Repeated exposure to nanosecond high power pulsed microwaves increases cancer incidence in rat

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Abstract

High-power microwaves are used to inhibit electronics of threatening military or civilian vehicles. This work aims to assess health hazards of high-power microwaves and helps define hazard threshold levels of modulated radiofrequency exposures such as those emitted by the first generations of mobile phones.

Rats were exposed to the highest possible field levels, under single acute or repetitive exposures for eight weeks. Intense microwave electric fields at 1 MV/m of nanoseconds duration were applied from two sources at different carrier frequencies of 10 and 3.7 GHz. The repetition rate was 100 pps, and the duration of train pulses lasted from 10 s to twice 8 min. The effects were studied on the central nervous system, by labelling brain inflammation marker GFAP and by performing different behavioural tests: rotarod, T-maze, beam-walking, open-field, and avoidance test. Long-time survival was measured in animals repeatedly exposed, and anatomopathological analysis was performed on animals sacrificed at two years of life or at death if earlier. One group was sham exposed.

Few effects were observed on behaviour. With acute exposure, an avoidance reflex was shown at very high, thermal level (22 W/kg); GFAP was increased some days after exposure. Most importantly, with repeated exposures, survival time was 4-month shorter in the exposed group, with eleven animals exhibiting a large sub-cutaneous tumour, compared to two in the sham group. A residual X-ray exposure was also present in the beam (0.8 Gy), which is not a bias for the observed result.

High power microwaves below thermal level in average, can increase cancer incidence and decrease survival time in rats, without clear effects on behaviour. The parameters of this effect need to be explored further, and a more precise dosimetry to be performed.

Introduction

High power microwaves (HPM) are used to inhibit the electronic systems of threatening vehicles. Concern has arisen as to whether HPM could lead to health hazards for operators of emitting systems and for personnel exposed in targeted vehicles. The health effects of HPM have been studied since the discovery of radar in the middle of the past century. Many experiments have been performed with microsecond pulses at levels of several hundred kilovolts per meter. Some studies have been published, but many others have been presented only as reports or at scientific meetings.

Studies performed with a specific absorption rate (SAR) above the thermal threshold of 4 W kg^{-1} have shown biological effects. Below 4 W kg^{-1}, for studies showing effects, the pulse duration of single pulses was between 40 ns and 10 µs, and peak-SAR was from 5 to 20 MW kg^{-1}. Half of the studies on HPM addressed behavioral endpoints, reviewed by D'Andrea [1]. Others bear on the cardio-vascular [2,3], visual [4], and auditory systems [5]. Only sparse work concerned blood-brain-barrier permeability [6,7], DNA damage [8,9], carcinogenesis [10-12], or cellular or sub-cellular mechanisms [13]. Concerning cancer, Zhang [14] and Devyatkov et al. [10] reported protective effects at levels above thermal threshold, with smaller tumors and a 30% increase of survival rate in exposed animals. Several years after Seaman' article [15], a recent review by Schunck reported only one new paper in 2009, and no other effects on cancer were reported [16]. However, durations of exposure in those studies were often short.

Using a realistic source of HPM, this study looked for whether the highest possible exposure levels could produce behavioural or functional effects in rats acutely exposed, or chronic pathological effects with a repetitive exposure for two months. We assessed effects of 3.7 and 10 GHz nanosecond pulsed HPM around 1 MV/m on the health and lifespan of male Sprague-Dawley rats.

Methods

Literature survey

We looked at the scientific and medical literature (NCBI-PubMed, Current Contents and Science Direct, more recently Web of Science) and at specialized databases of papers, scientific meetings and reports (EMF Database, IEEE ICES EMF Literature Database and WHO- EMF-Portal). The following keywords were used: HPM, high power microwave, high peak, electromagnetic pulse, microwave radiation, high exposure microwave, HPPP, EHPP, high intensity microwave.

Exposure systems

The sources of high-power microwaves (HPM) were two superradiance generators, one in X-band at 10 GHz (SRX) with pulses of 1 ns, the other in S-band at 3.7 GHz (SRS) with pulses of 2.5 ns. The strongest possible microwave electric fields were applied, of about 1 MV m-1, at a repetition rate of 100 pps (Table 1). The “SINUS type” electron accelerator of this system is made of a Tesla generator and a continuous formation line. A great advantage of this system is its small size. The superradiance source is derived from the back-wave oscillator, with the following characteristic features: ultrashort microwave pulses, and very high peak power.

