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Effects of co-exposure to 900 MHz radiofrequency electromagnetic fields and high-level noise on sleep, weight, and food intake parameters in juvenile rats

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1 Effects of co-exposure to 900 MHz radiofrequency electromagnetic
2 fields and high-level noise on sleep, weight, and food intake
3 parameters in juvenile rats

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Abstract

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Objective: Electrohypersensitive people attribute various symptoms to exposure of radiofrequency electromagnetic fields (RF-EMF); sleep disturbance is the most frequently cited. However, laboratory experiments have yielded conflicting results regarding sleep alterations. Our hypothesis was that exposure to RF-EMF alone would lead to slight or non-significant effects but that co-exposure to RF-EMFs and other environmental constraints (such as noise) would lead to significant effects.

Methods: 3-week-old male Wistar rats (4 groups, n=12 per group) were exposed for 5 weeks to continuous RF-EMF (900 MHz, 1.8 V/m, SAR=30 mW/kg) in the presence or absence of high-level noise (87.5 dB, 50-20000 Hz) during the rest period. After 5 weeks of exposure, sleep (24 h recording), food and water intakes, and body weight were recorded with or without RF-EMF and/or noise. At the end of this recording period, sleep was scored during the 1h resttime in the absence of noise and of RF-EMF exposure.

Results: Exposure to RF-EMF and/or noise was associated with body weight gain, with hyperphagia in the noise-only and RF-EMF+noise groups and hypophagia in the RF-EMF-only group. Sleep parameters recording over 24 h highlighted a higher frequency of active wakefulness in the RF-EMF-only group and a lower non-rapid eye movement/rapid eye movement sleep ratio during the active period in the noise-only group. There were no differences in sleep duration in either group. During the one-hour, constraint-free sleep recording, sleep rebound was observed in the noise-only group but not in the RF-EMF-only and RF-EMF+noise groups.

Conclusion: Our study showed effects of RF-EMF, regardless of whether or not the animals were also exposed to noise. However, the RF-EMF+noise group presented no exacerbation of those effects. Our results did not support the hypothesis whereby the effects of RF-EMF on physiological functions studied are only visible in animals exposed to both noise and RF-EMF.

Keywords: RF-EMF, noise, juvenile rat, sleep, food intake

1. Introduction

50 Various symptoms (headaches, fatigue, difficulty concentrating, skin manifestations, and
51 tinnitus) have been reported with regard to (and then attributed to) radiofrequency
52 electromagnetic field (RF-EMF) exposure in people living close to mobile phone base stations
53 (Santini et al., 2002) and in people suffering from electrohypersensitivity (EHS) (Johansson et
54 al., 2010; Knave et al., 1992). EHS is a phenomenon characterized by the perception from
55 subjects of non-specific symptoms, without biological explanations and attributed by the
56 subjects themselves at electromagnetic fields exposure. Although sleep disorders are the most
57 frequently mentioned symptoms (Berg-Beckhoff et al., 2009), a possible effect of RF-EMF on
58 sleep is subject to debate (Mohler et al., 2012). There is also controversy with regard to the
59 results of intermittent exposure to RF-EMF (i.e. 15 on-off cycles at 900 MHz over 8 h) on
60 adult in laboratory, which was associated with a significant decrease in rapid eye movement
61 (REM) sleep but no changes in the other sleep states. In rodents, chronic RF-EMF exposure (5
62 weeks at 900 MHz 1.0 V/m) induced REM fragmentation (Pelletier et al., 2013), whereas
63 acute exposure (at 1.8 or 2.45 GHz for 24 h) did not alter sleep (Crouzier et al., 2007).

64 Some studies involved co-exposure to RF-EMF and another environmental physical
65 constraint (Pelletier et al., 2013) or chemical constraint (Occelli et al., 2018). The results
66 showed that the effects of RF-EMF were amplified (to a variable extent) by the other factors,
67 relative to RF-EMF exposure alone. In view of these observations and the ongoing debate, we
68 checked that exposure to RF-EMFs alone, at the level typically encountered in everyday life
69 (measurements in France showed a mean level around 0.36 V/m and 90% of measurements
70 not exceeded 1.6 V/m, (ANFR 2018)), leads to small or non-significant effects, whereas
71 synergy between RF-EMF and another environmental constraint would led to statistically
72 significant physiological effects.

73 In our study, the other environmental constraint chosen was the noise. In fact, noise is a major
74 everyday environmental constraint. People may be exposed to noise (and RF-EMFs) in the
75 workplace and/or during leisure activities. It has been reported that noise disturbs sleep (Blois
76 et al., 1980; Fruhstorfer et al., 1988) and metabolism (Axelsson and Lindgren, 1985; Pyko et
77 al., 2015). Indeed, sleep perturbation may cause metabolic disorders (Spiegel et al., 1999).

