Research report

Spatial learning, monoamines and oxidative stress in rats exposed to 900 MHz electromagnetic field in combination with iron overload

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HIGHLIGHTS

• Rats exposed to 900 MHz electromagnetic field (EMF) combined or not with iron supplementation.
• Rats trained in spatial tasks, biogenic monoamines and oxidative stress measured.
• EMF: deficits in the exploration task only, monoamines altered and no global oxidative stress.
• EMF + iron overload: no greater behavioral and neurochemical deficits than EMF alone.
• EMF affects cognitive and neurochemical processes but no synergistic effects between EMF and iron.

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ABSTRACT

The increasing use of mobile phone technology over the last decade raises concerns about the impact of high frequency electromagnetic fields (EMF) on health. More recently, a link between EMF, iron overload in the brain and neurodegenerative disorders including Parkinson's and Alzheimer's diseases has been suggested. Co-exposure to EMF and brain iron overload may have a greater impact on brain tissues and cognitive processes than each treatment by itself. To examine this hypothesis, Long-Evans rats submitted to 900 MHz exposure or combined 900 MHz EMF and iron overload treatments were tested in various spatial learning tasks (navigation task in the Morris water maze, working memory task in the radial-arm maze, and object exploration task involving spatial and non spatial processing). Biogenic monoamines and metabolites (dopamine, serotonin) and oxidative stress were measured. Rats exposed to EMF were impaired in the object exploration task but not in the navigation and working memory tasks. They also showed alterations of monoamine content in several brain areas but mainly in the hippocampus. Rats that received combined treatment did not show greater behavioral and neurochemical deficits than EMF-exposed rats. None of the two treatments produced global oxidative stress. These results show that there is an impact of EMF on the brain and cognitive processes but this impact is revealed only in a task exploiting spontaneous exploratory activity. In contrast, there are no synergistic effects between EMF and a high content of iron in the brain.

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1. Introduction

The global system for mobile communications (GSM) standard operating in the 900 MHz frequency band is largely used in Europe, Africa, Middle East, and Asia. Considerable increase in the use of mobile phone technology over the last decade raises concerns about the impact of electromagnetic fields (EMF) on health. Because part of the electromagnetic energy emitted by a mobile phone is absorbed by the head and brain, a growing amount of human and animal studies has investigated the athermal effects of EMF on brain activity, behavior and learning. These studies are quite heterogeneous in terms of species, specific absorption rate (SAR), frequency, intensity, duration of irradiation but they show that neural mechanisms both in animals and humans can be altered following EMF exposure. For example, EMF have been found to affect electrophysiological activity (e.g., [1–6]), neurotransmitter systems (e.g.,
markers (150 g) (tial, in and initial induction, in neurodegenerative disorders including Parkinson’s and Alzheimer’s diseases has been suggested. Iron is essential for many physiological processes in the brain [21] and is actively transported across the blood brain barrier. Systemic iron excess in newborn or adult rats results in iron concentration increase in the brain and in learning deficits [22–31]. It has been suggested that EMF increases blood-brain barrier permeability ([13,32,33], see [34] for an alternative view) therefore resulting in greater accumulation of iron in the brain [21,35,36]. As iron catalyzes the formation of highly reactive hydroxyl radicals, high concentrations of iron would induce oxidative stress mechanisms [26,37] in brain areas that are affected in neurodegenerative disorders [21,38–40] which would lead to cognitive and behavioral disorders [26]. Consistent with this hypothesis, it has been shown that combining EMF irradiation and iron administration induces production of reactive oxygen species in in vitro studies [41], 50 Hz; [42], 930 MHz). There are however a lack of in vivo studies examining the effects of combined 900 MHz EMF and iron overload treatment on oxidative stress in the brain and cognitive processes.

The objective of the study was thus to examine whether combined 900 MHz EMF exposure and systemic iron overload produces greater cognitive and brain biochemical effects than each treatment separately. We thus addressed this hypothesis by training rats that received both iron overload and EMF treatments in different spatial cognitive tasks including a navigation task (Morris water maze task), a spatial working memory task (radial-arm maze task) and a spontaneous object exploration task involving spatial and nonspatial information processing. We also looked at various biochemical parameters including monoamine neurotransmitter systems and oxidative stress by measuring (1) Dopamine (DA) and serotonin (5HT) content in various brain areas and (2) Thiobarbituric acid reactive substances (TBARS) content, a marker of lipid peroxidation, and antioxidant enzyme activity (superoxide dismutase, SOD and catalase, CAT) that contribute to degradation of reactive oxygen species. In previous studies we had investigated the effects of 150 KHz EMF and iron overload separately or in combination on behavior and biochemical parameters [31,37]. In the present study, we used the same iron administration protocol, tested the animals in the same behavioral tasks, and measured similar biochemical markers to look at the impact of 900 MHz EMF in combination with iron overload. We therefore did not include an iron-treated group but referred to the iron-treated group of the Maaroufi et al’s study [31].

2. Material and methods

2.1. Subjects

Twenty-four one-month-old male Wistar rats, purchased from a commercial supplier (Janvier, Le Genest-St-Isle, France) and weighing 130–150 g (4 weeks old) served as subjects. Upon arrival, they were housed by groups of two with food and water ad libitum and kept in a temperature-controlled room (20 ± 2°C) with a 12/12 light/dark cycle. Three groups of rats were tested: (1) 900 MHz EMF–exposed (EMF, n = 8), (2) 900 MHz EMF–exposed in combination with iron overload (EMF-IO, n = 8), and (3) Sham–EMF exposed and not treated with iron, (SHAM-EMF, n = 8). Iron administration and EMF exposure started one week after arrival.

2.2. Iron overload

Rats received daily one i.p. injection of ferrous sulfate (FeSO4·7H2O, Sigma–Aldrich, France) dissolved in sodium chloride 0.9% or vehicle (i.p.; 3 mg of FeSO4 per kg of body) during 21 consecutive days. As previously shown, such a dose of iron administered in adult rats during 5 days results in significant accumulation of iron in the hippocampus, cerebellum, and basal ganglia, 16 days after treatment [22]. Based on this result, longer treatment using the same dose is expected to produce also long-term iron accumulation in the brain. In parallel, rats were exposed to 900 MHz EMF.

