

Paper presented at “The Biological Effects, Health Consequences and Standards for Pulsed Radiofrequency Field”, an international seminar sponsored by the International Commission on Nonionizing Radiation Protection and World Health Organization, at the Ettoll Majorare, Centre for Scientific Culture, Erice, Sicily, Italy, November 21-25, 1999.

## **Memory and Behavior**

**Henry Lai**

**Bioelectromagnetics Research Laboratory,  
Department of Bioengineering,  
University of Washington,  
Seattle, Washington,  
USA**

The nervous system is very sensitive to environmental disturbance. In the proceedings of an international symposium on the “Biological Effects and Health Hazard of Microwave Radiation” held in Warsaw, Poland in 1973, it was stated in a summary section that ‘the reaction of the central nervous system to microwaves may serve as an early indicator of disturbances in regulatory functions of many systems’ [Czerski et al., 1974].

Disturbance to the nervous system leads to behavioral changes. On the other hand, alteration in behavior would imply a change in function of the nervous system. Studies on the effect of radiofrequency radiation (RFR) on behavior have been carried out since the beginning of Bioelectromagnetics research. Some of these studies are briefly reviewed below.

It has been speculated that a pulsed RFR is more potent than its continuous-wave (CW) counterpart in causing biological effects [e.g., Barenski, 1972; Frey et al., 1975; Oscar and Hawkins, 1977]. To evaluate this, it is necessary to compare the effects of pulsed RFR with those of CW radiation. Thus, studies on both CW and pulsed (and frequency-modulated) RFRs are included in this review. Comparing the effects of CW and pulsed RFR can actually be related to the popular debate on the distinction between ‘thermal’ and ‘non-thermal/athermal’ effect. If an effect is elicited by a pulsed RFR but not by a CW RFR of the same frequency and intensity under the same exposure conditions, it may imply the existence of ‘non-thermal/athermal’ effect.

Behavior is generally divided into two main categories: spontaneous and learned. Effects of RFR exposure on both types of behavior have been investigated.

### **Spontaneous Behavior**

Spontaneous behaviors are generally considered to be more resistant to disturbance. The most well studied spontaneous behavior in Bioelectromagnetics research is motor (locomotor) activity. Change in motor activity is generally regarded as an indication of change in the arousal state of an animal.

Hunt et al. [1975] reported decreased motor activity in rats after 30 min of exposure to pulsed 2450-MHz RFR (2.5 msec pulses, 120 pps, SAR 6.3 W·kg<sup>-1</sup>). Mitchell et al. [1988] also observed a decrease in motor activity in rats after 7 hr of exposure to CW 2450-MHz RFR (10 mW·cm<sup>-2</sup>, average SAR 2.7 W·kg<sup>-1</sup>).

Roberti [1975] reported no significant change in locomotor activity in rats after long-term (185-408 h) exposure to RFR of different frequencies (10.7-GHz CW; 3-GHz CW; 3-GHz with 1.3 μs

pulses and 770 pps) and various intensities (SAR 0.15-7.5 W·kg<sup>-1</sup>). Mitchell et al. [1977] reported an increase in motor activity on a small platform of rats exposed to 2450-MHz RFR (CW, average SAR 2.3 W·kg<sup>-1</sup>, 5 hr/day, 5 days/week for 22 weeks). Motor activity of the RFR exposed rats increased during the first week of exposure and stayed higher than controls throughout the period of the experiment. D'Andrea et al. [1979, 1980] reported decreased motor activity on a stabilimetric platform and no significant change in running wheel activity measured overnight in rats exposed to a 2450-MHz RFR (CW, 5 mW·cm<sup>-2</sup>, SAR 1.2 W·kg<sup>-1</sup>, exposed 5 day/week with a total exposure time of 640 hrs, activity was measured every 2-weeks). However, they reported no significant effect in both behaviors in rats similarly exposed to a 915-MHz RFR even at a higher energy absorption rate (CW, 5 mW·cm<sup>-2</sup>, SAR 2.5 W·kg<sup>-1</sup>). Moe et al. [1976] reported a decrease in motor activity of rats exposed to 918 MHz RFR (CW, SAR 3.6-4.2 W·kg<sup>-1</sup>) during the dark period of the light-dark cycle in a chronic exposure experiment (10 hr/night for 3 weeks). Lovely et al. [1977] repeated the experiment using a lower intensity (2.5 mW·cm<sup>-2</sup>, SAR 0.9 W·kg<sup>-1</sup>, 10 hr/night, 13 weeks) and found no significant change in motor activity in the exposed rats. Thus, the threshold of response under their exposure conditions is between 1 and 4 W·kg<sup>-1</sup>.

The results from the above studies indicate that it would need a rather high energy absorption rate (>1 W·kg<sup>-1</sup>) to affect motor activity in animals. However, there are two studies reporting effects on motor activity at relatively low SARs. In a long-term exposure study, Johnson et al. [1983] exposed rats to pulsed 2450-MHz RFR (10 μs pulses, 800 pps) from 8 weeks to 25 months of age (22 hr/day). The average whole body SAR varied as the weight of the rats increased and was between 0.4-0.15 W·kg<sup>-1</sup>. Open field activity was measured in 3-min sessions with an electronic open-field apparatus once every 6 weeks during the first 15 months and at 12-week intervals in the final 10 weeks of exposure. They reported a significantly lower open field activity only at the first test session, and a rise in the blood corticosterone level was also observed at that time. The authors speculated that RFR might be 'minimally stressful' to the rats. Rudnev et al. [1978] studied the behavior of rats exposed to CW 2375-MHz RFR at 0.5 mW·cm<sup>-2</sup> (SAR 0.1 W·kg<sup>-1</sup>), 7 h/day for 1 month. They reported a decrease in balancing time in a treadmill and inclined rod and motor activity in an open-field after 20 days of exposure. The open-field motor activity was found to be increased at 3 months post-exposure. Interestingly, Frey [1977] also reported a decrease in motor coordination on a motor-rod in rats exposed to a 1300-MHz pulsed RFR (0.5 ms pulses, 1000 pps, average power density of 0.65 or 0.2 mW·cm<sup>-2</sup>).