61 **Table 1. Exposure parameters of the two exposure sources.**

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	SRX	**SRS acute**	**SRS avoidance**	**SRS repeated**
Beam diameter at output	13 cm	22 cm		
Frequency	10 GHz	3.7 GHz		
Total emitting power	350 MW	500 MW		
Pulse duration	1 ns	2.5 ns		
Train duration	10 s	Continuous		
Emission duration	Every 5 min for 1 h	2 x 8 min	14 min	2 x 8 min
Peak surface power at output	20 GW m^{-2}	2 GW m^{-2}		
Distance from the horn		0.60 m	0.13 m	3.0 m
Peak E-field	3 MV m^{-1}	1.7 MV m^{-1}	2.9 MV m^{-1}	0.56 MV m^{-1}
Peak SAR	95 MW kg^{-1}	31 MW kg^{-1}	90 MW kg^{-1}	3.33 MW kg^{-1}
Average SAR over total exposure	0.34 W kg^{-1}	4.7 W kg^{-1}	22 W kg^{-1}	0.83 W kg^{-1}

Animals

Six-weeks-old Sprague Dawley male rats were purchased from Charles River, L'Arbresle, France. They were either exposed or sham-exposed. The protocol was reviewed and approved by INERIS ethical committee. Animals were monitored clinically and for mortality once a day. Closer surveillance was performed in case of clinical observations, such as behaviour changes, dull hair or upon appearance of a tumor. After a repeated exposure, the criteria to determine when animals should be ethically euthanized during the follow-up were: weight loss (more than 20% as compared to the week before), ulceration of a tumor, tumor size larger than 8 cm, impaired movement, loss of spontaneous activity or loss of reactions to stimulus.

Exposure protocol

Two types of acute exposures were carried out. The SRX exposure lasted 10 s every 5 min for one hour, and the SRS exposure lasted 2 x 8 min with 10 min interval (26 min total). For acute exposures, rats were exposed one by one directly at the horn output (168 animals in total, 12 per group). Besides, one protocol of repeated exposures was used with SRS source. The 26 minutes-exposure was repeated each day, 5 days/week for 8 weeks. When performing mean term repetitive exposures every day with a realistic source that cannot easily be duplicated, there is a need for optimization of the design to expose many animals at the same time. The circular beam produced by the TM01 mode of the waves was adapted to this goal, with a beam width large enough to simultaneously expose 12 animals at 2.5 m from the SRS output. Animals were exposed 2 by cage in six cages placed each day at different positions on the circle. Then every day, 2 series of 12 animals were exposed, alternating with 2 series of sham exposure in-between to allow time for the

85 equipment to cool down between two successive exposure sessions. In total, two groups of 24 rats
86 received a repeated exposure, either real, either sham (Table 2).

87 **Table 2. Global design of the study.**
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	SRX	**SRS**	
Exposure	**acute**	**acute**	**repeated**
Emission duration	10 s every 5 min for 1 h	2 x 8 min with 10mn interval Avoidance: 14 min continuous	2 x 8 min with 10 mn interval 5 days/week for 8 weeks
Age at experiment	6 weeks	6 weeks	6 weeks
Nb animals /exposition	1	1	12
Behavioural tests	Beam walking (n=13/11[a]), rotarod (n=12/12), T-maze (13/11), open field (n=12/12), avoidance (n=11/10)	Beam walking, rotarod, T-maze, avoidance (n=12/12)	T-maze (n=8/8, wk14[b]), Beam walking (n=12/12, wk15), rotarod (n=24/24, wk16)

GFAP staining (J=exposition day n=nb animals)	J+2 n=12/12 J+7 n=11/11	J+2 n=12/12	
Anatomo-pathology (HES)	104 weeks or at death	104 weeks or at death	104 weeks or at death
Lifespan recording (up to 2 years)			n=24/24

[a]n=n1/n2=[number of exposed animals] / [number of sham animals].
[b]Wk = age of animals at the date of test.

After the end of repeated exposure, the animals were observed and followed up to 2 years of age. Lifespan was recorded, and anatomo-pathological examination was performed at the animal death.

For each test, a group of 12 exposed animals was compared to a similar-sized group of sham-exposed animals, put in the same place and under the same ambient conditions than the exposed animals, but without emission from the source.

Investigations on the central nervous system

After one acute or the last repetitive exposure, different behavioural tests were performed: beam-walking, rotarod, T-maze, open-field. An avoidance test was also performed during an acute SRS exposure, applied continuously for 14 minutes (Table 2). In the avoidance test, animals can choose to spend time in two parts of a box. One part is protected against the beam (shielded), the other is not. The time spent in the non-protected side is recorded.