78 Although exposure to noise or RF-EMF in everyday life is continuous (i.e. chronic), most
79 laboratory studies investigated acute exposure. Moreover, most of these studies were
80 performed on adults. In fact, juvenile life is a vulnerable period because of (i) the ongoing
81 development of physiological and neurologic functions, and (ii) the greater RF-EMF

82 penetration into a juvenile' tissues (including the brain), relative to the adult (Wiart et al.,
83 2011). To the best of our knowledge, the present study is the first to analyze the impact of co-
84 exposure to RF-EMF and high-level noise. Other novel aspects of the present study are the
85 chronic nature of the exposure, the fact that the experiments were performed on juvenile
86 animals, and the recording different periods (including a non-exposed period after period of
87 exposure).

88

2. Material and Methods

89 2.1 Animals

90 Experiments were conducted on 48 male Wistar rats (Janvier Labs, Le Genest Saint Isle,
91 France) weighing between 55 and 85 g and aged 3 weeks at the time of their arrival in our
92 facility (on day (D)1). Four groups of 12 animals were formed: a group exposed to noise only,
93 a group exposed to an RF-EMF only, a group co-exposed to RF-EMF and noise, and a control
94 (non-exposed) group. The groups were housed in anechoic chambers with a 12 h:12 h
95 dark/light cycle (lights on at 6 am and off at 6 pm) and controlled thermoneutral air
96 temperature (24 ± 1 °C), relative air humidity (mean \pm standard deviation (SD): $39 \pm 12\%$), and
97 air velocity (<0.2 m/s). Rats were individually housed in plastic cages (425 mm x 266 mm x
98 185 mm) within the chamber. Standard chow (3436EXF12, Serlab, Montataire, France) and
99 water were available *ad libitum*. Daily animal care was performed between 5 and 6 pm; the
100 RF-EMF and noise were turned off during this period. All experiments were performed in
101 accordance with the European guidelines (2010/63/EU) and the French governmental decree
102 2013-118 on the care and use of laboratory animals. The study protocol was approved by the
103 nationally accredited Regional Directorate for Health, Animal and Environment Protection
104 (Amiens, France) and the French Ministry of Research (license number: APAFIS#3735-
105 2016012017118094 v3).

106 Depending on the group, exposure began on D5 and ran for 5 weeks until sacrifice (i.e.
107 exposure from D5 to D41).

108 2.2 Exposure to an RF-EMF

109 The RF-EMF exposure started after four days of acclimatization. The climatic chambers were
110 equipped with RF-EMF antennas (model 800–10465, KATHREIN-Werke KG, Rosenheim,
111 Germany) powered by a generator (model RFS 900–64, RFPA, Artigues-près-Bordeaux,
112 France) emitting a 900 MHz continuous-wave EMF. Antennas were located horizontally in
113 the climatic chamber, 80 cm above the exposed rats' boxes. The generator's power was set to
114 obtain a field intensity of 1.8 ± 0.6 V/m. The animals' specific absorption rate was calculated
115 with a mean value of 30 mW/kg (calculated at 3 and 8 weeks of age for three parts of the
116 animal: head, body, and tail). The level of RF-EMF exposure was checked once a week with a
117 radiofrequency probe (PMM EP600, Narda Safety Test Solution, Hauppauge, NY, USA) and
118 monitored with computer software (Win EP 600, Narda Safety Test Solution). The RF-EMF
119 exposure ran for 23 h per day.

120

121 *2.3 Noise exposure*

122 Noise-exposed animals were subjected to a 24 h soundtrack divided into two main periods: a
123 noisy light period (the rat's rest period) from 6 am to 5 pm, and a noise-free dark period from
124 6 pm to 6 am. During the noise-free period and the handling period, the intensity of the
125 background noise was 65 dB.

126 The noise exposure period was split into 10 min segments, each of which had a unique
127 combination of noise types, frequencies and intensities. The noise types were urban sounds
128 (traffic, roadworks, sirens, etc.), music, and artificial sounds (white noise, red noise,
129 sinusoids, etc.). The mean \pm SD (range) sound level was 87.5 ± 3.7 dB (59–111). The
130 frequencies ranged from 50 to 20,000 Hz. To avoid habituation, four different 24 h noise
131 exposure files were built out of 10-minute segments arranged in a pseudo-random order. Each
132 day's noise exposure file was chosen in a pseudo-random manner. The noise was regulated by
133 an amplifier (Combo 130, Audiophony Hit Music SAS, Fontanes, France) and delivered
134 through loudspeakers (K50-8 Ohm, JBSystems, Groot-Bijgaarden, Belgium).

135 *2.4 Protocol*

136 After 4 days of habituation to the housing conditions, animals were exposed (or not) to noise
137 and/or RF-EMF for 5 weeks (until D41). On D26 or D27, a telemetric sensor was surgically
138 implanted in each animal. From D33 to D39, food intake, water intake and body weight data
139 were recorded. Electroencephalography (EEG) and electromyography (EMG) were recorded
140 for one day (D34) with exposure and for one hour (D39) without exposure. At D41, the rats
141 were sacrificed by a heart puncture under general anesthesia with a mixture of air and 2.5%
142 isoflurane (Iso-Vet 1000 mg/g, Piramal Healthcare UK Ltd, Morpeth, United Kingdom).

