the

transverse magnetization rapidly decays, while the longitudinal magnetization is growing at a slower rate. The magnetic field strength at each proton is not identical due to inhomogeneities in B_o and B_1, different magnetic environments (chemical shifts), and spin-spin exchange processes. These magnetic inhomogeneities cause the protons to precess at different rates, so that after an appropriate amount of time the nuclear spins are spread out, or dephased, over the entire xy plane. This process of dephasing is often called spin-spin or transverse relaxation because it is caused by the interaction of spinning particles.

On the macroscopic level, spin-spin relaxation causes the transverse magnetization to oscillate, resulting in a transient NMR signal, called the free induction decay (FID), that will decay toward zero with a characteristic time constant T_2^*. If the inhomogeneities in B_1 and B_0 are removed the time constant will depend only on tissue properties and it is called T_2. The significance of T_2^* is that it is the time required for 63% of the initial transverse magnetization to decay. This dephasing of spins in the xy plane due to magnetic inhomogeneities can be reversed by applying a 180^o pulse. The 180^o pulse reverses the relative positions of the spins so that the faster spins are placed behind the slower ones. The spins will begin to rephase as the faster spins catch up to the slower spins. This rephasing causes the transverse magnetization to grow. However, once the faster spins pass the slower ones, the spins will dephase again and the transverse magnetization will decay. This growth and decay of the transverse magnetization following a 180^o pulse is called the spin echo.

While the dephasing of spins in the xy plane is occurring, the protons in an "antiparallel" orientation begin to flip back to the more stable, "parallel" orientation. This type of spin relaxation is called spin-lattice or longitudinal relaxation, because it is caused by the interaction of protons with their surroundings, or lattice. As the protons re-align "parallel" to the field, the longitudinal magnetization grows exponentially with a time constant T_1.The spin-lattice time constant (T_1) is the time required for the longitudinal magnetization to reach 63% of its initial value. Since the longitudinal magnetization is in the same plane as the pick-up coil, it induces no voltage and is therefore not detectable.

Both the spin-spin (T_2) and spin-lattice (T_1) relaxation times depend on the tissue being studied. However, for a given tissue $T_1 >> T_2$, and a tissue with a long T_1 usually has a long T_2. Typically, spin-lattice relaxation times are on the order of a few seconds, whereas spin-spin relaxation times are on the order of a few tenths of a second. This comparison of typical T_1 and T_2 values shows that the transverse magnetization decays much faster than the longitudinal magnetization grows.

3. Measure NMR Signals

During the next step, which is data acquisition, the NMR signal (FID or spin echo) is measured. The intensity of the NMR signal depends on the RF pulse sequence used to excite the tissue. Two common pulse sequences are inversion recovery and spin echo.

The inversion recovery technique involves applying a 180° pulse followed after a time interval (TI) by a 90° pulse. The 180° pulse reverses the longitudinal magnetization so that it is antiparallel to the main field. Then during the time interval (TI), the longitudinal magnetization undergoes exponential growth characterized by the spin-lattice time constant T_1. Next, the 90° pulse rotates the longitudinal magnetization that has grown during TI into transverse magnetization. The magnitude of the transverse magnetization and the strength of the NMR signal depend on the ratio T_1/ TI. Since $3T_1$ is the time required for the longitudinal magnetization to reach 95% of its original value, a TI much less than $3T_1$ would produce a weak NMR signal.

In the spin echo technique, first a 90° pulse is applied followed after a time TE/2 by a 180° pulse. As previously discussed, this pulse sequence produces a spin echo at a time TE after the 90° pulse. The 180° pulse may be repeated several times with each one producing a spin echo. The strength of the NMR signal depends on the ratios TE/T_2^* and TR/T_1, where TR is the repetition time or the time between the first pulse of a cycle and the first pulse of the following cycle. The relationship between these ratios and the intensity of the NMR signal will be discussed later.