Another type of spontaneous behavior studied was consummatory behavior. In the Rudnev et al. [1978] study, the authors reported a decrease in food intake in their animals after long term exposure to CW RFR at 0.1 W·kg<sup>-1</sup>. Ray and Behari [1990] also reported a decrease in eating and drinking behavior in rats exposed for 60 days (3 hr/day) to a 7.5-GHz RFR (10-KHz square wave modulation) at an SAR of 0.0317 W·kg<sup>-1</sup> (average power density 0.6 mW·cm<sup>-2</sup>).

## **Learned behavior**

Several psychological studies have been carried out to investigate whether animals can detect RFR. One of the early studies was that of King et al. [1971] in which RFR was used as the cue in a conditioned suppression experiment. In conditioned suppression, an animal is first trained to elicit a certain response (e.g., bar-press for food). Once a steady rate of response is attained, a stimulus (e.g., a tone) will be presented to signify the on coming of a negative reinforcement (e.g., electric foot shock). The animal will soon learn the significance of the stimulus and a decrease in responding (conditioned suppression) will occur immediately after the presentation of the stimulus. In the experiment of King

et al. [1971], rats were trained to respond at a fixed-ratio schedule for sugar water reward. In a 2-hr session, either a tone or RFR would be presented and occasionally followed by an electric foot shock. Radiofrequency radiation of 2450 MHz, modulated at 12 and 60 Hz and at SARs of 0.6, 1.2, 2.4, 4.8, and 6.4  $\text{W}\cdot\text{kg}^{-1}$  was used as the conditioned stimulus. With training, consistent conditioned suppression was observed with the radiation at 2.4  $\text{W}\cdot\text{kg}^{-1}$  and higher. This indicates that rats can detect RFR at 2.4  $\text{W}\cdot\text{kg}^{-1}$ . Monahan and Henton [1977] also demonstrated that mice could be trained to elicit a response in order to escape or avoid RFR (CW, 2450-MHz, 40  $\text{W}\cdot\text{kg}^{-1}$ ). In another experiment, Carroll et al. [1980] showed that rats did not learn to go to a 'safe' area in the exposure cage in order to escape exposure to RFR (918-MHz, pulse modulated at 60 Hz, SAR 60  $\text{W}\cdot\text{kg}^{-1}$ ) (i.e., entering the 'safe' area resulted in an immediate reduction of the intensity of the radiation), whereas the animals learned readily to escape from electric foot shock by going to the 'safe' area. In a further study from the same laboratory, Levinson et al. [1982] showed that rats could learn to enter a 'safe' area, when the RFR was paired with a light stimulus. Entering the area would turn off both the radiation and light. They also showed that rats could learn to escape by entering the 'safe' area when RFR was presented alone, but learned at a lower rate than when the RFR was paired with a light. All these studies indicate that animals can detect RFR, probably as a thermal stimulus.

One of the most well established effects of pulsed RFR is the 'auditory effect'. Neurophysiological and psychological experiments indicate that animals can probably perceive microwave pulses as a sound stimulus [Chou et al., 1982a; Lin, 1978]. In a series of experiments, Frey and his associates [Frey and Feld, 1975; Frey et al., 1975] demonstrated that rats spent less time in the unshielded compartment of a shuttlebox, when the box was exposed to 1200-MHz pulsed RFR (0.5-ms pulses, 1000 pps, average power density 0.2  $\text{mW}\cdot\text{cm}^{-2}$ , peak power density 2.1  $\text{mW}\cdot\text{cm}^{-2}$ ) than during sham exposure. When a CW RFR (1200-MHz, 2.4  $\text{mW}\cdot\text{cm}^{-2}$ ) was used, rats showed no significant preference to remain in the shielded or unshielded side of the box. Hjeresen et al. [1979] replicated this finding using pulsed 2880-MHz RFR (2.3  $\mu\text{s}$  pulses, 100 pps, average power density 9.5  $\text{mW}\cdot\text{cm}^{-2}$ ) and showed that the preference to remain in the shielded side of a shuttlebox during RFR exposure could be generalized to a 37.5-kHz tone. Masking the 'radiation-induced auditory effect' with a 10-20 kHz noise also prevented shuttlebox-side preference during pulsed RFR exposure. These data indicate that the pulsed RFR-induced 'avoidance' behavior is due to the auditory effect.

The question is why rats avoid pulsed RFR? Is the 'auditory effect' stressful? This question was recently raised by Sienkiewicz [1999]. In an attempt to replicate our radial-arm experiment (Lai et al., 1989), he exposed mice to 900-MHz radiation pulsed at 217 Hz for 45 min a day for 10 days at a whole body SAR of 0.05  $\text{W}\cdot\text{kg}^{-1}$ . He didn't observe any significant effect of RFR exposure on maze learning, but reported that 'some of the exposed animals in our experiment appeared to show a stress-like response during testing in the maze. The animals tested immediately after exposure showed a more erratic performance, and were slower to complete the task compared to the animals tested after a short delay following exposure. This pattern of behavior may be consistent with increased levels of stress.' He also reported that exposed animals showed increased urination and defecation. He speculated that these behavioral effects were caused by the 'auditory effect' of the pulsed RFR.

Many studies investigated the effects of RFR exposure on schedule-controlled behavior. A schedule is the scheme by which an animal is rewarded (reinforced) for carrying out a certain behavior. For example, an animal can be reinforced for every response it makes, or reinforced intermittently upon responding according to a certain schedule (e.g., once every ten responses). Schedules of different complexity are used in psychological research. The advantage of using reinforcement schedules is that they generate in animals an orderly and reproducible behavioral pattern that can be maintained over a long period of time. This allows a systematic study of the effect

of RFR. Generally speaking, more complex behaviors are more susceptible to disruption by environmental factors. However, the underlying neural mechanisms by which different schedules affect behavior are poorly understood.