2.3. EMF exposure

The apparatus allowing to irradiate rats with a 900 MHz electromagnetic field was built by Dr Philippe Pignet from the “Centre Commun de Ressources en Micro-ondes” (Marseille, France). A signal generator of the R&S SMY02 family (Rohde & Schwarz, Meudon, France) was used to generate a 900 MHz EMF. The apparatus incorporated a dipole antenna and a chamber formed by an absorbent material that limited stray reflections (Fig. 1A). The antenna was placed at a 50 cm distance of the chamber center and powered with 20 dBm (100 mW) current at 900 MHz. The electric field generated in the chamber was estimated by splitting the resonating dipole in a series of elementary dipoles and adding up their contributions. The resulting field was homogenous at all levels, bottom, antenna axis and top, of the chamber. The specific absorption rate (SAR) was estimated using the following formula: SAR = (W/kg) = (d/dt)(dW/dm) + (d/dt)(dW/pdV) = (σE²/ρ), where E is the generated electric field (rms), σ the specific tissue conductivity (S/m), and ρ the tissue density (kg/m³). Using σ = 1 S/m and ρ = 1046 kg/m³ as rough estimations of global body conductivity and density, respectively, we obtained SAR = 0.05 W/kg minimum and 0.18 W/kg maximum depending on the position of the rat in the field.

The apparatus was placed in a 1.7 m × 3.2 m, temperature controlled (20°C), ventilated, dimly lit room, on a table. A 40 cm × 26 cm × 16 cm cage with a Plexiglas cover that contained six rats (always the same rats) for each assay was placed in the chamber. Rats were exposed to 900 MHz EMF, 1 h/day (between 9 and 12 h) during 21 consecutive days. During irradiation, animals were very quiet in their cage. The sham exposed control rats were placed in the cage using the same procedure as treated rats except that they did not receive irradiation.

2.4. Behavioral testing

Rats were tested after completion of EMF exposure and/or iron administration. They were trained in (1) A reference memory task in the Morris water maze (post-treatment days 1–8), (2) A working memory task in the eight-arm radial maze (post-treatment days 14–20), (3) An object exploration task in a circular arena (post-treatment day 21, 22 or 23, although each rat was tested once, one could not test all rats on a single day).

2.4.1. Water maze navigation task

The water maze was an elevated circular pool (diameter 1.80 m) located in the middle of a room containing a large variety of cues. The pool was filled with 20°C water that was made opaque by addition of 2 kg of white chalk powder. A white-painted circular platform (diameter 8 cm) was placed inside the pool, 30 cm away from the wall. Its top surface was 1 cm below the surface of the water and was therefore invisible to the animals. A camera positioned above the apparatus and connected to a DVD recorder and to a monitor allowed tracking the trajectory of the animal. The
animals received four trials per day for 8 days. A typical trial consisted of releasing gently a rat in the water, its head facing the wall, from one of four possible starting places (N, E, S, and W) around the perimeter of the pool. The four starting positions were used in a pseudo random order within a four trial block. Once in the water, the rats swam until they eventually came across and climbed on the escape platform that was always located in the middle of the NW quadrant (reference memory task). When a rat had not reached the platform after 60 s swimming, it was gently guided by hand toward the platform. After the last daily trial, rats were dried in a towel and put back in their home cage. After all trials had been run on day 8, each rat was given a probe trial. The platform was removed and the rats were allowed to swim until 60 s have elapsed. All trials were processed on line by a Videotrack tracking system (ViewPoint, Champagne-au-Mont-d’Or, France). Raw data were processed using custom-made computer programs to calculate navigation parameters. Escape latency was used to measure learning and memory in rats. For the probe trial, we calculated the amount of time spent in the quadrant where the platform had been located (goal quadrant) and in the three other quadrants of the pool.

2.4.2. 8-Arm radial maze working memory task

The apparatus was a black-painted elevated (50 cm above the floor) stainless steel radial maze with eight arms (100 cm long, 10 cm wide) radiating from an octagonal shaped central platform (30 cm large). The maze was located in a small experimental room (3 m × 2 m) that provided numerous environmental cues. A food cup was placed 0.5 cm from the distal part of each arm. All eight cups were baited with one 45 mg sucrose pellet. After completion of the navigation task in the water maze, rats were submitted to a deprivation schedule which lasted 5 days in order to reduce their body weight to 85% of their initial weight. The animals were then placed by groups of two on the maze and were trained to eat 45 mg sucrose pellets scattered over the central platform and arms during 15 min daily sessions (familiarization phase). Training consisted in two daily trials with a 60 min inter-trial interval for 8 days. For each trial, an animal was placed on the central platform and was allowed to visit all 8 baited arms to eat the pellets. After the animal found all pellets or after 15 min have expired, it was removed from the maze. An arm entry was registered if the rat placed its four paws within the alley. Re-entries in already visited arms were counted as working memory errors. The mean number of errors in each day was used for analysis.