Before an image can be generated, the origin of each NMR signal must be encoded into the signal. This encoding of position into the NMR signal can be accomplished by superimposing magnetic field gradients (G) onto the main magnetic field (B_o). For example, a field gradient (G_z) applied in the z direction (parallel to B_o) produces a magnetic field, $B_o + zG_z$, that varies

linearly along the z axis. This gradient allows the z coordinate of a proton to determine the magnetic field it experiences.

As shown earlier, the RF absorbed by a proton is proportional to the magnetic field strength, and the RF absorbed is also equal to the frequency of the NMR signal that the proton emits as it relaxes. Since the frequency of the NMR signal depends on the magnetic field from where it originates and this field is determined by the z coordinate, the position of the NMR signal is encoded into the signal as its frequency. Therefore, the gradients allow frequency encoding of position.

To encode the x and y coordinate of the signal, gradients along the x and y axes are introduced. Applying both gradients simultaneously would merely produce another gradient that is the sum of the two, so no image can be created. However, by switching the gradients on and off at various times, an image may be produced.

Typically, the z gradient is turned on when the RF pulses are delivered, so that a thin slice through the body is selectively excited. Next, the x and y gradients are used to divide this slice into tissues voxels (volume elements). The x gradient is turned on between RF pulses, so that the signal from each tissue voxel having a different x coordinate has a different frequency. Because the x gradient frequency encodes position, it is often called the frequency encoding gradient.

The y, or phase encoding, gradient affects the amplitude of the signal that arises from each column, because it alters the phasing between the

protons in the xy plane. The net effect of the three gradients is to produce a single NMR signal consisting of many frequencies, with each frequency corresponding to a different position along the x axis. So far, the data generated is called a view. To create an image many views must be collected, usually 256 views. The only difference between views is the strength of the y gradient.

4. Image Reconstruction

Since the y gradient is changed 256 times during data acquisition, there are 256 different NMR signals produced by the tissue slice. These signals are received by a computer which performs a two dimensional fourier transform on the data to generate an image. The image consists of 256 pixels with each one corresponding to a different voxel in the tissue slice.

The fourier transform (FT) is a mathematical procedure to separate out the frequency components of a signal from its amplitudes as a function of time, and vice versa. Thus, the FT changes each NMR signal (amplitude vs. time) from a time domain to a frequency domain (amplitude vs. frequency). The first FT is performed on each of the 256 signals, and the resulting amplitude vs. frequency data from each signal is stored in the memory of a computer.

After a FT has been performed on each of the 256 NMR signals, a second FT, called an inverse FT, is performed on the data stored in the computer memory. The inverse FT rebuilds the original signal of the tissue slice by

adding together its frequency (position) components weighted by their amplitudes. Therefore, an image mapping signal amplitude (brightness) versus position is created.

As noted previously, the amplitude of the signal from each tissue voxel determines the brightness of its corresponding image pixel, so any factor altering the signal amplitude from each voxel affects the image contrast. The tissue properties affecting the amplitude of a NMR signal are the proton concentration and the characteristic time constants (T_1 and T_2). However, the extent to which each property affects the amplitude is controlled by the operator through the pulse sequence parameters (TI, TR, and TE).

For spin echo images the pulse parameters TE and TR control the contribution of each tissue property to the image's contrast. The TR length affects the contribution of T_1 differences between tissue voxels to the image contrast. If TR is long enough for the longitudinal magnetization of each voxel to reach its initial value as determined by its proton concentration, then the amplitude of the FID from each voxel would be independent of its T_1 value and proportional to its proton concentration. A short TR will have the opposite effect; the amplitude of the FID from each voxel will depend primarily on its T_1 value.