In a study by D'Andrea et al. [1977], RFRs of different frequencies and intensities were studied on their effects on bar-pressing rate on a variable-interval schedule. It was found that the latency time of stoppage to respond after the radiation was turned on correlated with the rate of rise in body temperature of the animal. Lebovitz [1980] also studied the effects of pulsed 1300-MHz RFR (1  $\mu$ s pulses, 600 pps) on rats bar-pressing on a fixed-ratio schedule for food reinforcement. A 15-minute 'rewarded' period, when bar pressing was rewarded with food, was followed by a 10-min 'unrewarded' period. Both food reinforced bar presses and unrewarded bar presses during the periods were studied. No significant effect was detected in both types of response at SAR of 1.5  $W \cdot kg^{-1}$ . However, at 6  $W \cdot kg^{-1}$ , there was a slight reduction in rewarded bar presses and a large reduction in unrewarded bar presses. The authors concluded that the unrewarded behavior was more susceptible to the effect of RFR than the rewarded behavior. However, Hunt et al. [1975] trained rats to bar press for saccharin water rewards in the presence (5- second duration) of a flashing light and not to respond in the presence of a tone. After 30 min of exposure to 2450-MHz RFR (modulated at 20 Hz, SAR of 6.5 or 11.0  $W \cdot kg^{-1}$ ), rats made more misses at the presence of the light, but there were no significant changes in the incidences of bar-pressing error when the tone was on (unrewarded). Gage [1979] trained rats to alternate responses between 2 levers at 11-30 times for a food reinforcement. Decrement in response rates was observed after 15 hrs of exposure to CW 2450-MHz RFR at 10, 15, and 20  $mW \cdot cm^{-2}$  (0.3  $W \cdot kg^{-1}$  per  $mW \cdot cm^{-2}$ ).

Effects of RFR on more complex operant response sequence and reinforcement schedules were studied in various experiments. de Lorge and Ezell [1980] tested rats on an auditory vigilance (observing-response) behavioral task during exposure to pulsed 5620-MHz (0.5 or 2  $\mu$ s, 662 pps) and 1280-MHz (3  $\mu$ s, 370 pps) RFR. In this task, rats had to discriminate two tones in order to press one of two bars appropriately for food reinforcement. The task required continuous sensory-motor activities in which the animal had to coordinate its motor responses according to the stimulus cues (tone) presented. Behavioral decrement was observed at a SAR of 3.75  $W \cdot kg^{-1}$  with the 1280-MHz radiation, and at 4.9  $W \cdot kg^{-1}$  with the 5620-MHz radiation. The authors concluded that '...the rat's observing behavior is disrupted at a lower power density at 1.28 than at 5.62 GHz because of deeper penetration of energy at the lower frequency, and because of frequency-dependent differences in anatomic distribution of the absorbed microwave energy.' In another experiment, de Lorge [1984] studied rhesus monkeys trained on the auditory vigilance (observing-response) task. After the training, the effects of exposure to RFR of different frequencies (225, 1300, and 5800 MHz) were studied [225-MHz-CW; 1300-MHz- 3  $\mu$ s pulses, 370 pps; 5800-MHz- 0.5 or 2  $\mu$ s pulses, 662 pps]. Reduction in performance was observed at different power density thresholds for the frequencies studied: 8.1  $mW \cdot cm^{-2}$  (SAR 3.2  $W \cdot kg^{-1}$ ) for 225 MHz, 57  $mW \cdot cm^{-2}$  (SAR 7.4  $W \cdot kg^{-1}$ ) for 1300 MHz, and 140  $mW \cdot cm^{-2}$  (SAR 4.3  $W \cdot kg^{-1}$ ) for 5800 MHz. de Lorge concluded that the behavioral disruption under different frequencies of exposure was more correlated with change in body temperature. Disruption occurred when the colonic temperature of the animal had increased by 1°C.

Thomas et al. [1975] trained rats to bar press on two bars: a fixed ratio of 20 on the right bar (20 bar presses produced a food pellet reward) and differential reinforcement of low rate (DRL) on the left bar (bar presses had to be separated by at least 18 sec and no more than 24 sec to produce a reward). There was a time-out period between schedules, i.e., no reinforcement available for responding. Animals were tested 5-10 min after 30 min of exposure to either CW 2450-MHz, pulsed 2860-MHz (1  $\mu$ s pulses, 500 pps) or pulsed 9600-MHz (1  $\mu$ s pulses, 500 pps) RFR at various power

densities. An increase in DRL response rate was observed with 2450-MHz radiation  $>7.5 \text{ mW}\cdot\text{cm}^{-2}$  (SAR  $2.0 \text{ W}\cdot\text{kg}^{-1}$ ), 2860-MHz RFR  $>10 \text{ mW}\cdot\text{cm}^{-2}$  ( $2.7 \text{ W}\cdot\text{kg}^{-1}$ ), and 9600-MHz RFR  $>5 \text{ mW}\cdot\text{cm}^{-2}$  (SAR  $1.5 \text{ W}\cdot\text{kg}^{-1}$ ). A decrease in the rate of response at the fixed ratio schedule was seen in all three frequencies when the power density was greater than  $5 \text{ mW}\cdot\text{cm}^{-2}$ . In addition, an increase in response rate was observed during time-out periods under irradiation of the three frequencies of RFR at greater than  $5 \text{ mW}\cdot\text{cm}^{-2}$ . This indicates a disruption of the animals' ability to discriminate the different schedule situations.