2.4.3. Object exploration task

The apparatus was a square arena (80 cm on a side) with a painted flat white wooden floor. It contained four objects (A, B, C, D). Object A was a plastic house-shaped object (11 cm × 8 cm × 20 cm), object B a dark gray painted bottle (8 cm diameter, 20 cm high), object C a Rubik’s cube (5.5 cm on each side) placed on a cylindrical metal base (total height, 22.5 cm), and object D a white plastic cylinder (8 cm diameter, 12 cm high). In the last session, a novel object was used to replace a familiar object. The novel object (E) was a cylindrical spray-paint container (6.5 cm diameter, 20 cm high). The initial arrangement of the four objects (A, B, C, D) was a tetragon (Fig. 1B). The distance between objects A, B, and C was approximately 30 cm, and object D was located approximately 20 cm from the three other objects. The open field was surrounded by a white curtain so that the environment was visually uniform. Each rat was submitted to eight successive 4-min sessions separated by 4-min intervals during which the animal was returned to its home cage. From sessions 1 to 5, the rats were exposed to the initial configuration of objects. In session 6 (spatial change), object D had been displaced toward the peripheral wall therefore yielding a new spatial configuration (see Fig. 1B). Note that all objects were at the same distance from the wall (approximately 17 cm). Session 7 was similar to session 6. In session 8 (non-spatial change), one of the familiar objects (A) was replaced by a novel object (E). This protocol allowed to measure the ability of rats to habituate (session 1–5), detect a spatial change (session 6) and detect a non spatial change (session 8). All sessions were recorded on DVD for off line analysis. To evaluate object exploration, we measured the time spent by the rat’s snout in a circular area centered on the object. The diameter of this area was determined for each object so that detection corresponded to a contact or close proximity (<2 cm) of the snout with the object. The reaction to spatial change and non-spatial change was quantified by calculating two scores, a re-exploration score for the spatial change and a re-exploration score for the non-spatial change. For the spatial change, we considered separately the displaced object (D) and the non-displaced objects (A, B, C). The spatial re-exploration score was the contact duration for object D during sessions 5 subtracted from the contact duration for the

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Fig. 1. (A) 900 MHz irradiation apparatus. (B) Protocol of the object exploration task. Rats are submitted to eight successive sessions (S1 to S8). From S1 to S5, the arena contained 4 objects. In S6, the object configuration was modified by displacing object C (spatial change). In S7 the configuration was the same as in S6. In S8, a novel object (E) replaced a familiar object (A) (non-spatial change).
same object during sessions 6. A similar re-exploration score was calculated for the non-displaced objects, the difference of contact duration between sessions 6 and 5 being calculated for each object and averaged across the 3 objects for each rat. The amount of reaction to non-spatial change was quantified by calculating an object re-exploration score defined as the average contact duration for the familiar objects (B, C, D) subtracted from the novel object (E) contact duration in S8.

2.5. Biochemical measurements

At the end of the experimental period, the rats were given a lethal dose of sodium pentobarbital (80 mg/kg) and then decapitated. Brain was removed, rapidly washed with cold phosphate buffered saline (PBS), then immediately frozen on dry ice and finally stored at 80 °C. Thick coronal slices (2–3 mm) of various brain regions (cortex: 5.16–2.16 mm; striatum: 2.16–0.48 mm; hippocampus: 2.52–4.56 mm and cerebellum: 9.36–14.04 mm) relative to bregma [43] were made at 20 °C using a cryostat (Leica CM3050). Micropunches were taken from these different brain regions. Samples were weighed and homogenized (1/10, w/v) in cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EDTA, (HEPES/EDTA) a Potter–Elvehjem homogenizer fitted with a pestle for microtubes. Part of homogenate was immediately prepared for measurement of monoamines while the remaining part was stored on ice for measurement of oxidative stress.

2.5.1. Monoamines

Sample contents in monoamines (DA and 5HT) and their respective catecholamines, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5HIAA), respectively were measured as described previously [44]. Briefly, homogenate was sonicated in 1 volume of 0.1 M perchloric acid, centrifuged at 10,000 × g for 15 min at 4 °C, and supernatants were kept at −80 °C until being assayed for monoamines and catecholamines by high performance liquid chromatography with electrochemical detection. The mobile phase consisted of 0.1 M sodium acetate, 0.17 mM octyl sulfate, 8% methanol, 0.7 mM EDTA, pH 4.5, and was delivered through a LC-10ADvp Shimadzu pump (Kyoto, Japan) into a C-18 (ODS2, 4.6 mm × 150 mm) Spherisorb column (Waters, Milford, MA, USA). Twenty microliter samples were injected and analyzed using a Coulombel II, ESA detector (Chelmsford, MA, USA). The limit of detection was 20 fmol/sample.

2.5.2. Oxidative stress parameters

Homogenates were centrifuged at 1600 × g for 10 min at 4 °C. Part of the resulting supernatant (SN1) was kept at −80 °C until used to determine protein and TBARS contents as well as total SOD activity. The remaining part of SN1 was further centrifuged at 10,000 × g for 15 min at 4 °C and supernatant (SN2) was kept at −80 °C until used to determine CAT activity. Protein content was determined in SN1 by the Bradford’s method [45] using the Coomassie Protein Assay Kit (Pierce, Rockford, IL, USA) and bovine serum albumin as standard. TBARS content was measured by the method of Richard et al. [46]. Briefly, samples were incubated for 1 h at 95 °C in 0.75 volume of a mixture made of 2 volumes of 55.36 mM thiobarbituric acid and 1 volume of 7% perchloric acid. The samples were then chilled on ice to stop the reaction and 2 volumes of butanol-1 were added to extract the TBARS. After the phases have been separated by centrifugation (1500 × g, 10 min, 4 °C), the TBARS content was determined in the butanol-1 extract using a fluorescent plate reader (Fluoroskan Ascent FL; λex = 530 nm, λem = 590 nm). Malondialdehyde (MDA; 1,1,3,3-tetrahydroxypropane) standards were included with each assay and TBARS contents were expressed in nmoles of MDA/mg of proteins. SOD was assayed using the SOD assay kit (Cayman) according to manufacturer instructions. This assay uses SOD ability to inhibit the reduction of a tetrazolium salt by superoxide anions, generated by xanthine oxidase. Formation of the formazan product was monitored at 450 nm using a microplate reader. One unit of SOD is defined as the amount of enzyme necessary to have 50% of inhibition. SOD activity was expressed as units/mg of proteins. CAT was assayed using the catalase assay kit (Cayman) according to manufacturer instructions. This assay utilizes the peroxidatic function of CAT for determination of enzyme activity. Formaldehyde production from methanol in presence of optimal concentration of H2O2 was monitored at 540 nm on a microplate reader using purpald as chromogen. CAT activity was expressed as nmol/min/mg of proteins.

2.6. Data analysis

Behavioral data were analyzed using two-way analyses of variance (ANOVA) with repeated measures. For objects exploration, in addition to a between-group comparison, the re-exploration scores were compared to a zero value that would indicate that no re-exploration occurred after the spatial change or the non-spatial change using t tests. A factorial ANOVA with objects (displaced objects vs. non-displaced objects) and groups as factors was used to analyze the re-exploration score for the spatial change. One-way ANOVAs were used to analyze the biochemical data. Post hoc Newman–Keuls (NK) tests were used for specific comparisons.