The TE length influences the contribution of T_2 differences to the image contrast. A short TE does not allow the transverse magnetization of any voxel to decay significantly, so T_2 differences between voxels do not contribute to image contrast. A long TE allows the amplitude of the NMR signal from

each voxel to be affected by its T_2 value. By altering TR and TE, the operator controls the relative contribution of each tissue property to the image. The image is said to be weighted by the main tissue property affecting the the NMR amplitude. For instance, the contrast of a T_1-weighted image is determined by the differential T_1 distribution between tissue voxels, and it can be generated by selecting a short TR and a short TE. Likewise, a T_2-weighted image can be produced by a long TR and a long TE, and a proton concentration-weighted image is created by a long TR and a short TE.

Chapter Two: Summary of Adey's Studies on the Interaction of Electromagnetic Radiation with Neural Tissue

A) BACKGROUND INFORMATION

Prior to the 1970's, most research on the biologic effects of nonionizing electromagnetic radiation (EMR) focused on the gross thermal effects caused by the absorption of EMR. Thermal effects are those cellular responses that are caused by an increase in tissue temperature. In general, thermal effects are independent of the mode of heating, so in many of these early studies the researchers neglected to record the wave parameters of the EMR used to heat the tissues.

In the early 1970's, researchers began to explore the possibility of non-thermal effects by exposing tissue to weak (less than 100 mW/ cm^2) EM fields that did not cause an increase in tissue temperature. Unlike thermal effects, the non-thermal effects of EMR depend on its wave parameters. These athermal studies clearly show that weak, nonionizing electromagnetic (EM) fields can produce a variety of responses in organisms without causing significant heating of tissue (less than 0.1 degree Celsius). For instance, rays and migrating birds use the earth's weak magnetic field (0.00003 tesla) as an aid in navigation; rays can also sense the bioelectric fields produced by their prey [Ref. 1].

Non-thermal effects are also exhibited by the nervous system of several species. The nervous system's sensitivity to weak oscillating EM fields has

been shown by studies on the biorhythms of birds and monkeys, the estimation of time passage by monkeys, and the electroencephalogram (EEG) patterns of monkeys and cats. The circadian rhythm in birds and humans is lengthened when they are shielded from environmental EM fields. In both species, the imposition of a 10 Hz, 2.5 V / m EM field restored the circadian rhythm close to its normal length of 24 hours [Ref. 2]. Clearly, both the environmental and the 10 Hz EM fields contain some essential cue, interpreted by the brain, for the maintenance of normal biorhythms.

A series of experiments conducted in Adey's laboratory have shown that weak oscillating EM fields can alter the subjective time estimates of monkeys [Ref. 2]. Only within a certain frequency and intensity range were the EM fields effective in altering time estimates, with the maximal change in time estimates occurring when the monkeys were exposed to a 7 Hz Em field of 56 V / m.

The EEG is produced by the leakage of waves from neurons into the fluid surrounding them, and the rhythmic EEG patterns are generated by the extracellular summation of these waves. Whether EEG waves are brain signals or merely the noise of the brain's activity is unknown. However, several studies suggest that the EEG waves contain information and are functional as brain signals.

In various experiments, the EEG and behavioral responses are strongly correlated, so that certain EEG patterns may be used to predict specific behavioral responses [Ref. 3]. Furthermore, electrical communication within the central nervous system (CNS) has already been shown to occur; some neurons in the mammalian spinal cord use extracellular electrical signals to

interact at a distance [Ref. 4]. Thus, at least some neurons are capable of electrical communication by sensing the electric gradient in their vicinity.

Additional evidence that EEG waves are brain signals comes from the studies of EMR-CNS interaction cited above. In each case, the oscillating EM fields that altered behavioral responses had a frequency or an amplitude-modulation frequency near signature EEG frequencies (5-25 Hz.), whereas modulation frequencies or field frequencies far from the EEG frequency did not produce any behavioral responses. In addition, the effective field strengths were close to naturally occurring EEG-level electric gradients.

Assuming EEG waves are brain signals, external oscillating EM fields (at EEG frequencies or amplitude-modulated at brain wave frequencies) can alter neuronal activity by two means. First, the external fields can summate with the EEG waves to change the coded signal before the waves are decoded by the neurons. The second possibility is that the external EM fields contain information that is directly decoded by neurons without prior summation with EEG waves. In both situations, the effect of the oscillating EM fields is to alter the electric gradient in the vicinity of neurons so the coded information is scrambled and altered neuronal responses produced.