143 *2.5 Surgery*

144 At the beginning of the 4th week, a subcutaneous telemetric EEG and EMG sensor (F20-EET,
145 Data Sciences International, St. Paul, MN, USA) was implanted under general anesthesia with
146 isoflurane (5% for induction, and then 2.5% during surgery). To record the EEG signal, two
147 gold-plated screws (15 SURTEX, Dentatus AB, Spånga, Sweden) were inserted into the
148 animal's skull, just above the dura matter. Dental cement (Dentalon, Henri Schein, Alfortville,
149 France) was used to fix the probe and isolate the EEG signal. The EMG signal was recorded
150 from two probes were inserted into the dorsal muscles of the neck. After surgery, animals
151 were allowed to recover for 5 days with or without exposure in accordance with their group.

152

153 *2.6 Data acquisition and analysis*

154 *2.6.1 Body weight*

155 The animals' body mass was measured every day using scales (Scaltec SPO-62, Scaltec
156 Instruments, Göttingen, Germany; sensitivity: 0.1 g). Due to difference of weight between the
157 4 groups of animals, the weight gain (in grams) was calculated as follows: weight gain =
158 Weight at D_x – Weight at D_1 .

159 *2.6.2 Food and water intakes*

160 Daily food intake was scored automatically with individual scales (Sartorius TE601, Sartorius
161 Lab Instruments GmbH; sensitivity: 0.1 g). The time, the duration (in seconds) and the
162 quantity of each meal (in grams) were recorded using in-house software. Water intake was
163 measured daily, using individual nursing bottles (sensitivity: 5 mL). The ratios between the
164 total quantity of food eaten and the animal's body weight was calculated.

165 Due to technical problems, food and water intakes was not calculated for 1 of the 12 animals
166 in the RF-EMF group. The food and water intakes were calculated for the other 11 animals in
167 the RF-EMF group, and for all animals in the noise-exposed, RF-EMF+noise, and control
168 groups.

169 *2.6.3 Sleep*

170 The EEG and EMG sensors were connected wirelessly to a receiver (RPC-1, Data Sciences
171 International) and a computer via a matrix (Data Exchange Matrix, Data Sciences
172 International). Data were recorded using Ponemah software (Data Sciences International).

173 Recordings were scored visually every 4 seconds with Spike2 software (version 7.01,
174 Cambridge Electronic Design, Cambridge, UK) as active wakefulness (AW), quiet
175 wakefulness (QW), non-rapid-eye-movement (NREM) sleep, and REM sleep. Quiet
176 wakefulness was defined as a short episode (lasting between 8 s and 2 min) of wakefulness
177 within a sleep episode, and during which the animal did not move or eat (Pelletier et al.,
178 2013). For NREM and REM sleep, the total amount (in min per 24 h), episode frequency (per
179 hour), mean episode duration (in min) and proportion of total sleep time (TST, in %) were
180 calculated.

181 Due to occasional technical problems, sleep parameters were calculated for 11 of the 12
182 animals in the RF-EMF-only group, 10 of the 12 animals in the noise-only group, all 12
183 animals in the RF-EMF+noise group, and all 12 animals in the controls.

184 *2.6.4 Statistical analysis*

185 Data (expressed as the mean \pm SD) were analyzed using Statview software (version 5.0, SAS
186 Institute Inc., Cary, NC, USA). When the data were normally distributed (according to a
187 Kolmogorov-Smirnov test), a two-way analysis of variance (with Fisher's partial least-squares
188 difference post-test) was applied. Simple effects of RF-EMF exposure (i.e. RF-EMF \pm noise
189 vs no RF-EMF \pm noise), of noise exposure (i.e. noise \pm RF-EMF vs no noise \pm RF-EMF) and
190 interaction RF-EMF x noise were analyzed. If the data were non normal (e.g. food and water
191 intake parameters), a Kruskal-Wallis and a Mann-Whitney tests were used. Comparisons of
192 the RF-EMF+noise group vs. the control group were also performed using a non-parametric
193 test. The threshold for statistical significance was set to $p < 0.05$.

194

3. Results

195 *3.1 The animals' body weight*

196 The body weight gain (related to weight on the day of arrival) of the RF-EMF-exposed, noise-
197 exposed and RF-EMF+noise animals was higher than that in controls (control: 210.5 ± 25.5 g,
198 RF-EMF-exposed: $231.9.5 \pm 27.6$ g, $p < 0.001$ vs controls; noise-exposed: 222.7 ± 28.8 g,
199 $p = 0.018$ vs controls; and RF-EMF+noise: 223.5 ± 23.3 g, $p = 0.005$ vs controls). Body weight
200 gain was significantly lower in the noise-exposed group than in the RF-EMF exposed group
201 ($p = 0.037$).

202 *3.2 Food and water intake on 24 h*

203 **Animals exposed to noise only** ate more than controls did, regardless of the nycthemeral
204 period. This result was due to a higher total quantity of food, a greater number of meals, and a
205 longer mean meal duration. Moreover, the noise-exposed animals drank more than controls
206 did (Table 1).