3. Results

3.1. Behavior

3.1.1. Water maze navigation task

Fig. 2A shows the time-course of escape latency across sessions in SHAM-EMF, EMF, and EMF-IO groups. A two way ANOVA with repeated measures showed no effect of group (F(2,21) = 0.66, P > 0.05), an effect of session (F(7,147) = 31.91, P < 0.001) and no group × session interaction (F(14,147) = 1.35, P > 0.05), indicating that all groups learned the task at similar rate. Moreover, all groups reached similar performance level at the end of training (session 8, NK tests, all Ps > 0.05).

As shown in Fig. 2B, during the probe test, all three groups showed a preference for the goal quadrant relative to the other quadrants (SHAM, t(7) = 5.43, P < 0.001; EMF-IO, t(7) = 8.93, P < 0.001; EMF, t(7) = 8.30, P < 0.001).

3.1.2. Eight-arm radial maze working memory task

Fig. 2C shows the number of errors across days in EMF, EMF-IO, and SHAM-EMF groups. An ANOVA with repeated measures revealed no effect of group (F(2,21) = 2.76, P > 0.05), an effect of day (F(6,126) = 7.09, P < 0.001) and no significant group × day interaction (F(12,126) = 1.36, P > 0.05). Thus, all groups similarly improved their performance across days.

3.1.3. Object exploration task

Habituation was assessed by measuring locomotor activity (distance run) and object exploration (duration of contacts with the objects). Fig. 3A left panel shows the time course of distance run across habituation sessions (S1–S5). All groups exhibited a decrease in locomotor activity (ANOVA with repeated measures, no effect of group, F(2,21) = 2.49, P > 0.05; significant effect of session F(4,84) = 54.87, P < 0.001). A significant group × session interaction (F(8,84) = 3.14, P < 0.05) indicated that all 3 groups did not decrease their locomotor activity at similar rate, however. As shown in Fig. 3A, EMF-IO and EMF showed a greater decrease than the SHAM group. The 3 groups did not exhibit different locomotion on the last habituation session (NK tests, EMF-IO vs. SHAM: P = 0.87; EMF vs. SHAM: P = 0.89). All groups showed similar habituation
Fig. 2. Water maze navigation task. (A) Escape latency to reach the platform during acquisition. (B) Time spent in the goal quadrant and the other quadrants (averaged over the 3 quadrants) during the probe test in SHAM EMF, EMF-IO and EMF groups. (C) Eight arm radial maze task. Number of errors (visits to an already visited arm) during acquisition in SHAM EMF, EMF-IO and EMF groups. ***<i>P</i>&lt;0.001.

Fig. 3. Object exploration task. (A) Left panel. Distance run from S1 to S5 (cm). Right panel. Duration of contacts (s) with the objects from S1 to S5. (B) Re-exploration score for the displaced and the non-displaced objects (contact duration for object D in S5 subtracted from the contact duration for the same object in S6). (C) Re-exploration score for the non-spatial novelty (average contact duration for the familiar objects B, C, D) subtracted from the novel object (E) contact duration in S8. NK tests: *<i>P</i>&lt;0.05, ***<i>P</i>&lt;0.001; <i>t</i>-tests comparing the re-exploration score to a zero value: +<i>P</i>&lt;0.05, ++<i>P</i>&lt;0.01, +++<i>P</i>&lt;0.001.
of object exploration (Fig. 3A right panel, duration of contacts, ANOVA with repeated measures, no effect of group, $F(2,21) = 1.19$, $P > 0.05$; significant effect of session, $F(4,84) = 24.27$, $P < 0.001$, no group $\times$ session interaction, $F(8,84) = 1.32$, $P > 0.05$).

In S6, the arrangement of the objects in the arena was modified by moving one object to a new location. Fig. 3B shows the re-exploration score for the displaced and non-displaced objects in the 3 groups. A factorial ANOVA revealed significant effects of group ($F(2,42) = 9.02$, $P < 0.001$), and object (displaced vs. non-displaced objects, $F(1,42) = 6.22$, $P < 0.05$) but no group $\times$ object interaction ($F(2,42) = 1.25$, $P > 0.05$). NK tests and t-tests comparing the re-exploration score to a zero value (see data analysis) showed that, consistent with our previous work (e.g., [47]), SHAM rats reacted to the spatial change by selectively re-exploring the displaced object (t-test for displaced object, $t(7) = 6.09$, $P < 0.001$); NK test for displaced vs. non-displaced objects, $P > 0.05$) indicating that they accurately detected and identified the change. In contrast, EMF rats did not show any re-exploration of the displaced objects between S5 and S6 (t-test, $t(7) = 0.20$, $P > 0.05$), did not show any preference for the displaced object during S6 (NK test displaced vs. non-displaced objects, $P < 0.05$), and differed from SHAM rats (displaced object, NK tests, $P > 0.01$), indicating that they were unable to detect the spatial change. EMF-IO rats increased their exploration of the displaced object (t-test, $t(7) = 2.93$, $P < 0.05$) but did not show a preference for the displaced objects during S6 (NK test for displaced vs. non-displaced objects, $P > 0.05$). In addition, there was a marginally significant difference between the re-exploration score in SHAM and EMF-IO groups for the displaced object (NK tests, $P = 0.063$). This suggests that although EMF-IO rats detected that some change occurred in the environment, they were not able to accurately detect the change. Combining the displaced and the non-displaced objects, we confirmed that EMF-IO rats displayed a global reactivation of their exploratory activity in S6 and did not discriminate the two kinds of objects (One way ANOVA on the re-exploration score calculated for the whole set of objects, $F(2,21) = 4.42$, $P < 0.05$; NK, SHAM $>$ EMF, $P < 0.05$, EMF-IO $>$ EMF, $P = 0.05$, SHAM $=$ EMF-IO, $P > 0.05$).