Although the precise mechanism by which EM fields produce neuronal responses is not known, the neuronal membrane is known to play a crucial role in the transduction of EM fields. The resistance of the neuronal membrane is greater than the resistance of the extracellular fluid, so the preferred pathway for EM fields is the extracellular fluid. Consequently, the extracellular EM signals must cross the membrane in order to alter intracellular metabolic activity.

On the macroscopic level, there are two methods for the extracellular EM fields to produce intracellular changes. In the first method the EM fields directly cross the neuronal membrane and the attenuated intracellular waves interact with intracellular structures to alter activity. The other possibility is for the EM field to interact with the neuronal membrane to trigger a transmembrane signal which in turn alters neuronal activity. Since the second mechanism parallels other types of cellular excitation, such as neural excitation with hormones or LSD, most studies have searched for the transmembrane messenger responsible for the transduction of EM fields.

The most probable transmembrane messenger is the calcium ion. It is known to mediate several transmembrane signals, including hormone release, neurotransmitter release, and protein kinase activation. Calcium ions compete with protons for binding sites formed by the acidic terminals of glycoproteins attached to the extracellular surface of the membrane. Bass and Moore have developed a model of excitability and transductive coupling through the interaction of protons and calcium ions on the cell-surface glycoproteins that may be the mechanism by which the neuronal membrane transduces EM fields [Ref. 2].

B) Adey's Studies on the Effects of ELF Fields and Modulated RF Fields on the Calcium Ion Efflux From Neural Tissue

Since the calcium ion is intimately involved in many neuronal responses, Ross Adey chose to assess the effects of field interactions with brain tissue by

measuring calcium ion fluxes. His first study examined the effects of pulsed electrical stimulation on brain chemistry in the awake cat [Ref. 4]. After the radioactive tracers, $^{45}Ca^{2+}$ and [^{3}H]GABA, were incorporated into the cerebral cortex, agar electrodes were used to directly stimulate the cortex by establishing a relatively uniform voltage gradient of 20-60 mV / cm in the cortex. Adey found that using a square waveform and a stimulus train with 200 pulses per second (duration 1.0 msec) produced about a 20% increase in the efflux of both calcium and the amino acid neurotransmitter gammaaminobutyric acid (GABA). The mean result was a 1.29-fold increase in calcium efflux and a 1.21-fold increase in the efflux of GABA. It was also determined that a square waveform of the applied pulses is essential, because other waveforms were ineffective at changing the efflux of calcium and GABA.

From the results of this experiment Adey drew two main conclusions. First, an amplification mechanism is necessary for such a weak extracellular field to increase GABA release from within the synaptic terminal, because the field was less than one ten thousandth of the 50 mV membrane potential of the synaptic terminal. Since the applied fields are within the range of naturally occurring EEG gradients, Adey believes that the experiment lends support to the suggestion that electrical interaction exists between cortical cells. That is , the electric field generated by activity in one cortical neuron may influence the activity in a nearby cell.

After showing that direct stimulation of the cat's cortex with weak pulsed electric currents triggers calcium release, Adey examined the effects of weak imposed fields on the calcium flux from brain tissue. First he tested the

effects of a weak (1 mW / cm^2), radiofrequency (147 MHz) EM field amplitude-modulated by slow sinusoidal waves on the calcium efflux from the isolated forebrain of neonatal chicks [Ref. 3]. The RF field was amplitude-modulated at various frequencies between 0.5 and 35 Hz. In addition, some of the chick brains were poisoned with cyanide to inhibit metabolism.

The results from this experiment show that the unmodulated RF field and the RF fields modulated at 0.5, 3, and 35 Hz did not cause a significant change in the calcium ion efflux. In contrast, there was a significant increase in the calcium efflux from samples exposed to fields at modulation frequencies between 6 and 20 Hz, with a maximal effect at 16 Hz (18% increase in calcium ion efflux). The samples treated with cyanide showed the same field effects as the untreated brains.