207 In contrast, **animals exposed to an RF-EMF only** ate less than controls due to a lower
208 number of meals and a shorter mean meal duration. However, the quantity of food relative to
209 the body weight is similar to controls. As in the noise-only group, the animals in the RF-
210 EMF-only group drank more than controls did.

211 **The animals in the RF-EMF+noise** group tended to eat more than controls did, due to a
212 higher mean meal quantity, a longer mean meal duration but a lower number of meals over 24
213 h. Compared with the noise-only group, animals in the RF-EMF+noise group ate less food in
214 total and had fewer meals. Compared with animals exposed to RF-EMF only, the total
215 quantity and meal duration were higher in animals in the RF-EMF+noise group. The water
216 intake for RF-EMF+noise animals did not differ significantly from that of the controls, but
217 was lower than in animals exposed to noise only or an RF-EMF only.

218 *3.3 Sleep measured over 24 h with exposure*

219 The **effects of noise** (independently of RF-EMF exposure, significant interaction between
220 noise exposure and light/dark periods) were only significant during the dark period (i.e.
221 during the recovery from noise exposure) (Table 2): the total durations of NREM sleep and
222 QW were lower in the noise group (-22.6 min, $p = 0.01$ and -6.8 min, $p = 0.03$, respectively) and
223 tended to be compensated by a higher total duration of AW and REM ($+24.2$ min, $p = 0.08$ and
224 $+5.2$ min, $p = 0.1$, respectively), so that the relative duration of NREM sleep (relative to the
225 TST) fell and the relative duration of REM sleep rose ($p = 0.004$).

226 **Exposure to RF-EMF** (independently of noise exposure, significant effect of RF-EMF
227 exposure alone) significantly induced a higher frequency ($p=0.029$) and a shorter mean
228 duration ($p=0.012$) of AW episodes, so that the total duration did not differ significantly. In
229 contrast to the effects of noise, the results in the RF-EMF group were not dark/light-
230 dependent.

231 **The parameters in the RF-EMF+noise group** did not significantly differ from those
232 measured in the three other groups. Interestingly, the only sleep parameters influenced by a
233 significant interaction between the noise exposure and the RF-EMF exposure was the mean
234 duration of NREM sleep episodes: in contrast to the RF-EMF+noise group, the RF-EMF
235 exposure alone and the noise exposure alone had longer NREM sleep episodes (respectively
236 $p=0.048$ and $p=0.006$ vs the control group) (Table 2).

237 *3.4 Sleep measured over 1 h without exposure*

238 **Noise exposure** (independently of RF-EMF exposure, significant effect of noise exposure
239 alone) induced significant effects during the 1 h-long, noise- and RF-EMF-free in the daytime
240 (Table 3). In the noise-only group, the TST was significantly longer ($p=0.036$) as a
241 consequence of a longer duration of NREM ($p=0.05$) and REM ($p=0.05$) and a shorter AW
242 duration ($p=0.02$), despite a slight increase in the total duration of QW ($p=0.014$). There were
243 no statistically significant differences in REM and NREM but there was a trend towards a
244 higher percentage of REM sleep and a lower percentage of NREM sleep, relative to animals
245 not exposed to noise.

246 **Exposure to an RF-EMF alone** did not lead to differences vs. the control group.

247 **Sleep variables in the RF-EMF+noise group** did not differ from those measured in the
248 noise-only or RF-EMF-only groups.

249

4. Discussion

250 *4.1 Weight*

251 Our results showed that whatever the type of exposure (RF-EMF and/or noise), exposed
252 animals gained more body weight than controls. The RF-EMF-only group gained
253 significantly more weight than the noise-only group. The association between higher body
254 weight and noise exposure has already been described in rodent models (Mavanji et al., 2013;
255 Parrish and Teske, 2017). Likewise, RF-EMF exposure induced weight variations (+6%) in an
256 adult rodent model after chronic exposure (280 days) to a 900 MHz RF-EMF (intensity not
257 indicated) (Sommer et al., 2004); however, the RF-EMF literature is very sparse in this
258 respect. Despite a greater body weight gain in animals in the RF-EMF+noise group, we did
259 not observe a specific effect of co-exposure to an RF-EMF and noise; since there were no
260 significant differences between the RF-EMF+noise group on one hand and the RF-EMF-only
261 group and noise-only groups on the other. Several literature studies have demonstrated that
262 stress and/or loss of sleep can lead to weight gain and changes in food intake (von Kries et al.,
263 2002; Michel et al., 2003; Spiegel et al., 2004). Even though the sleep alterations associated
264 with our environmental exposures were small (see below), we cannot rule out a possible
265 involvement in the greater body weight in the RF-EMF+noise group.