In session S8, we tested the ability of the rats to detect a non spatial change by replacing a familiar object by a novel object. As shown in Fig. 3C, all groups showed a preference for the novel object relative to the familiar objects (t-tests against zero, SHAM, $t(7) = 5.53$, $P < 0.001$; EMF-IO, $t(7) = 5.06$, $P < 0.01$; EMF, $t(7) = 2.38$, $P < 0.05$). However, EMF rats displayed a much lower re-exploration of the novel object that both SHAM and EMF-IO rats (one-way ANOVA, significant effect of group, $F(2,21) = 6.68$, $P < 0.01$; NK tests, SHAM $>$ EMF, $P < 0.01$; EMF-IO $>$ EMF, $P < 0.05$).

To sum up, both EMF and EMF-IO groups did not show altered performance in the water maze navigation and radial arm maze tasks. In contrast these two groups were impaired in the object exploration task: EMF rats were not able to detect the spatial change and showed a degraded reaction to the non-spatial change. EMF-IO rats were not as efficient as SHAM rats to discriminate the spatial change but were able to detect the non-spatial change.

#### 3.2. Biochemical measurements

#### 3.2.1. DA, DOPAC and DOPAC/DA ratio

Only samples diluted so that both monoamines and metabolites were detected were taken into account. Thus, the number of samples included in the analysis may vary across areas (see Table 1). Levels of DA and DOPAC measured in 4 brain areas, the cerebellum, the striatum, the hippocampus and the prefrontal cortex, along with the calculated DOPAC/DA ratio are shown in Table 1. A difference in DA content was found in the striatum and the hippocampus

was higher in EMF-IO rats than in both SHAM and EMF rats in the striatum (NK tests, both EMF-IO vs. EMF and EMF-IO vs. SHAM, $P < 0.01$). For the hippocampus, DA content was lower in EMF rats than in the two other groups (NK tests, both EMF vs. EMF-IO and EMF vs. SHAM, $P < 0.01$).

A significant effect of group was found for the DOPAC in the hippocampus (one-way ANOVA, $F(2,20) = 3.08$, $P < 0.05$). Similarly to the pattern of DA content, EMF rats had less DOPAC than both SHAM and EMF-IO rats (NK tests, both EMF vs. EMF-IO and EMF vs. SHAM, $P < 0.05$). There was a marginally significant difference in the DOPAC/DA ratio in both the striatum and the hippocampus (One way ANOVA, striatum, $F(2,20) = 3.09$, $P = 0.067$; hippocampus, $F(2,16) = 3.10$, $P = 0.073$).

#### 3.2.2. 5HT, 5HIAA, 5HIAA/5HT ratio

Levels of 5HT and 5HIAA measured in 4 brain areas, the cerebellum, the striatum, the hippocampus and the cortex, along with the calculated 5HIAA/5HT ratio are shown in Table 1. 5HT content was modified in the cerebellum and the hippocampus (one-way ANOVA, cerebellum, $F(2,21) = 7.80$, $P < 0.01$; hippocampus, $F(2,21) = 7.80$, $P < 0.05$) in treated rats. EMF and EMF-IO groups had more 5HT than the SHAM group in the cerebellum (NK, $P < 0.05$, $P < 0.1$, respectively) but were not different from each other ($P > 0.05$). In the hippocampus, 5HT content was significantly lower in the EMF group than in the EMF-IO group (NK, $P < 0.05$) and marginally higher in the EMF-IO group than the SHAM group (NK, $P = 0.078$). Alteration of 5HIAA content was also found in the cerebellum ($F(2,21) = 12.74$, $P < 0.001$) but was marginally significant in the hippocampus ($F(2,21) = 3.08$, $P = 0.067$). In the cerebellum, this difference was due to a lower content of 5HIAA in both EMF-IO and EMF groups than in the SHAM group (NK, both $P < 0.001$). The 5HIAA/5HT ratio was altered in the cerebellum ($F(2,21) = 15.66$, $P < 0.001$), due to a lower value in both EMF-IO and EMF rats than in SHAM rats (NK, both $P < 0.001$).

Overall, the monoamines were affected in both treated groups. DA content and metabolism was altered in the striatum and the hippocampus. 5HT content and metabolism was altered in the cerebellum and the hippocampus. Globally, although EMF and EMF-IO rats were both impaired, degradation of the monoamine systems was more pronounced in EMF rats than in EMF-IO rats.

#### 3.2.3. Oxidative stress parameters

Only samples where enough material was present to perform duplicate were analyzed. Thus, the number of samples included in the analysis may vary across areas (see Table 2). Oxidative stress in the brain was evaluated by measuring the production of TBARS, a marker of lipid peroxidation and the activity of two antioxidant enzymes, SOD and CAT, in the cerebellum, striatum, hippocampus and prefrontal cortex.

TBARS content was affected in the striatum (one-way ANOVA, $F(2,18) = 6.38$, $P < 0.01$), hippocampus ($F(2,18) = 4.28$, $P < 0.05$) and prefrontal cortex ($F(2,16) = 5.21$, $P < 0.05$). More specifically, EMF rats had a lower TBARS content than both SHAM and EMF-IO rats in the striatum (NK, $P < 0.05$, and $P < 0.01$, respectively) and the hippocampus (NK, both $P < 0.05$). In contrast, in the prefrontal cortex TBARS content was lower in EMF-IO rats than in both SHAM and EMF rats (NK, both $P < 0.05$).