Since the unmodulated RF field did not change the calcium ion efflux, Adey believes that the 147 MHz carrier wave by itself does not alter calcium ion movements. Rather it is the amplitude-modulation frequency that is the critical factor determining calcium ion fluxes. Because the modulation frequency window of 6 - 20 Hz corresponds to naturally occurring brain wave frequencies, Adey suggests that this experiment also supports the hypothesis that electrical interaction exists between neurons. That cyanide treatment did not affect the calcium ion flux strongly suggests that the release of calcium from brain tissue is independent of any ongoing metabolism. This observation indicates that the released calcium came from anionic binding sites on the external surface of the membrane, because the release of intracellular calcium requires metabolic energy. Adey suggests that the modulation of the radiofrequency energy alters the extracellular electric

gradients which in turn disrupt the electrochemical equilibrium that exists in cerebral tissue between Ca^{2+} and its anionic binding sites at the acidic terminals of the membrane glycoproteins. Furthermore, the modulated RF induced displacement of Ca^{2+} at one site may lead to the displacement of calcium from several adjacent sites, thus establishing a cascade reaction capable of propagating and amplifying local electrical events.

Next Adey searched for an electric field strength window over which modulated RF fields are capable of modifying calcium ion effluxes. Using a 450 MHz RF field, amplitude-modulated at 16 Hz, Adey found that only field strengths between 0.1 and 1.0 mW / cm^2 (10-100 mV/ cm) caused a significant increase in the Ca^{2+} efflux from the isolated forebrains of neonatal chicks [Ref. 2].

The significance of this experiment is that the effective field strengths correspond to naturally occurring EEG-level gradients. That both the amplitude-modulation frequencies and the electric field strengths required to produce an increase in the calcium ion efflux correspond to natural EEG frequencies and field strengths strongly supports the hypothesis that the EEG contains information. Because both a 147 MHz and a 450 MHz RF field, amplitude-modulated at 16 Hz, causes an increase in Ca^{2+} efflux, the hypothesis that the Ca^{2+} flux is independent of the carrier wave is supported.

Since the slow sinusoidal modulations of the carrier wave seem to be the critical factor determining calcium ion fluxes, Adey tested the effects of extra-low frequency (ELF) fields (under 100 Hz) on the calcium ion fluxes from isolated cat and chick brains. Although the ELF studies are not directly related to magnetic resonance imaging, which uses pulsed RF fields, the ELF studies

may be used to find the mechanism by which the neuronal membrane demodulates the RF signal.

To test the effects of weak, ELF, sinusoidal electric fields on the calcium ion efflux from nervous tissue, Adey exposed isolated cat and chick cerebral tissues to fields at 1, 6, 16, 32, or 75 Hz with electric field strengths of 5, 10, 56, and 100 V/m [Ref. 5]. The release of preincubated $^{45}Ca^{2+}$ from exposed samples was compared to its release from unexposed (control) samples.

Adey found that the calcium flux from cat and chick cerebral tissues is sensitive to both the frequency and amplitude of the electric field. In contrast to modulated RF fields which increase Ca^{2+} efflux, Adey found that ELF fields decrease the calcium ion efflux under most conditions. Significant decreases (11-15%) in the calcium ion efflux from chick cerebral tissues were induced by fields at 6 and 16 Hz with field strengths of 10 or 56 V/m. For cat cerebral cortexes, only the 56 V/m field at 6 or 16 Hz caused a significant decrease in the Ca^{2+} efflux.