Schrot et al. [1980] trained rats to learn a new daily sequence of pressing of three bars for food reinforcement. An increased number of errors and decreased learning rates were observed in the animals after 30 min of exposure to pulsed 2800-MHz RFR (2  $\mu\text{s}$  pulses, 500 pps) at average power densities of 5 and  $10 \text{ mW}\cdot\text{cm}^{-2}$  (SAR 0.7 and  $1.7 \text{ W}\cdot\text{kg}^{-1}$ , respectively). No significant effect on performance was observed at power densities of 0.25, 0.5, and  $1 \text{ mW}\cdot\text{cm}^{-2}$ .

D'Andrea et al. [1989] studied the behavioral effects of high peak power RFR pulses of 1360-MHz. Rhesus monkeys performing on a complicated reinforcement-schedule involving time-related behavioral tasks (inter-response time, time discrimination, and fixed interval responses) were exposed to high peak power RFR ( $131.8 \text{ W}\cdot\text{cm}^{-2}$  rms, pulse repetition rate 2-32 Hz). No significant disturbance in performance was observed in the monkeys. Akyel et al. [1991] also studied the effects of exposure to high peak power RFR pulses on behavior. In their experiment, rats pre-trained to bar-press for food reinforcement on either fixed ratio, variable interval, or DRL schedule were exposed for 10 min to 1250-MHz pulses. Each pulse (10  $\mu\text{s}$  width) generated a whole body specific absorption of  $2.1 \text{ J}\cdot\text{kg}^{-1}$ , which corresponds to a whole body average SAR of  $0.21 \text{ mW}\cdot\text{kg}^{-1}$ . The pulse rate was adjusted to produce different total doses (0.5-14  $\text{kJ}\cdot\text{kg}^{-1}$ ). Only at the highest dose (14  $\text{kJ}\cdot\text{kg}^{-1}$ ), stoppage of responding was observed after exposure, when the colonic temperature was increased by  $\sim 2.5^\circ\text{C}$ . Responding resumed when colonic temperature returned to within  $1.1^\circ\text{C}$  above the pre-exposure level. When responding resumed, the response rates on the fixed ratio and variable interval schedules were below the pre-exposure base line level. Responses on the DRL schedule were too variable to allow a conclusion to be drawn. The authors concluded that the effect of the high peak power RFR pulses on schedule-controlled behavior was due to hyperthermia.

Several studies investigated the effects of long-term RFR exposure on schedule controlled-behavior. Mitchell et al. [1977] trained rats to respond on a mixed schedule of reinforcement (FR-5 EXT-15 sec), in which 5 responses would give a reward and then a 15 sec lapse time (extinction period) was required before a new response would be rewarded. In addition, the schedule of reinforcement was effective when a lamp was on, while no reinforcement was given when the lamp was off. Rats were then exposed to CW 2450-MHz RFR (average SAR  $2.3 \text{ W}\cdot\text{kg}^{-1}$ ) for 22 weeks (5 hr/day, 5 days/week) and tested at different times during the exposure period. The RFR-exposed rats showed higher responses during the extinction period, indicating poorer discrimination of the response cues. Navakatikian and Tomashevskaya [1994] described a complex series of experiments in which they observed disruption of a behavior (active avoidance) by RFR. In the study, rats were first trained to perform the behavior and then exposed to either CW 2450-MHz RFR or pulsed 3000-MHz RFR (400-Hz modulation, pulse duration 2  $\mu\text{s}$ , and simulation of radar rotation of 3, 6, and 29 rotations/min) for 0.5-12 hrs or 15-80 days (7-12 hr/day). Behavioral disruption was observed at a power density as low as  $0.1 \text{ mW}\cdot\text{cm}^{-2}$  ( $0.027 \text{ W}\cdot\text{kg}^{-1}$ ).

Two series of well-designed experiments were run by D'Andrea and his colleagues to investigate the effects of chronic RFR exposure on behavior. In one experiment [D'Andrea et al., 1986 a], rats were exposed for 14 weeks (7 hr/day, 7 days/week) to CW 2450-MHz RFR at  $2.5 \text{ mW}\cdot\text{cm}^{-2}$  (SAR  $0.7 \text{ W}\cdot\text{kg}^{-1}$ ). After exposure, the rats were trained to bar press on an interresponse time

criterion (IRT). In this schedule, the animals had to respond within 12 to 18 sec after the previous response in order to receive a food reward. Radiofrequency radiation exposed rats emitted more responses during the training period. When the training was completed, the RFR-exposed rats had lower efficiency in bar-pressing to obtain food pellets, i.e., they made more inappropriate responses and received fewer food pellets than the sham-exposed rats during a session. In a signalled two-way active avoidance shuttlebox test, the RFR-exposed rats showed less avoidance response than the sham-exposed rats during training; however, no significant difference in responses in the shuttlebox test was detected at 60 days after exposure between the RFR- and sham-exposed animals. In this experiment, a decrease in the threshold of electric foot shock detection (i.e., increase in sensitivity) was also observed in the irradiated rats during the exposure period, and an increased open-field exploratory behavior was observed in the rats at 30 days post-exposure. It may be interesting to point out that Frey [1977] also reported a decrease in tail pinch-induced aggressive behavior in RFR-exposed rats. Increased latency, decrease in duration, and episodes of fighting after tail pinching were observed between two rats being irradiated with RFR. This could be due to a decreased sensitivity or perception of pain and the RFR-induced activation of endogenous opioids described below.