SOD activity was affected in the striatum only (one-way ANOVA, striatum, $F(2,21) = 10.39$, $P = 0.001$) resulting from a higher activity in EMF rats than in EMF-IO and SHAM rats (NK, $P < 0.001$ and, $P < 0.05$, respectively). A marginally significant difference was found in the prefrontal cortex, $(F(2,19) = 3.27$, $P = 0.060$). Similar to SOD, CAT activity was also affected in the striatum (one-way ANOVA, $F(2,18) = 21.75$, $P < 0.001$). However this was due to a lower activity in EMF-IO rats (NK, EMF-IO vs. EMF and vs. SHAM, both $P < 0.001$).
Table 1
Content of DA, DOPAC, DOPAC/DA ratio, content of 5HT, 5HIAA, and 5HIAA/5HT ratio (pmol/mg of tissue, mean ± s.e.m.), in various brain regions (cerebellum, striatum, hippocampus and prefrontal cortex) in SHAM, EMF-IO and EMF groups.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>DA (pmol/mg)</th>
<th>DOPAC (pmol/mg)</th>
<th>DOPAC/DA</th>
<th>5HT (pmol/mg)</th>
<th>5HIAA (pmol/mg)</th>
<th>5HIAA/5HT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHAM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.10 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.42 ± 0.08</td>
<td>0.67 ± 0.07</td>
<td>2.28 ± 0.30</td>
<td>3.78 ± 0.66</td>
</tr>
<tr>
<td>Striatum</td>
<td>51.41 ± 5.44</td>
<td>10.68 ± 1.85</td>
<td>0.20 ± 0.02</td>
<td>1.53 ± 0.17</td>
<td>3.65 ± 0.38</td>
<td>2.43 ± 0.18</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.41 ± 0.25</td>
<td>0.30 ± 0.09</td>
<td>0.19 ± 0.03</td>
<td>1.50 ± 0.09</td>
<td>4.48 ± 0.33</td>
<td>3.04 ± 0.28</td>
</tr>
<tr>
<td>Prefrontal cort.</td>
<td>0.43 ± 0.06</td>
<td>0.14 ± 0.02</td>
<td>0.32 ± 0.04</td>
<td>2.26 ± 0.09</td>
<td>2.81 ± 0.13</td>
<td>1.25 ± 0.06</td>
</tr>
<tr>
<td><strong>EMF-IO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.12 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.38 ± 0.05</td>
<td>1.06 ± 0.07*</td>
<td>1.20 ± 0.08*</td>
<td>1.14 ± 0.04*</td>
</tr>
<tr>
<td>Striatum</td>
<td>75.20 ± 5.53**</td>
<td>12.46 ± 1.40</td>
<td>0.16 ± 0.01</td>
<td>1.72 ± 0.12</td>
<td>3.61 ± 0.15</td>
<td>2.16 ± 0.18</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.73 ± 0.33</td>
<td>0.29 ± 0.10</td>
<td>0.16 ± 0.02</td>
<td>1.64 ± 0.14</td>
<td>4.33 ± 0.32</td>
<td>2.71 ± 0.17</td>
</tr>
<tr>
<td>Prefrontal cort.</td>
<td>0.93 ± 0.27</td>
<td>0.29 ± 0.10</td>
<td>0.28 ± 0.02</td>
<td>2.30 ± 0.20</td>
<td>3.17 ± 0.11</td>
<td>1.44 ± 0.11</td>
</tr>
<tr>
<td><strong>EMF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.11 ± 0.007</td>
<td>0.04 ± 0.00</td>
<td>0.34 ± 0.03</td>
<td>0.92 ± 0.07*</td>
<td>1.05 ± 0.08*</td>
<td>1.15 ± 0.04*</td>
</tr>
<tr>
<td>Striatum</td>
<td>44.46 ± 4.46</td>
<td>8.76 ± 1.02</td>
<td>0.19 ± 0.01</td>
<td>1.44 ± 0.15</td>
<td>3.19 ± 0.18</td>
<td>2.32 ± 0.17</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.35 ± 0.11**</td>
<td>0.04 ± 0.01*</td>
<td>0.10 ± 0.03</td>
<td>1.22 ± 0.08*</td>
<td>3.55 ± 0.17</td>
<td>2.96 ± 0.17</td>
</tr>
<tr>
<td>Prefrontal cort.</td>
<td>0.68 ± 0.15</td>
<td>0.21 ± 0.04</td>
<td>0.32 ± 0.03</td>
<td>2.35 ± 0.29</td>
<td>2.75 ± 0.29</td>
<td>1.23 ± 0.11</td>
</tr>
</tbody>
</table>

The number of samples (n) in each group used for analysis is indicated in the first column (n1 for DA and DOPAC, n2 for 5HT and 5HIAA analyses). * indicates a significant difference (irrespective of the sign) between the EMF-IO group and the EMF group, # between the EMF-IO group and the SHAM group, and $ between the EMF group and the SHAM group. Differences are only indicated once. For P values see text.

Table 2
TBARS content (nmol MDA/mg proteins), SOD (U/mg protein) and CAT (nmol/min/mg proteins) activity in different brain structures (cerebellum, striatum, hippocampus, prefrontal cortex) SHAM, EMF-IO and EMF groups (mean ± s.e.m.).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>TBARS</th>
<th>SOD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHAM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.42 ± 0.04</td>
<td>0.53 ± 0.04</td>
<td>1.44 ± 0.19</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.83 ± 0.06</td>
<td>0.34 ± 0.03</td>
<td>0.50 ± 0.05</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.99 ± 0.09</td>
<td>0.43 ± 0.04</td>
<td>0.44 ± 0.07</td>
</tr>
<tr>
<td>Prefrontal cort.</td>
<td>0.97 ± 0.08</td>
<td>0.33 ± 0.01</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td><strong>EMF-IO</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.47 ± 0.05</td>
<td>0.43 ± 0.03</td>
<td>1.61 ± 0.17</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.96 ± 0.08</td>
<td>0.27 ± 0.02</td>
<td>0.28 ± 0.03**</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.04 ± 0.11</td>
<td>0.47 ± 0.04</td>
<td>0.43 ± 0.08</td>
</tr>
<tr>
<td>Prefrontal cort.</td>
<td>0.73 ± 0.05**</td>
<td>0.28 ± 0.01</td>
<td>0.51 ± 0.05</td>
</tr>
<tr>
<td><strong>EMF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.39 ± 0.05</td>
<td>0.58 ± 0.12</td>
<td>1.85 ± 0.11</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.60 ± 0.07**</td>
<td>0.50 ± 0.05*</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.62 ± 0.07*</td>
<td>0.53 ± 0.09</td>
<td>0.49 ± 0.10</td>
</tr>
<tr>
<td>Prefrontal cort.</td>
<td>0.98 ± 0.06</td>
<td>0.35 ± 0.03</td>
<td>0.52 ± 0.11</td>
</tr>
</tbody>
</table>

The number of samples (n) in each group used for analysis is indicated in the first column (n1 for TBARS, n2 for SOD, n3 for CAT analyses). * indicates a significant difference (irrespective of the sign) between the EMF-IO group and the EMF group, # between the EMF-IO group and the SHAM group, and $ between the EMF group and the SHAM group. Differences are only indicated once. For P values see text.