From this data Adey concluded that both frequency and amplitude windows exist for the inhibition of calcium release from cerebral tissue. The threshold amplitude for chick cerebral tissue was 10 V/m, whereas for cat cerebral tissue it was 56V/m. The frequency window for both tissues was between 6 and 16 Hz. Once again, Adey uses the result that the calcium ion flux from cerebral tissue is sensitive to brain wave frequencies as evidence for the hypothesis that cerebral neurons can interact electrically. Adey believes that the ELF-induced inhibition of calcium release can occur by a cooperative mechanism similar to the one by which modulated RF fields stimulate calcium release. For instance, the interaction between a specific site on the

neuronal membrane and the ELF field may trigger conformational changes at adjacent sites which inhibit calcium release .

Several years after Adey's ELF studies, Carl Blackman and associates performed similar experiments, but Blackman's group obtained different results. Blackman exposed isolated chick forebrains to 16 Hz sinusoidal ELF fields at electric field gradients of 1, 2, 3.5, 5, 6, 7.5, 10, 20, 30, 35, 40, 45, 50, 60, or 70 V/m [Ref. 6]. The results indicate that a 16 Hz field within the field strength ranges 5 to 7.5 V/m and 35 to 50 V/m enhances the calcium ion efflux, whereas field strengths 1 to 2, 10 to 30, and 60 to 70 V/m had no effect on the Ca^{2+} efflux.

In contrast to Adey's studies, which showed that ELF fields, at the correct frequency and amplitude, decrease the calcium efflux from brain tissue, Blackman found that ELF fields increase the calcium efflux. Furthermore, Blackman found that a 16 Hz field enhanced the Ca^{2+} efflux over two intensity regions (5-7.5 and 35-50 V/m) separated by a region of no effect (10-30 V/m), whereas Adey's less detailed studies disclosed only one intensity window (10-56 V/m) for decreased calcium ion efflux. A final difference between the results of the two studies was the field strengths at which a 16 Hz field could modify the calcium ion efflux. Adey found that a 10 or 56 V/m field at 16 Hz could alter the calcium ion efflux, but Blackman found no effect on the Ca^{2+} efflux at these two field strengths.

C) REFERENCES

1) Adey, W.R. : Tissue Interactions With Nonionizing Electromagnetic Fields. *Physiological Reviews* vol. 61, No. 2, p. 435-514, (April 1981).

2) Adey, W.R. : Neurophysiologic Effects of Radiofrequency and Microwave Radiation. *Bulletin of the New York Academy of Medicine* vol. 55, No. 11, p. 1079-1093, (December 1979).

3) Bawin, S.M., Kaczmarek, L.K. & Adey, W.R. : Effects of Modulated VHF Fields on the Central Nervous System. *Annals of the New York Academy of Science* vol. 247, p. 74-79, (1975).

4) Kaczmarek, L.K. & Adey, W.R. : Weak Electric Gradients Change Ionic and Transmitter Fluxes in Cortex. *Brain Research* vol. 66, p. 537-540, (1974).

5) Bawin, S.M. & Adey, W. R. : Sensitivity of Calcium Binding in Cerebral Tissue to Weak Environmental Electric Fields Oscillating at Low Frequency. *Proceedings of the National Academy of Science U.S.A.* vol. 73, No. 6, p. 1999-2003, (1976).

6) Blackman, C.F., Benane, S. G., Kinney, L.S., Joines, W.T. & House, D.E. : Effects of ELF Fields on Calcium-ion Efflux from Brain Tissue in Vitro. *Radiation Research* vol. 92, p. 510-520, (1982).

Chapter Three: Safety of Magnetic Resonance Imaging on Corneal Endothelial Health

Background Information

Magnetic Resonance Imaging (MRI) is a relatively new medical diagnostic tool that, among other things, is useful in detecting tumors and blood clots. Unlike x-rays which use ionizing radiation, MRI uses magnetic field gradients and pulsed RF radiation to obtain images. So far no serious side effects have been associated with this procedure; however, studies done on animals have shown that radiowaves may damage the corneal endothelium (Ref. 1).