266 *4.2 Food and water intake*

267 Depending on the type of exposure, greater body weight was not always related to
268 hyperphagia. We observed that the noise exposure alone induced hyperphagia; this is
269 consistent with the literature (such as exposure of rodents to 85 dB for 9 days, described by
270 Mavanji et al. These effects are probably due to the intensity or chronicity of the exposure,
271 given that acute exposure (for 12 h) in another study did not induce modifications in the food
272 intake (Parrish and Teske, 2017).

273 Conversely, RF-EMF exposure alone did not induce a variation in the total quantity of food
274 ingested – only the pattern of intake was modified. In a previous study in our laboratory, we
275 did not observe any significant variations in the total quantity of food ingested with RF-EMF
276 exposure in the same condition (Pelletier et al., 2013). Thus, a chronic exposure to RF-EMF
277 alone did not modify the food intake. Interestingly, in this previous study, the total food intake
278 was greater when RF-EMF exposed animals were housed at an ambient temperature of 31 °C
279 during one day (relative to non-exposed animals housed at the same temperature). However,
280 in our present study, the total food intake in the group chronically exposed to RF-EMF and
281 noise was similar to that in the control group - even though the food intake pattern was

282 different. The intake in the RF-EMF+noise group showed some characteristics of noise
283 effects: i.e. a lower number of meals and a longer meal duration. In contrast to our starting
284 hypothesis, exposure to both an RF-EMF and noise was not associated with accentuation of
285 the effects of noise alone or of RF-EMF alone with regard to food intake variables.

286 Exposure to noise alone or to an RF-EMF alone induced a greater water intake than in
287 controls. Alterations in water intake have not previously been studied in RF-EMF-exposed
288 animals. Surprisingly (and as for the total food intake), the water intake of animals exposed to
289 both an RF-EMF and noise was similar to that of controls and was lower than that of animals
290 exposed to noise alone or an RF-EMF alone. To the best of our knowledge, this antagonistic
291 effect of RF-EMF vs. noise has not previously been described for food intake or weight gain
292 parameters.

293 *4.3 Sleep parameters*

294 Surprisingly, noise exposure (with and without RF-EMF exposure) did not induce any
295 significant sleep alterations during the daytime-resting period (i.e. when noise was present) –
296 suggesting that the sleep need was predominant and/or the environmental constraint was not
297 great enough to reduce sleep duration. However, during the nighttime (active, non-noisy)
298 period, the animals in the noise-only group displayed a lower mean duration of NREM sleep
299 (with no change in TST), a shorter QW duration, a lower NREM/REM ratio and a higher AW
300 and REM duration. Noise exposure alone induce a higher mean duration of NREM episodes,
301 independently of the light and dark periods. The absence of a difference in TST is surprising,
302 given that two other studies of noise-exposed rats observed a lower TST during the daytime-
303 resting period (i.e. when noise was present) (Mavanji et al., 2013; Parrish and Teske, 2017).
304 Even though these studies used much the same protocol as we did (i.e. noise during the light
305 period, and sleep assessment during the dark and light periods), the overall period of exposure
306 (85 dB, for 9 h during the daytime) was shorter (1 to 9 days).

307 During the second recording period (a one-hour period outside the noise exposure chamber),
308 the noise-exposed group displayed a higher TST (8 min more than in a control group) and
309 lower amount of AW (-10.2 min less than control group). This is a typical sleep rebound
310 phenomenon, and indicates greater sleep pressure in this group even though it was not
311 statistically significant during the light period with noise exposure. The absence of sleep
312 perturbation might have been due to a strong circadian influence independently of sleep
313 (Borbely 1982). It might also have been due to the time distribution of the sleep during the
314 dark (resting) period; a greater TST was observed during the first part of the night - as soon as

315 noise exposure stopped – but did not achieve statistical significance over the whole 12 h dark
316 period. The lack of significant active time after-effects on TST and the greater TST during the
317 one hour-recording without noise exposure might be due to the difference in the time interval
318 between sleep onset and the end of the noise exposure (30 min for the one-hour session but 60
319 min for the 24 h recording). Averaging TST over the 12 h active period might mask effects
320 occurring in the first few hours of the following active period (Bach et al. 1991). The
321 nighttime effects on the chosen sleep parameters also evidenced (in line with a rebound
322 effect) the invisible effects of noise exposure during the daytime.

323 Regardless of the noise exposure, the animals exposed to RF-EMF with or without noise,
324 showed a slight fragmentation of AW (a higher episode frequency and a lower mean duration
325 of episodes) but no change in the total AW duration. However, the animals exposed to RF-
326 EMF alone has a higher mean duration for NREM sleep episodes. Some literature data have
327 highlighted changes in sleep architecture with RF-EMF exposure, although REM was mainly
328 affected. Acute RF-EMF exposure (900 MHz for 8 h) in humans was associated with a shorter
329 sleep latency and a significant lower duration of REM sleep (Mann and Röschke, 1996;
330 Wagner et al., 1998). The chronic exposure performed in our laboratory (5 weeks) was
331 associated with REM sleep fragmentation (Pelletier et al., 2013). However, the effects of RF-
332 EMF on sleep remain subject to debate.