Overall, the parameters reflecting oxidative stress were affected in a complex way. EMF rats exhibited decreased TBARS concentration in the striatum, hippocampus, and increased SOD in the striatum. EMF-rats exhibited decreased TBARS in the prefrontal cortex, and decreased CAT in the striatum.

4. Discussion

4.1. Behavioral findings

The objective of this study was to examine the impact of 900 MHz radiofrequency exposure and of combining iron overload and 900 MHz exposure on spatial cognition and a number of biochemical parameters including monoamine content and oxidative stress markers. The main findings are (1) iron overload did not amplify the effects of 900 MHz EMF, (2) the behavioral effects of 900 MHz EMF were task-dependent, (3) EMF exposure (but not combined EMF exposure and iron treatment) affected monoamine neurotransmission (DA and 5HT) mainly in the hippocampus, (4) none of these two treatments produced global oxidative stress.

Rats exposed to 900 MHz EMF showed no deficit in the water maze navigation and radial arm maze tasks but were impaired in the object exploration task. Since rats were not able to detect both spatial or non-spatial novelty, our results suggest a general memory deficit [47,48]. The deficits may also result from increased anxiety. We did not specifically address this issue but the data are not consistent with such an explanation. The amount of locomotion and object exploration which are generally affected in anxiety-like behavior [49,50] were not different from those of control rats in habituation sessions. In addition, thigmotaxis behavior that is associated with anxiety [51] was not seen in this task as well as in the water maze navigation task. It is somewhat surprising that EMF-exposed rats were not impaired in the water maze navigation and radial arm maze tasks which also require general memory processes. Since the animals were overtrained in these tasks it is possible that redundant storage of information in the brain allowed preserved performance. In contrast, in the object exploration task, the memory deficit would be expressed because the amount of exposure to the environment was not sufficient for inducing compensatory processes. An alternative hypothesis is that the 900 MHz exposure would affect processes that are specific to the detection of novelty but are not necessary in the water maze or radial-arm maze tasks. We cannot strictly rule out this possibility but the formation of memory in these tasks is thought to involve common general processes (encoding of spatial and non-spatial information, match-mismatch processes underlying detection of novelty [e.g., [52–54]]). In addition, the hippocampus has been shown to be crucial for performing all these tasks [47,55,56]. The nature of motivation underlying learning in these tasks may be also an important factor that would influence learning and memory formation. The objects exploration task which involves spontaneous exploratory activity appears to be more sensitive to various harmful agents and may therefore be more suitable to detect cognitive deficits than other learning tasks. Consistent with this hypothesis, we found similar differential behavioral impact of a combination of iron overload treatment and 150 KHz EMF exposure [31]. Overall, our results suggest that 900 MHz EMF exposure disrupted general
memory processes in a spontaneous spatial and non-spatial object recognition task. Comparing these results with those of the electromagnetic literature is difficult since variations in the exposure and other parameters (species, strain, etc.) can account for differences. For example, GSM EMF have been shown to spare memory in an object recognition task in rats ([19] 900 MHz, Sprague-Dawley rats, acute head-only exposure) but not in mice ([57], 1800 MHz, C57BL/6 mice, chronic whole-body exposure). A deleterious effect of EMF on working memory in a radial-maze task was found in some studies ([14], 2450 MHz; [58], 916 MHz) but not in others ([17], 2450 MHz; [19], 900 MHz; [59], 900 MHz), EMF exposure induced place navigation learning deficits in the Morris water maze ([15], 2450 MHz, post-natal exposure; [60], 840 MHz, adult exposure) but here we show a lack of effects.

Interestingly, in a previous study rats that received an iron overload treatment were also impaired in the object exploration task but their deficit was different from that of EMF-exposed rats in the present study as they were not able to detect the spatial change but did detect the non-spatial change [31]. Here, combining 900 MHz EMF with iron overload did not induce a degradation of the performance in all 3 tasks relative to iron-treated rats (referring to the rats of the Maaroufi et al’s study [31]) or 900 MHz EMF-exposed rats. Surprisingly, in the object exploration task the combined treatment induced even less pronounced deficits than 900 MHz EMF-exposed rats since they detected the spatial change (although they were unable to identify the precise object displacement). Interestingly, a similar paradoxical effect was found when combining 150 kHz EMF and iron overload. Rats with combined treatment showed weaker deficits than iron treated rats [31]. Overall the results suggest that not only combining EMF and iron overload did not induce potentiated effects but it also neutralized the deleterious effects of each treatment separately. To our knowledge, such an effect has not been previously described in the literature. How could an interaction between EMF and iron overload have beneficial effects on learning? Possible mechanisms may involve a direct effect on learning and memory perhaps through synaptic plasticity but may also involve an effect on anxiety, stress, attention or some other mechanism. The existing literature on EMF and iron overload is poor and does not allow us to propose a realistic hypothesis to account for this effect. It remains that the object exploration task that favors the expression of a learning and memory restoration as we showed here and in the Maaroufi et al.’s study [31] could be further exploited to investigate the possible cellular and molecular correlates of such a restoration.