In a second experiment [D'Andrea et al., 1986 b], rats were exposed to 2450-MHz RFR at  $0.5 \text{ mW}\cdot\text{cm}^{-2}$  (SAR  $0.14 \text{ W}\cdot\text{kg}^{-1}$ ) for 90 days (7 hr/day, 7 days/week). Open-field behavior, shuttlebox performance, and schedule-controlled bar-pressing behavior for food pellets were studied at the end of the exposure period. A small deficit in shuttlebox performance and an increased rate of bar-pressing were observed in the RFR exposed rats. Summarizing the data from these two series of experiments [D'Andrea et al., 1986 a,b], D'Andrea and his co-workers concluded that the threshold for the behavioral and physiological effects of chronic RFR exposure in the rats studied in their experiments occurred between the power densities of  $0.5 \text{ mW}\cdot\text{cm}^{-2}$  (SAR  $0.14 \text{ W}\cdot\text{kg}^{-1}$ ) and  $2.5 \text{ mW}\cdot\text{cm}^{-2}$  (SAR  $0.7 \text{ W}\cdot\text{kg}^{-1}$ ).

In a further experiment, DeWitt et al. [1987] also reported an effect on an operant task in rats after exposure for 7hr/day for 90 days to CW 2450-MHz RFR at a power density of  $0.5 \text{ mW}\cdot\text{cm}^{-2}$  ( $0.14 \text{ W}\cdot\text{kg}^{-1}$ ).

Little work has been done to investigate the effects of RFR on memory functions. We [Lai et al., 1989] studied the effect of short-term (45 min) RFR exposure (2450-MHz, 2  $\mu\text{sec}$  pulses, 500 pps,  $1 \text{ mW}\cdot\text{cm}^{-2}$ , SAR  $0.6 \text{ W}\cdot\text{kg}^{-1}$ ) on the rats' performance in a radial-arm maze, which measures spatial working (short-term) memory function. The maze consists of a central circular hub with arms radiating out like the spokes of a wheel. In this task, food-deprived animals are trained to explore the arms of the maze to obtain food reinforcement at the end of each arm. In each session they have to enter each arm once and a reentry is considered as an error. This task requires 'working memory', i.e., the rat has to remember the arms it has already entered during the course of a session. We found that short-term (45 min) exposure to RFR before each session of maze running significantly retarded the rats' abilities to perform in the maze. They made significantly more errors than the sham-exposed rats. In a further experiment [Lai et al., 1994], we found that the RFR-induced working memory deficit in the radial-arm maze was reversed by pretreating the rats before exposure with the cholinergic agonist physostigmine or the opiate antagonist naltrexone, whereas pretreatment with the peripheral opiate antagonist naloxone methiodide showed no reversal of effect. These data indicate that both cholinergic and endogenous opioid neurotransmitter systems inside the central nervous system are involved in the RFR-induced spatial working memory deficit. Spatial working memory requires the functions of the cholinergic innervations in the frontal cortex and hippocampus. The behavior result agrees with our previous neurochemical findings that RFR exposure decreased the activity of the cholinergic systems in the frontal cortex and hippocampus of the rats [Lai et al., 1987]. Endogenous

opioids [Lai et al., 1992] and the 'stress hormone' corticotropin-releasing factor [Lai et al., 1990] are also involved. Our hypothesis is that radiofrequency radiation activates endogenous opioids in the brain, which in turn cause a decrease in cholinergic activity leading to short-term memory deficit. Related to this that there is a report by Kunjilwar and Behari [1993] showing that long-term exposure (30-35 days, 3 hrs/day, SAR 0.1-0.14 W/kg) to 147-MHz RFR and its sub-harmonics 73.5 and 36.75 MHz, amplitude modulated at 16 and 76 Hz, decreased acetylcholine esterase activity in the rat brain, whereas short-term exposure (60 min) had no significant effect on the enzyme. There is another report by Krylova et al. [1992] indicating that 'cholinergic system plays an important role in the effects of electromagnetic field on memory processes'. There are also two studies suggesting the involvement of endogenous opioids in the effects of RFR on memory functions [Krylov et al., 1993; Mickley and Cobb, 1998].

In a more recent experiment, we [Wang and Lai, 2000] studied spatial long-term memory using the water maze. In this test, rats are trained to learn the location of a submerged platform in a circular water pool. We found that rats exposed to pulsed 2450-MHz RFR (2  $\mu$ s pulses, 500 pps, 1.2 W $\cdot$ kg $^{-1}$ , 1 hr) were significantly slower in learning and used a different strategy in locating the position of the platform.

## Comments

- (1) From the data available, it is not apparent that pulsed RFR is more potent than CW RFR in affecting behavior in animals. Even though different frequencies and exposure conditions were used in different studies and hardly any dose-response study was carried out, there is no consistent pattern that the SARs of pulsed RFR reported to cause an effect are lower than those of CW RFR. For example, the Thomas et al [1975] study showed that the thresholds of effect of CW 2450-MHz (2.0 W $\cdot$ kg $^{-1}$ ) and pulsed 2860-MHz (2.7 W $\cdot$ kg $^{-1}$ ) radiation on DRL bar-pressing response are quite similar.
- (2) Thermal effect is definitely a factor in the effects reported in some of the experiments described above. A related point is that most psychoactive drugs also affect body temperature. Stimulants cause hyperthermia, barbiturates cause hypothermia, and narcotics have a biphasic effect on body temperature (hyperthermia at low doses and hypothermia at high doses). It is not uncommon to observe a change of 2-3°C within 30 min after a drug is administered. However, in reviewing the literature, there is no general correlation between the effects of psychoactive drugs on body temperature and schedule-controlled behavior. Thus, body temperature may not be a major factor in an animal's responding under schedule-controlled behavior, at least in the case of psychoactive drugs. On the contrary, some of the experiments described above strongly suggest the role of hyperthermia on the RFR effect on the behavior. Perhaps, a sudden and large increase in body temperature as in the case of RFR can have a major effect on responding.
- (3) Generally speaking, when effects were observed, RFR disrupted schedule-controlled behavior in animals such as in the cases of discrimination responding [de Lorge and Ezell, 1980; Hunt et al., 1975; Mitchell et al., 1977], learning [Schrot et al., 1980], and avoidance [D'Andrea et al., 1986 a,b]. This is especially true when the task involved complex schedules and response sequence. In no case has an improvement in behavior been reported in animals after RFR exposure. It is puzzling that only disruptions in behavior by RFR exposure are reported. In the studies on EEG, both excitation (desynchronization) and depression (synchronization) have been reported after exposure to RFR [Bawin et al., 1973; Chizhenkova, 1988; Chou et al., 1982b; Dumansky and Shandala, 1974; Goldstein and Sisko, 1974; Takeshima et al., 1979]. Motor activity has also been