After the behavioural tests were performed, animals were sacrificed, and an immunohisto-chemical labelling of the brain inflammation marker GFAP was achieved on 40 µm thick slices for 5 areas of the brain: frontal cortex, gyrus dentate, putamen, pallidum and cerebellar cortex, 2 days after the SRX and the SRS exposures, and 7 days after the SRX exposure. (Table 2).

The global design of this study and the sample size in each test are summarized in Table 2.

Anatomopathology

After the end of repeated exposures, the animals were followed up to 2 years of age. For animals needing an ethical sacrifice, the lifespan was recorded. Either at this time or at 104 weeks, animals were sacrificed with a lethal overdose of pentobarbital (5.0 ml kg-1 IP), organs were collected and fixed in 4% isotone buffered formalin for 48 to 72h. Organs larger than 5 mm were cut for an optimized fixation and all tissue samples were included in paraffine blocks.

Five µm slices were cut with a microtome and 6 slides per organ were prepared. An anatomo-pathological examination was performed on two slices per organ. One slide was coloured with haematoxylin-eosin stain (HES), the second was stored in case of need for any other specific labelling.

Dosimetry

Electric field has been measured at the actual exposure distance of 2.2 m from the source output with a germanium detector and calculated for closer distances. As numerical computation of specific absorption rate (SAR) by FDTD has not been available, the whole-body specific absorption rates (SAR) (defined as electromagnetic power absorbed per unit of tissue mass) were calculated for each condition from the rat's position and size as described by Gandhi [17] and Durney et al. [18]. Time-averaged SARs were 0.8 W kg-1 for the repeated exposure, and between 0.34 and 22 W kg-1 for acute exposures. Peak SARs during the pulses were between 3.3 and 95 MW kg-1 (Table 1). Some residual X-rays came out from the device, for 20 mGy/day (total 0.8 Gy). Numerical and experimental dosimetry and thermometry need to be performed to reinforce the results of this study.

Statistics

Percentages of time spent in the exposed or in the blinded box were compared by two-way ANOVA with two factors: exposure (exposed or sham) and period (habituation or exposure). Percentages of labelled areas for GFAP were compared by two-way ANOVA with two factors: exposure (exposed or sham) and localisation (brain area). Survival rates of repetitively exposed animals were compared with Prism 5 v5.02 by the log-rank test (Mantel-Cox), with calculation of two-tail p value, of the median survival and of the hazard ratio between both groups by the Mantel-Haenszel method.

Results

Behavioural tests

First, behavioural tests to evaluate cognitive and sensori-motor functions were performed. No effects were observed after acute or repetitive exposures on behavioural results in beam-walking, T-maze and open-field tests. After repeated exposure to a superradiance S source (SRS) of HPM, rotarod performance was assessed: rats had to stay for three minutes on an axis rotating at 16 turns per minute. They underwent one training session and one test session. In the test session, rotarod performance was significantly enhanced in exposed animals: 41/72 exposed animals succeeded, compared to 23/72 sham animals - $p < 0.001$. Also, during an acute SRS exposure, with a choice for rats to stay in an exposed or a shielded compartment (avoidance reflex), exposed animals spent 3.7% of time on the exposed side, compared to 21.9% for the sham group – $p < 0.001$.

Brain inflammation

Then, brain inflammation was assessed by measuring glial fibrillary acidic protein (GFAP) levels, indicative of damaged or dysfunctional cerebral tissue. With a superradiance X source (SRX) of HPM, expression of GFAP was not increased two days (D2), but was increased seven days (D7), after an acute exposure, in all brain areas, except the cerebellum cortex (+50.0% - $p < 0.02$). With SRS, GFAP expression was increased two days after acute exposure (D2 - +115% - $p < 0.001$) (Fig 1).

Fig 1. GFAP expression after repeated exposure to SRS source. GFAP immunohistochemical labelling in different brain areas two days after exposure with Superradiance S source (% labelled area - Mean ± SEM). White = sham (n=12); black = exposed (n=12). One slice per area per animal. *** $p < 0.001$

Lifespan

Most strikingly, six exposed animals deceased early between 33 and 47 weeks, leading to a 4-months decrease of lifespan in the repetitively exposed group (n=24) compared to the sham group (n=24) (Fig 2). The median lifespan was 590 days for the exposed group, compared to 722 days for the sham group – $p < 0.0001$. One sham rat was used as sentinel for sanitary control, eleven sham animals survived at the end of the experiment, whereas only two animals survived in the exposed group. The hazard ratio was 4.1 [CI = 2.0-8.6].