4.2. Neurochemical findings

Analysis of monoamines in four brain regions (cerebellum, striatum, hippocampus, and prefrontal cortex) in the rats revealed an effect of treatments. EMF exposure induced a decrease in DA (while the turnover ratio, an index of dopaminergic activity, was not affected), and 5HT levels in the hippocampus. The DA innervation of the hippocampus has been shown to be involved in synaptic plasticity [61] and at a behavioral level, to be implicated in the encoding of spatial information in a novel environment [62–64]. The 5HT system is considered to influence cognitive processes via its interactions with other neurotransmission systems, i.e. cholinergic, glutamatergic, dopaminergic or GABAergic systems [65,66]. Thus, some manipulations of the serotoninergic system have little impact on memory [66]. However, it has been shown in studies using the same type of task as here, i.e. spontaneous object/spatial recognition task, that manipulations of the 5HT system have a clear effect, either beneficial or deleterious, on object recognition ([67] for a review, [68]). In addition, a correlation between 5HT level alterations in the hippocampus and deficits in spatial and non-spatial processing has been recently reported [69]. These data thus support the hypothesis of an influence of 5HT on cognitive processes although the mechanisms underlying this influence are still unclear. The results also show an alteration of the 5HT system (5HT content was increased and the turn-over was decreased) in the cerebellum in both rats with EMF and combined EMF and iron overload. However, there is so far no evidence of a relationship between an alteration of cerebellar 5HT and the observed behavioral deficits and this remains to be clarified.

Combining EMF and iron overload treatments reversed the decrease in DA and 5HT observed in the hippocampus of 900 MHz EMF-exposed rats, an effect which may be paralleled with their attenuated behavioral deficits in the object exploration task. DA level was higher in the striatum, an alteration that may perhaps explain the inaccurate novelty detection [70]. To our knowledge few studies have measured DA and 5HT metabolism following EMF exposure. Inaba et al. [7] found no modification of DA content in any of the brain regions but an increase of the turnover ratio. The lower 5HT content in the hippocampus was not reported either. Overall, the main finding is that the EMF-exposed group showed a decrease in both DA and 5HT in the hippocampus whereas the rats with combined treatment showed levels similar to control rats. Our results are consistent with the hypothesis of a link between the monoamine system in the hippocampus and the ability to detect and process spatial and non-spatial novelty. Further studies are nevertheless required to investigate this possibility.

EMF may have adverse effects on brain tissues by increasing free radicals which enhance lipid peroxidation. Oxidative stress may then affect cognitive processes and result in behavioral deficits. Evaluation of oxidative stress in the brain involved measurement of TBARS concentration reflecting Malondialdehyde content, a product of lipid peroxidation, and measurement of antioxidant defense systems including SOD and CAT activity. SOD catalyzes the degradation of the endogenously produced superoxide anion in oxygen and hydrogen peroxide which is then degraded by CAT in oxygen and water. Most animal studies that tested the impact of microwave EMF on the brain have reported an increase in lipid peroxidation and a decrease in antioxidant activity, which is indicative of oxidative stress [71–76]. Our study can be distinguished from the studies of the literature since we evaluated oxidative stress in different brain regions (cerebellum, striatum, hippocampus, and prefrontal cortex) rather than in the whole brain. Doing this we did not observed an increase but instead a decrease in lipid peroxidation that was restricted to the striatum and hippocampus in EMF-IO rats and to the prefrontal cortex in EMF rats. Note that this decrease was larger in EMF rats. Antioxidant activity was diminished in the striatum for the two treated groups: SOD was lower in EMF rats and CAT in EMF-IO rats. A possible mechanism is that EMF would induce reductive stress, an aberrant increase in reducing power that produces metabolic alterations leading to several pathologies [77,78]. When EMF is combined with iron overload, most of the oxidative stress effects of iron overload would be neutralized, suggesting that under reductive stress condition, iron overload is no longer able to induce oxidative stress. This hypothesis remains to be specifically examined as it has never been shown so far that reductive stress can occur in the brain. Overall, our results indicate that although EMF-exposed rats and EMF-IO rats showed some alteration of their oxidant status, they did not show general oxidative stress in the brain as described in the existing literature. Subsequently, it appears difficult to explain the observed behavioral deficits on the basis of this alteration.

It is also difficult to explain why we did not obtain similar oxidative stress as in previous studies. A first possibility would be related to the differences between studies in SARs and duration of daily or overall EMF exposure. Some studies report oxidative stress following a similar [72] or even shorter [73] exposure duration. Another critical parameter may be the species or the strain. We used Long Evans rats whereas other studies used Wistar rats
[73,79], guinea pigs [74] or rabbits [80] that may show different neurochemical responses to similar treatments. There may be also species–dependent differences in SARs.

Hence, our results show that 900 MHz EMF induces cognitive deficits but these deficits are not revealed in all spatial tasks. Rats were impaired in the object exploration task only. Iron overload did not potentiate the effects of EMF in any of the tasks but produced attenuated deficits in the object exploration task. In addition our results suggest a relationship between the behavioral deficits and the EMF-induced changes in biogenic monoamines. In contrast, EMF had a weak impact on oxidative stress. These results do not of course demonstrate and a fortiori characterize the relationship between cellular/molecular alterations and cognitive/behavioral deficits which needs further work. Our study suggests that it is crucial to test animals in multiple behavioral tasks and in parallel evaluate different biological parameters to elucidate the mechanisms which determine the occurrence of cognitive deficits following EMF exposure. It is also necessary to investigate more systematically the effects of the exposure parameters on behavior and biochemical markers. These parameters usually differ from a study to another, impeding generalization of the exposure effects. Manipulating these parameters as the animals are submitted to a behavioral protocol taxing various aspects of memory and cognition should allow to improve our understanding of the impact of mobile phone EMF on cognition.

Acknowledgements

The authors thank Dr Philippe Pignet of the Centre Comm de Ressources en Micro-ondes (CCM), Laboratoire d’Electromagnétisme, Compatibilité Electromagnétique et Hyperfréquences in Marseille for kindly providing the irradiation apparatus and Dr René de Sèze from the Institut National de l’Environnement Industriel et des Risques (Verneuil-en-Halatte, France) for advice on SAR calculation. Funding was provided by the CNRS and Aix-Marseille University.

References


Save E, Pouret B, Foreman N, Buhot M-C. Objects exploration and reaction to a spatial and a non-spatial change in the rat following damage to the posterior parietal cortex or the dorsal hippocampus. Behav Neurosci 1992;106:447–56.


Pruit L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003;463:3–33.


Lemon N, Manahan-Vaughan D. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. J Neurosci 2006;26:7723–9.