333 Co-exposure to both environmental constraints (RF-EMF and noise) resulted in values similar
334 to those observed in controls for the 24 h-sleep recording and the 1 h (noise- and RF-EMF-
335 free) sleep period. There are several possible explanation for this finding. Firstly, one can
336 hypothesize that RF-EMFs protect against the harmful effects of noise exposure on sleep.
337 Secondly, the addition of a second factor might increase the overall level of stress and thus
338 trigger defense mechanisms that reduce sleep and homeostatic perturbations. A spectral
339 analysis might facilitate the analysis of these putative effects and could provide additional
340 insights.

5. Conclusion

341
342 Noise and RF-EMF are both environmental constraints but acted in different ways on the
343 parameters studied here. Firstly, noise exposure was associated with a greater body weight
344 gain and a higher food intake. Sleep parameters were altered indirectly (i.e. during the
345 nighttime noise-free period), with a lower NREM/REM balance but no change in the TST.
346 However, a rebound effect was observed (a higher TST, more REM sleep, and less AW)
347 when animals slept during a 1 h noise- and EMF-free period - indicating that sleep pressure
348 may have been increased.

349 The RF-EMF-only group showed a greater body weight gain. An assessment of sleep
350 parameters during the 24 h recording highlighted the fragmentation of AW (even though no
351 such effect was observed during the 1 h constraint-free period); this fragmentation argued in
352 favor of a direct effect of the RF-EMF. Animals exposed to an RF-EMF appeared to reduce
353 their energy expenditure by limiting REM sleep and increasing food intake.

354 Animals simultaneously to both noise and an RF-EMF were also heavier than controls,
355 although there was no difference in the total food quantity. Hence, co-exposure to noise did
356 not exacerbate the effects of RF-EMF exposure on juvenile rats. Indeed, most of the effects on
357 sleep in animals exposed to noise alone or RF-EMF exposure alone disappeared in co-
358 exposed animals. In contrast to our hypothesis, co-exposure did not accentuate the effects
359 observed with single exposures; this contrasts with the results of other studies of co-exposures
360 (i.e. ambient temperature changes and neuroinflammatory reactions) (Ocelli et al., 2018;
361 Pelletier et al., 2013).

362

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371

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441

Food and water intake parameters	Control group	Noise-exposed group	RF-EMF exposed group	RF-EMF+noise group
Total quantity of food (g)	18.5±2.4	21.2±3.0 ***##	18.1±6.9 **	19.6±4.1***
Total quantity of food/body weight	0.066±0.006	0.073±0.008 ***###	0.066±0.007	0.068±0.007 trend
Number of meals	12.2±2.2	13.4±2.6 **###	10.3±4.1 **	10.6±2.8 ***
Mean meal duration (s)	667.3±228.7	819.9±324.9 **	545.9±285.1 *###	806.9±375.2 **
Total quantity of water (mL)/24 h	28.9±6.7	36.2±2.7 ***#	33.4±9.0 ***#	30.5±7.6

442 **Table 1:** Food and water intakes (mean ± SD) in control (n=12), noise-exposed (n=12), RF-EMF-
443 exposed (n=11) and RF-EMF+noise-exposed (n=12) animals per 24 h. The total quantity of food eaten
444 was expressed in grams and relative to the body weight. A Mann-Whitney test was used to compare
445 each group with the control group (* p<0.05 ** p<0.01, *** p<0.001, trend 0.1<p<0.05) and the RF-
446 EMF+noise-exposed group (# p<0.05 ## p<0.01, ### p<0.001).
447

Parameters	Stages of vigilance	Period	Control group	Noise group	RF-EMF group	RF-EMF+noise group	Statistical analysis ^a
Total duration (min)	TST	Light (rest) period	469.3±3 6.0	485.8±3 6.2	491.5±3 5.6	481.9±42.7	
		Dark (active) period	284.8±2 9.9	267.4±3 6.4	288.2±3 0.7	272.1±53.5	
	NREM	Light (rest) period	417.4±2 9.3	438.5±3 3.5	441.4±2 9.6	438.6±35.0	
		Dark (active) period	225.3±2 6.0	202.7±2 7.5	227.1±2 1.3	204.2±36.9	Noise effect

) period					p=0.01 ^b
	REM	Light (rest) period	51.9±14 .6	47.3±15 .1	50.2±17 .2	43.3±13.5	
		Dark (active) period	59.5±8. 3	64.7±14 .0 trend	61.1±15 .2	67.9±18.8	Noise effect p=0.1 ^b
	AW	Light (rest) period	152.3±2 5.4	138.8±2 8.3	149.1±3 0.1	139.2±37.1	
		Dark (active) period	394.9±3 2.5	419.1±4 7.0	393.9±2 9.0	415.7±61.1	Noise effect p=0.085 ^b
	QW	Light (rest) period	98.4±29 .2	95.4±20 .1	79.4±17 .8	98.8±18.4	
		Dark (active) period	40.3±10 .3	33.5±11 .7	37.9±6. 7	32.2±9.2	Noise effect p=0.029 ^b
Relative duration (% of TST)	NREM	Light (rest) period	89.0±2. 7	90.3±2. 9	89.9±3. 0	91.1±2.4	
		Dark (active) period	79.0±2. 3	75.9±3. 7	79.0±3. 9	75.6±4.3	Noise effect p=0.004 ^b
	REM	Light (rest) period	11.0±2. 7	9.7±2.9	10.1±3. 0	8.9±2.5	
		Dark (active) period	21.0±2. 3	24.1±3. 7	21.0±3. 9	24.4±4.3	Noise effect