reported to increase [D'Andrea et al., 1979, 1980; Frey et al., 1975; Hjeresen et al., 1979; Mitchell et al., 1977; Rudnev et al., 1978] and decrease [Hunt et al., 1975; Johnson et al., 1983; Mitchell et al., 1988; Moe et al., 1976; Rudnev et al., 1978] after RFR exposure. If these measurements can be considered as indications of electrophysiological and behavioral arousal and depression, improvement in behavior should occur under certain conditions of RFR exposure. This is especially true with avoidance behavior. Psychomotor stimulants that cause EEG desynchronization and motor activation improve avoidance behavior, whereas tranquilizers that have opposite effects on EEG and motor activity decrease avoidance behavior.

- (4) It is difficult to conclude from the effects of RFR on schedule-controlled behavior the underlying neural mechanisms involved. In general, the effects of the effect of RFR on schedule-controlled behavior is similar to those of other agents, e.g., psychoactive drugs. For example, the way that a certain drug affects schedule-controlled behavior depends on the base line level of responding. A general rule is that drugs tend to decrease the rate when the base line responding rate is high and vice versa. This is known as rate-dependency. Exposure to RFR caused a decrease in response rate when a variable interval schedule that produces a steady rate of responding was used [D'Andrea et al., 1976; 1977], and an increase in responding when the DRL-schedule of reinforcement, that produces a low base line of responding, was used [Thomas et al., 1975]. This may reflect a rate-dependency effect. The effect of an agent can also depend on the schedule of reinforcement. For example, amphetamine has different effects on responses maintained on DRL schedule and punishment-suppressed responding schedule, even though both schedules generate a similar low response rate. Stimulus control as a determinant of response outcome was seen in the study of Lebovitz [1980] when unrewarded responses were disrupted more by RFR than rewarded responses, and the study of Hunt et al. [1975] that showed the reverse relationship. In the former experiment a fixed interval schedule was used, whereas in the latter a discrimination paradigm was studied.
- (5) It is also interesting to point out that in most of the behavioral experiments, effects were observed after the termination of RFR exposure. In some experiments (e.g., Rudnev et al., 1978; D'Andrea et al., 1986 a,b), tests were made days after exposure. This suggests a persistent change in the nervous system after exposure to RFR.
- (6) In many instances, effects on learned behavior were observed at a SAR less than  $4 \text{ W}\cdot\text{kg}^{-1}$ . (D'Andrea et al [1986a,b]  $0.14$  to  $0.7 \text{ W}\cdot\text{kg}^{-1}$ ; DeWitt et al. [1987]  $0.14 \text{ W}\cdot\text{kg}^{-1}$ ; Gage [1979]  $3 \text{ W}\cdot\text{kg}^{-1}$ ; King et al.[1971]  $2.4 \text{ W}\cdot\text{kg}^{-1}$ ; Lai et al. [1989]  $0.6 \text{ W}\cdot\text{kg}^{-1}$ ; Mitchell et al. [1977]  $2.3 \text{ W}\cdot\text{kg}^{-1}$ ; Navakatikian and Tomashevskaya [1994]  $0.027 \text{ W}\cdot\text{kg}^{-1}$ ; Schrot et al. [1980]  $0.7 \text{ W}\cdot\text{kg}^{-1}$ ; Thomas et al. [1975]  $1.5$  to  $2.7 \text{ W}\cdot\text{kg}^{-1}$ ; Wang and Lai [2000]  $1.2 \text{ W}\cdot\text{kg}^{-1}$ ).
- (7) Does disturbance in behavior have any relevance to health? The consequence of a behavioral deficit is situation dependent and may not be direct. It probably does not matter if a person is playing chess and RFR in his environment causes him to make a couple of bad moves. However, the consequence would be much more serious if a person is flying an airplane and his response sequences are disrupted by RFR radiation.

## References

- Akyel, Y., Hunt, E.L., Gambrill, C., and Varga, C. Jr., 1991, Immediate postexposure effects of high-peak-power microwave pulses on operant behavior of Wistar rats, *Bioelectromagnetics* 12:183-195.