That radiofrequency (RF) waves may damage the corneal endothelium in rabbits and monkeys was observed in experiments conducted at Johns Hopkins University by Henry Kues and associates (Ref. 1). A single four hour exposure to continuous wave (CW) radiation at 2,450 MHz and a power of 10 or 20 mW / cm^2 caused distinct corneal changes in rabbits. These corneal perturbations were not immediately observable; it took 24 to 48 hours for these changes to be seen.

The same group obtained similar results when the experiment was repeated using cynomolgus monkeys instead of rabbits. CW radiation at 2,450 MHz and 30 mW / cm^2 produced corneal abnormalities in all 7 primates tested. One of them showed corneal changes at a power of 10 mW / cm^2. Once again, there was a 24 to 48 hour latency period before the corneal perturbations were observed.

This group further demonstrated that changing the waveform from a CW mode to a pulse mode increased corneal damage at the same average power. The monkeys were exposed to the same RF (2,450 MHz) but the radiation was pulsed (10 microsecond pulse, 100 pulses per second). The average power of the pulse mode was 10 mW / cm^2 with a peak power of 10,000 mW per cm^2. Using this procedure, 8 out of 11 primates showed corneal abnormalities, whereas with CW radiation at the same power only 1 out of 7 primates exhibited corneal changes.

Preliminary studies by the same group indicate that pilocarpine, a drug used to treat glaucoma, lowers the power threshold for effects in monkeys from 10 mW/cm^2 to 5 mW/cm^2. Since glaucoma patients being treated with pilocarpine may show RF sensitivities at power levels lower than normal, this group would be a good one to test for the effects of the RF pulses used in MRI.

The following three results from Henry Kues' experiments are pertinent to our study of the effect of MRI on the human corneal endothelium:

1) The observed corneal changes occurred below the "thermal" level, indicating that these changes are not attributable to the heating of the cornea.

2) After exposure to the RF radiation, there is a 24 to 48 hour latency period before the corneal endothelial cellular damage can be observed.

3) Pulsed RF radiation is more effective in causing corneal cellular changes than CW RF radiation.

Corneal endothelial cells in humans have two properties which make damage to these cells very critical: they do not regenerate and they keep the cornea properly hydrated and clear. Anatomically, the corneal endothelium is a monolayer of 350,000 to 500,000 hexagonal-shaped cells that separates the cornea from the aqueous humor. These cells can be observed in vivo with a specular endothelial microscope (SEM). This instrument also allows the corneal endothelium to be photographed, so that cell morphology and size can be studied at a later time.

Stressed and abnormal corneal endothelial cells excrete collagen which accumulates on the posterior surface of Descemet's membrane to form mushroom-like excrescences called corneal guttata. These guttata cause the overlying cornea endothelial cells to degenerate and lose metabolic function. Thus, the presence of corneal guttata is a sign of chemical and physical damage to the corneal endothelium.

Since corneal endothelial cells do not reproduce, there must be some mechanism to compensate for this lost metabolic activity and to maintain a continuous barrier between the cornea and the aqueous humor. According to Laing et. al., there are two mechanisms for the repair of corneal endothelial cell damage (Ref. 2). In one process, the damaged cells are shed into the aqueous humor and are replaced by adjacent cells which subsequently enlarge to fill in the defect. In the other mechanism, damaged cells coalesce with adjacent cells to form a single, large multinucleated cell. Both of these repair mechanisms produce larger cells which decreases the cell density. Consequently, the cell density may be used to quantify the damage to the

corneal endothelium. When the cell density falls below 300-500 cells / mm^2, the endothelium cannot keep the cornea properly hydrated, resulting in stromal and epithelial oedema and cloudiness (Ref. 3).

MATERIALS AND METHODS

First, prior to MRI scanning we used a Keeler-Konan Specular Microscope to photograph the corneal endothelium of the patients. Subjects who wear contact lenses were excluded, because contacts may perturb the corneal endothelium. Next, we performed an MR scan on the volunteers using a Picker 0.5 tesla MR scanner with 21.3 MHz RF pulses. Finally, 2 days after the MRI scan we took another photograph of the corneal endothelium.