) period					p=0.004 ^b
Frequency (ep)	NREM	Light (rest) period	22.3±3. 2	19.9±2. 8	19.4±3. 6	22.0±5.1	
		Dark (active)) period	11.2±1. 7	8.7±2.2	11.2±1. 2	9.7±2.2	
	REM	Light (rest) period	4.6±1.3	4.2±1.5	4.3±1.1	4.1±1.1	
		Dark (active)) period	3.9±0.7	3.8±0.8	4.0±0.9	4.4±1.3	
	AW	Light (rest) period	1.3±0.5	1.0±0.4	1.3±0.5	1.6±0.9	RF-EMF effect p=0.029 ^c
		Dark (active)) period	1.5±0.4	1.6±0.9	2.0±0.8	1.7±0.5	
	QW	Light (rest) period	21.0±3. 3	18.9±2. 6	18.1±3. 6	20.5±4.7	
		Dark (active)) period	9.7±1.6	7.3±2.1	9.3±1.0	8.0±2.3	
Mean episode duration (min)	NREM	Light (rest) period	1.6±0.3	1.8±0.3	2.0±0.4	1.7±0.4	RF-EMF and noise interaction p=0.01 ^d
		Dark (active)	1.7±0.2	2.0±0.5	1.7±0.3	1.8±0.3	

) period						
REM	Light (rest) period	1.0±0.2	1.0±0.3	1.0±0.3	0.9±0.2		
	Dark (active) period	1.3±0.3	1.4±0.2	1.3±0.2	1.3±0.2		
AW	Light (rest) period	10.9±3. 7	12.1±3. 6	10.8±3. 5	9.1±4.6	RF-EMF effect p=0.012 ^c	
	Dark (active) period	23.9±7. 9	27.8±13. .2	18.0±6. 0	22.2±6.8		
QW	Light (rest) period	0.4±0.0 8	0.4±0.0 7	0.4±0.0 4	0.4±0.07		
	Dark (active) period	0.3±0.0 7	0.4±0.0 6	0.3±0.0 6	0.3±0.06		

448

449 **Table 2:** Sleep parameters (mean ± SD) in control (n=12), noise-exposed (n=10), RF-EMF-exposed
450 (n=11) and RF-EMF+noise-exposed (n=12) animals per 24 h, and split into light (rest/noise exposure)
451 and dark (activity/no noise) periods. AW: active wakefulness; QW: quiet wakefulness; NREM: non-
452 rapid-eye-movement; REM: rapid-eye-movement; TST: total sleep time. Statistical results from the
453 analysis of variance.

454 ^a: Column indicate results from ANOVA analysis.

455 ^b: *p*-values represent significant effect of noise exposure, only during the dark period, whatever the
456 presence of RF-EMF exposure.

457 ^c: *p*-values represent significant effect of RF-EMF exposure, whatever the considered period and the
458 presence of noise exposure.

459 ^d: *p*-value represents significant interaction between the two factors: RF-EMF exposure and noise
 460 exposure.

461

462

463

			Control group	Noise group	RF-EMF group	RF-EMF+noise group	Effect of noise ^a	Effect of RF-EMF ^b
Total duration (min)	sleep	TST	32.5±13.9	43.2±7.1	33.8±15.7	39.2±11.5	p=0.03	NS
		NREM	31.5±13.1	40.4±7.0	32.6±14.8	37.7±10.9	p=0.05	NS
		REM	1.0±1.5	2.8±2.3	1.2±2.1	1.7±1.3	p=0.05	NS
	wake	AW	22.2±16.3	9.8±8.5	22.5±17.1	14.1±13.8	p=0.02	NS
		QW	5.3±3.3	7.0±3.3	3.7±1.6	6.6±3.1	p=0.01	NS
Relative duration (% of TST)	sleep	NREM	97.4±3.5	93.6±5.6	97.2±4.3	96.3±2.8	NS	NS
		REM	2.6±3.5	6.4±5.6	2.8±4.3	3.7±2.8	NS	NS
Frequency (ep/h)	sleep	NREM	15.4±7.4	16.0±6.9	13.7±4.3	17.7±6.9	NS	NS
		REM	1.2±1.7	3.8±2.6	2.0±2.4	3.2±2.1	p=0.007	NS
	wake	AW	2.4±1.6	1.4±0.8	2.8±2.3	1.5±1.2	p=0.01	NS
		QW	13.1±6.9	14.9±6.8	10.8±4.5	16.1±6.5	NS	NS
Mean episode duration (min)	sleep	NREM	2.0±0.9	3.0±1.3	2.5±1.3	2.4±1.1	NS	NS
		REM	0.7±0.6	0.7±0.4	0.5±0.4	0.7±0.7	NS	NS
	wake	AW	9.4±12.6	5.7±6.1	13.3±17.0	9.0±6.6	NS	NS
		QW	0.4±0.2	0.5±0.2	0.3±0.1	0.4±0.1	NS	NS