- Barenski, S., 1972, Histological and histochemical effects of microwave radiation on the central nervous system of rabbits and guinea pigs, *Am J Physiol Med* 51:182-190.
- Bawin, S.M., Gavalas-Medici, R.J., and Adey, W.R., 1973, Effects of modulated very high frequency fields on specific brain rhythms in cats, *Brain Res* 58:365-384.
- Carroll, D.R., Levinson, D.M., Justesen, D.R., and Clarke, R.L., 1980, Failure of rats to escape from a potentially lethal microwave field, *Bioelectromagnetics* 1:101-115.
- Chizhenkova, R.A., 1988, Slow potentials and spike unit activity of the cerebral cortex of rabbits exposed to microwaves, *Bioelectromagnetics* 9:337-345.
- Chou, C.K., Guy, A.W., and Galambos, R., 1982a, Auditory perception of radiofrequency electromagnetic fields, *J Acoust Soc Am* 71:1321-1334.
- Chou, C.K., Guy, A.W., McDougall, J.B., and Han, L.F., 1982b, Effects of continuous and pulsed chronic microwave exposure on rabbits, *Radio Sci* 17:185-193.
- Czerski, P., Ostrowski, K., Shore, M.L., Silverman, C.H., Sues, M.J., and Waldeskog, B., eds., 1974, *"Biological Effects and Health Hazard of Microwave Radiation: Proceedings of an International Symposium,"* Polish Medical Publisher, Warsaw.
- D'Andrea, J.A., Gandhi, O.P., and Kesner, R.P., 1976, Behavioral effects of resonant electromagnetic power absorption in rats. In: *"Biological Effects of Electromagnetic Waves,"* vol 1, C.C. Johnson and M.L. Shore, eds., HEW Publication (FDA) 77-8010, Rockville, MD.
- D'Andrea, J.A., Gandhi, O.P., and Lords J.L., 1977, Behavioral and thermal effects of microwave radiation at resonant and nonresonant wavelengths, *Radio Sci* 12:251-256.
- D'Andrea, J.A., Gandhi, O.P., Lords, J.L., Durney, C.H., Johnson, C.C., and Astle, L., 1979, Physiological and behavioral effects of chronic exposure to 2450-MHz microwaves, *J Microwave Power* 14:351-362.
- D'Andrea, J.A., Gandhi, O.P., Lords, J.L., Durney, C.H., Astle, L., Stensaas, L.J., and Schoenberg, A.A., 1980, Physiological and behavioral effects of prolonged exposure to 915 MHz microwaves, *J Microwave Power* 15(2):123-135.
- D'Andrea, J.A., DeWitt, J.R., Emmerson, R.Y., Bailey, C., Stensaas, S., and Gandhi, O. P., 1986a, Intermittent exposure of rat to 2450-MHz microwaves at 2.5 mW/cm<sup>2</sup>: behavioral and physiological effects, *Bioelectromagnetics* 7:315-328.
- D'Andrea, J.A., DeWitt, J.R., Gandhi, O. P., Stensaas, S., Lords, J.L., and Nielson, H.C., 1986b, Behavioral and physiological effects of chronic 2450-MHz microwave irradiation of the rat at 0.5 mW/cm<sup>2</sup>, *Bioelectromagnetics* 7:45-56.
- D'Andrea, J.A., Cobb, B.L., and de Lorge, J., 1989, Lack of behavioral effects in the rhesus monkey to high peak power microwave pulses at 1.3 GHz, *Bioelectromagnetics* 10:65-76.
- de Lorge, J.O. , 1984, Operant behavior and colonic temperature of *Macaca mulatta* exposed to radiofrequency fields at and above resonant frequencies. *Bioelectromagnetics* 5:233-246.
- de Lorge, J., and Ezell, C.S., 1980, Observing-responses of rats exposed to 1.28- and 5.62-GHz microwaves, *Bioelectromagnetics* 1:183-198.
- DeWitt, J.R., D'Andrea, J.A., Emmerson, R.Y., and Gandhi, O.P., 1987, Behavioral effects of chronic exposure to 0.5 mW/cm<sup>2</sup> of 2450-MHz microwaves. *Bioelectromagnetics* 8:149-157.
- Dumansky, J.D., and Shandala, M.G., 1974, The biologic action and hygienic significance of electromagnetic fields of super high and ultra high frequencies in densely populated areas. In: *"Biologic Effects and Health Hazard of Microwave Radiation: Proceedings of an International Symposium,"* P. Czerski, et al., eds., Polish Medical Publishers, Warsaw.

- Frey, A.H., 1977, Behavioral effects of electromagnetic energy. In: *"Symposium on Biological Effects and Measurement of Radio Frequency Microwaves,"* D.J. Hazzard, ed., HEW Publication (FDA), 77-8026, Rockville, MD.
- Frey, A.H., and Feld, S.R., 1975, Avoidance by rats of illumination with low power nonionizing electromagnetic energy, *J Comp Physiol Psychol* 89:183-188.
- Frey, A.H., Feld, S.R., and Frey, B., 1975, Neural function and behavior: defining the relationship. *Ann N Y Acad Sci* 247:433-439.
- Gage, M.I., 1979, Behavior in rats after exposure to various power densities of 2450 MHz microwaves, *Neurobehav Toxicol* 1:137-143.
- Goldstein, L., and Sisko, Z., 1974, A quantitative electroencephalographic study of the acute effect of X-band microwaves in rabbits. In: *"Biological Effects and Health Hazards of Microwave Radiation: Proceedings of an International Symposium,"* P. Czerski, et al., eds., Polish Medical Publishers, Warsaw.
- Hjeresen, D.L., Doctor, S.R., and Sheldon, R.L., 1979, Shuttlebox-side preference as mediated by pulsed microwaves and conventional auditory cue. In: *"Electromagnetic Fields in Biological System,"* S.S.Stuchly, ed., Ottawa, Canada.
- Hunt, E.L., King, N.W., and Phillips, R.D., 1975, Behavioral effects of pulsed microwave radiation, *Ann NY Acad Sci* 247:440-453.
- Johnson, R.B., Spackman, D., Crowley, J., Thompson, D., Chou, C.K., Kunz, L.L., and Guy, A.W., 1983, Effects of long-term low-level radiofrequency radiation exposure on rats, vol. 4, Open field behavior and corticosterone, USAF SAM-TR83-42, Report of USAF School of Aerospace Medicine, Brooks AFB, San Antonio, TX.
- King, N.W., Justesen, D.R., and Clarke, R.L., 1971, Behavioral sensitivity to microwave irradiation, *Science* 172:398-401.
- Krylova, I.N., Dukhanin, A.S., Il'in, A.B., Kuznetsova, E.Iu., Balaeva, N.V., Shimanovskii, N.L., Pal'tsev, Iu.P., and Iasnetsov, V.V., 1992, The effect of ultrahigh frequency electromagnetic radiation on learning and memory processes (article in Russian), *Biull Eksp Biol Med* 114:483-484.
- Krylov, I.N., Iasnetsov, V.V., Dukhanin, A.S., and Pal'tsev, Iu.P., 1993, Pharmacologic correction of learning and memory disorders induced by exposure to high-frequency electromagnetic radiation (article in Russian), *Biull Eksp Biol Med* 115:260-262.
- Kunjilwar, K.K., and Behari, J., 1993, Effect of amplitude-modulated radio frequency radiation on cholinergic system of developing rats, *Brain Res* 601:321-324.
- Lai, H., Horita, A., Chou, C.K., and Guy, A.W., 1987, Low-level microwave irradiation affects central cholinergic activity in the rat, *J Neurochem* 48:40-45.
- Lai, H., Carino, M.A., and Guy, A.W., 1989, Low-level microwave irradiation and central cholinergic systems, *Pharmac Biochem Behav* 33:131-138.
- Lai, H., Carino, M.A., Horita, A. and Guy, A.W., 1990, Corticotropin-releasing factor antagonist blocks microwave-induced changes in central cholinergic activity in the rat, *Brain Res Bull* 25:609-612.
- Lai, H., Carino, M.A., Horita, A. and Guy, A.W., 1992, Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. *Bioelectromagnetics* 13:237-246.
- Lai, H., Horita, A., and Guy, A.W., 1994, Microwave irradiation affects radial-arm maze performance in the rat, *Bioelectromagnetics* 15:95-104.