To assess the damage to the corneal endothelium caused by a single MR scan we compared the number of guttata in the pre-scan photograph with the number in the post-scan photograph. A Bourne-Kaufman, or fixed frame, approximation of cell density was also performed on the pre- and post-scan photographs using a calibrated grid according to the procedure given in reference three.

RESULTS

For all patients, no guttata were observed in either the pre-scan or post-scan photographs. Consequently, if the change in the number of guttata caused by a single MR scan is used as the criteria to assess corneal endothelial

damage, then our experiment indicates that a single MR scan does not damage the corneal endothelium.

The pre-scan and post-scan Bourne-Kaufman estimates of cell densities are summarized in the table on the next page.

TABLE I : Bourne-kaufman Estimations of Cell Densities

Patient number (gender, age)	Pre-scan (cells per mm^2)		Post-scan (cells per mm^2)	
	Left eye	Right eye	Left eye	Right eye
#1) Female, 38	3300	3225	3213	3312
#2) Male, 39	2794	2844	2800	3025
#3) Female, 18	3581	3465	3413	3450
#4) Male, 17	3478	3525	3450	3563
#5) Male, 29	2775	2843	2768	2940

DISCUSSION

No significant conclusions can be drawn from our cell density data. The average change in corneal endothelial cell density following a single MR scan was found to be +10.4 cells / mm^2 with a standard deviation of 98 cells / mm^2. Since no known mechanism causes an increase in corneal endothelial cell density in adults, our result of a positive change in cell density following a MR scan reflects both indeterminate and determinate errors. Two possible

explanations for our findings are the precision of the Bourne-Kaufman approximation of cell densities and the natural variability in cell densities for different areas of the eye.

If the experiment were performed on a statistically large number of patients, then cell density changes within two standard deviations of the average change should include 95.5% of the patients. Based on the average change and the standard deviation of our small data set, this implies that 95.5% of the changes in cell density should fall between -186 and +207 cells / mm^2. The lower limit is 5.85% of the average cell density in our data set (3188.2 cells / mm^2). This means that the data is insufficient to find a 6% or less decrease in cell density following a single MR scan. Since a 6% decrease per scan is very serious, the data are not sufficient to rule out the possibility of corneal endothelial damage caused by a MR scan. More complete studies must be done before any conclusions can be drawn on the effects of MRI on the corneal endothelium.

The direction for further investigation involves a more extensive study. First, more patients should be tested to improve the statistical significance of the results. The controls of the experiment should also be improved. This could be achieved by utilizing age and gender matched control subjects; the members of the control group have their cell densities counted at the same intervals as the other subjects but the control subjects do not go through a MR scan.

Another method to improve the experiment is to count the cell density several times following the MR scan. For instance, the cell density could be

determined at intervals of 2, 5, 10, and 15 days after the MR scan. The longer time delays would allow one to determine whether it takes longer than two days before corneal endothelial damage is observable.

Two variations of our study that might be useful are testing the effects of multiple MR scans and the effects of different radio frequencies. The possibility of accumulative or synergistic effects of exposure to the pulsed RF utilized in MRI can be evaluated by performing multiple MR scans on patients. Several different radio frequencies should be tested because although some radio frequencies utilized in MRI might be safe other radio frequencies may be harmful. We used 21.3 MHz RF pulses in our experiment, but other MR scanners have different strength magnets which require the use of different radio frequencies to obtain an image.

REFERENCES

1. Koslov, S. (1984) in Nonlinear Electrodynamics in Biological Systems, eds. Adey, W. R. & Lawrence, A. F. (Plenum Press, New York), 585-598.

2. Laing, R.A., Neubauer, L., Leibowitz, H.M. & Oak, S.S. : *Archives of Ophthalmology* vol 101, p. 1712-1715, (1983).

3. Mayer, D.J. (1984) : Clinical Wide-field Specular Microscopy, (Baillliere Tindall, London).