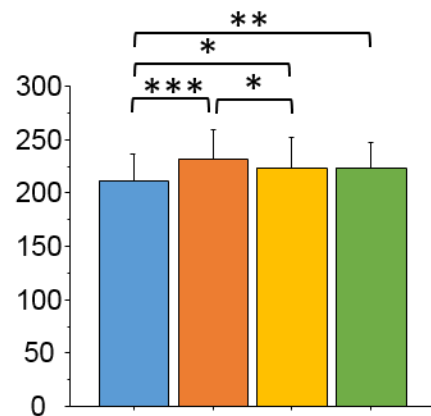
464 **Table 3:** Sleep parameters (mean ± SD) in control (n=12), noise-exposed (n=11), RF-EMF-exposed
 465 (n=10) and RF-EMF+noise-exposed (n=12) animals measured during a one-hour, RF-EMF- and noise-
 466 free. AW: active wakefulness; QW: quiet wakefulness; NREM: non-rapid-eye-movement; REM:
 467 rapid-eye-movement; TST: total sleep time. Statistical results from the analysis of variance.

468 ^a: *p*-values represent significant differences between the noise non-exposed group and the
 469 noise exposed group, whatever the presence of RF-EMF exposure (NS: non-significant).

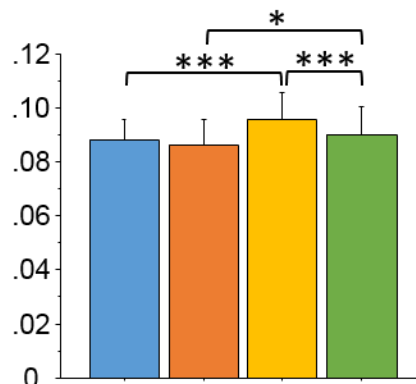
470 ^b: *p*-values represent significant differences between the RF-EMF non-exposed group and the
 471 RF-EMF exposed group, whatever the presence of noise exposure (NS: non-significant).

472

Body weight gain(g)

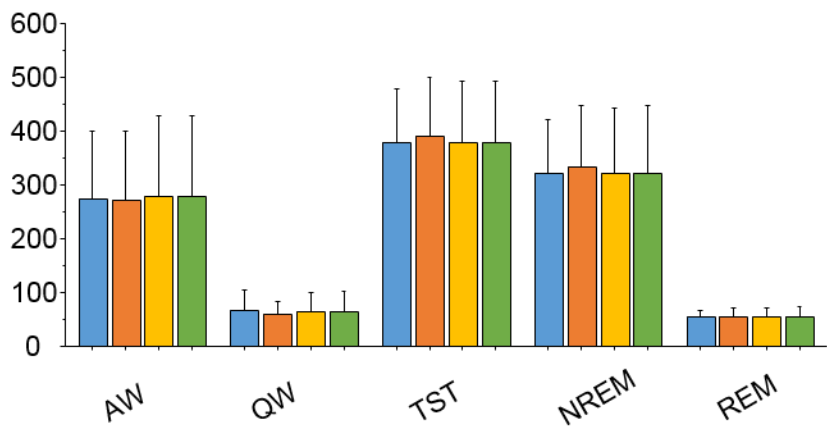


Total quantity of food ingested/
body weight gain per 24h

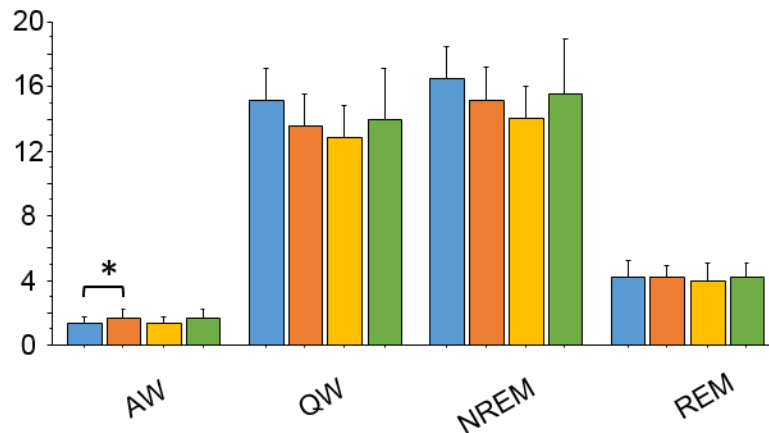


* p<0,05 ; ** p<0,01 ; *** p<0,001

Total duration of sleep stages (min)



Frequency of episodes (épisode/h)



Mean duration of episodes (s)