- Lebovitz, R.M., 1980, Behavioral changes during long-term microwave irradiation. In: *"Proceeding of the International Symposium on the Biological Effects of Electromagnetic waves,"* UNSI, CNFRS, Jouy-en-Josas, France.
- Levinson, D.M., Grove, A.M., Clarke, L.R., and Justesen, D.R., 1982, Photic cueing of escape by rats from an intense microwave field, *Bioelectromagnetics* 3:105-116.
- Lin, J.C., 1978, *"Microwave Auditory Effects and Applications"*, Charles C, Thomas, Springfield, IL.
- Lovely, R.H., Myers, D.E., and Guy, A.W., 1977, Irradiation of rats by 918-MHz microwaves at 2.5 mW/cm<sup>2</sup>: delineating the dose-response relationship, *Radio Sci* 12(6):139-146.
- Mickley, G.A. and Cobb, B.L., 1998, Thermal tolerance reduces hyperthermia-induced disruption of working memory: a role for endogenous opiates? *Physiol Beh* 63:855-865.
- Mitchell, C.L., McRee, D.J., Peterson, N.J., and Tilson, H.A., 1988, Some behavioral effects of short-term exposure of rats to 2.45-GHz microwave radiation, *Bioelectromagnetics* 9:259-268.
- Mitchell, D.S., Switzer, W.G., and Bronaugh, E.L., 1977, Hyperactivity and disruption of operant behavior in rats after multiple exposure to microwave radiation, *Radio Sci* 12(6):263-271.
- Moe, K.E., Lovely, R.H., Meyers D.E., and Guy, A.W., 1976, Physiological and behavioral effects of chronic low-level microwave radiation in rats. In: *"Biological Effects of Electromagnetic Waves,"* vol. 1, C.C. Johnson and M.L. Shore, eds., HEW Publication (FDA) 77-8010, Rockville, MD.
- Monahan, J.C., and Henton, W., 1977, Free operant avoidance and escape from microwave radiation. In: *"Symposium on Biological Effects and Measurement of Radio Frequency Microwaves"*, D.J. Hazzard, ed, HEW Publication (FDA) 77-8026, Rockville, MD.
- Navakatikian, M.A., and Tomashevskaya, L.A., 1994, Phasic behavioral and endocrine effects of microwaves of nonthermal intensity. In: *"Biological Effects of Electric and Magnetic Fields, vol. 1"*, D.O. Carpenter, ed., Academic Press, San Diego, CA.
- Oscar, K.J., and Hawkins, T.D., 1977, Microwave alteration of the blood-brain barrier system of rats, *Brain Res* 126:281-293.
- Ray, S., and Behari, J., 1990, Physiological changes in rats after exposure to low levels of microwaves. *Rad Res* 123:199-202.
- Roberti, B., Heebels, G.H., Hendricx, J.C.M., deGreef, A.H.A.M., and Wolthuis, O.L., 1975, Preliminary investigation of the effect of low-level microwave radiation on spontaneous motor activity in rats, *Ann NY Acad Sci* 247:417-424.
- Rudnev, M., Bokina, A., Eksler, N., and Navakatikyan, M., 1978, The use of evoked potential and behavioral measures in the assessment of environmental insult. In: *"Multidisciplinary Perspectives in Event-Related Brain Potential Research,"* D.A. Otto, ed., EPA-600/9-77-043, U.S. Environmental Protection Agency, Research Triangle Park, NC.
- Schrot, J., Thomas, J.R., and Banvard, R.A., 1980, Modification of the repeated acquisition of response sequences in rats by low-level microwave exposure, *Bioelectromagnetics* 1:89-99.
- Schwan, H.P., 1971, Interaction of microwave and radiofrequency radiation with biological systems, *IEEE Microwave Th Tech MTT-19*:146-150.
- Sienkiewicz, Z., 1999, Behavioural effects of radiofrequency fields. In *"Mobile Telephones and Health: an Update on the Latest Research"*, Gothenburg, Sweden.
- Takashima, S., Onaral, B., and Schwan, H.P., 1979, Effects of modulated RF energy on the EEG of mammalian brain, *Rad Environ Biophys* 16:15-27.
- Thomas, J.R., Finch, E.D., Fulk, D.W., and Burch, L.S., 1975, Effects of low level microwave radiation on behavioral baselines, *Ann NY Acad Sci* 247:425-432.
- Wang, B.M. and Lai, H., 2000, Acute exposure to pulsed 2450-MHz microwaves affects water-maze performance of rats, *Bioelectromagnetics* 21:52-56.