Fig 2. Survival proportion of rats after repeated daily exposure. Empty circles = sham; black circles = exposed. Survival curves were significantly different ($p < 0.001$).

Lethal tumours and anatomopathology

For tumours identified as the cause of death, eleven of the exposed animals showed one or two large sub-cutaneous tumours of different types (five were malignant – seven appeared before 20 months) (Table 3 and e.g. Fig 3), compared to only two such tumours in the sham group (both malignant, first one at 22 months).

Table 3. Lifespan to death or sacrifice and anatomopathological diagnostic of lesions.

Rat #	Age at death (weeks)	Exposed group	Sham group
4	33	Internal mass, adenopathies …	
10	38	Fibrosarcoma	
21	39	Internal mass, adenopathies, spleen	
8	43	Fibrosarcoma	
20	45	Fibroma, ulcerated	
5	47	Posterior limbs paralysed	
36	60		/[a]
3	66	Internal mass, spleen, pancreas	

6	66	Subcutaneous adenocarcinoma	
27	73		Large cystic kidneys
23	79	Fibroma, ulcerated	
17	82	/	
37	83		/
25	84		Posterior limbs paralysed
46	84		/
2	84	Pituitary tumour	
11	84	Spongy/granulous kidneys	
19	85	*Mesenteric mass*[b], ileon (lysed organs)	
45	86		Large preputial glands
34	88		Sentinel animal
24	88	Polycystic kidneys	
7	88	Osteosarcoma	
16	88	**Fibro-epithelial polype**[c], cystic and spongy kidneys	
14	91	Jejunal mass	
9	92	Mass: adrenal/kidney/spleen (internal bleeding)	
48	92		Large preputial glands
13	94	**Zymbal's gland adenoma**, ulcerated ear area, *pituitary tumour*, cystic and spongy kidneys	
1	94	**Fibro-adenoma**, cystic and spongy kidneys, tracheo-bronchial ganglions large and inflammatrory	
15	95	/	
29	97		Large cystic and spongy kidneys, *white lung masses*
35	98		Cystic and spongy kidneys, duodenum dark and spongy content
26	98		**Subcutaneous schwannoma**, *large spleen*, large left preputial gland
40	99		/
12	99	**Fibrosarcoma** Dark abdominal cavity, testes soft small and dark. Soft brain, small spleen, *large left adrenal gland*, external part of lungs grey/brown	
18	103	/	

22	103	Fibro-adenoma	
39	104		Fibrosarcoma
28, 30-33 41-44, 47	103- 104		Sacrificed, no tumor

Left column: exposed group (#=1-24); right column: sham group (#=25-48). [a]/: no macroscopic abnormality; [b]*italic*: internal masses found at death or at 24 months; [c]**bold**: large external masses leading to ethical sacrifice. Only two exposed rats survived at the end of the experiment at 103 weeks. Eleven rats of the sham group survived to the end of the experiment: #28, 30-33, 38, 41-44 and 47 were sacrificed at 103 or 104 weeks without any tumor.

Fig 3. (a) Picture of one exposed rat with two fibromas (left) – (b) macroscopic view of the femoral tumor (right). The two fibromas were in the axillary and the femoral area, the macroscopic view was taken at autopsy (death at 18.5 months).

One of the exposed animals with an external tumour also had a pituitary tumour, and at death, six other exposed animals had abdominal masses and one had a pituitary tumour. Tumour types and lifespan are detailed in Table 3.

Discussion

Although they are hugely far above environmental levels, the question has been raised whether intense and very short pulses (nanosecond range) could have health effects. Old studies considered typical radar modulation of 1/1000th (1 µs every ms, i.e. repetition rate of 1 kHz). Below the thermal level of 4W/kg, no specific effect of modulation had been proven, so this modulation factor of 1/1000th has been considered as safe in the public health standards [19]. Recent studies on high intensity nanosecond pulsed microwaves have been performed on cells and their electrophysiological properties, or on membrane permeability, but none on animals with repeated exposures [16].

Behavioural tests

No effect was seen in learning experiments, but a positive effect was found in the rotarod test, which mainly addresses a sensori-motor activity. This could be due to a slight heating at the SAR of 4.7 W kg^{-1} of the SRS source. Such a heating effect has been hypothesized by Preece who observed an increased reactivity (shorter reaction time) in human volunteers exposed to mobile phones at a SAR of 1.7 W kg^{-1} [20]. Avoidance of the SRS beam was significant in exposed animals subjected to a thermal SAR of 22 W kg^{-1}, which is high above the thermal threshold of 4 W kg^{-1} identified by ICNIRP [19]. This expected result actually confirms the relevance of this threshold.

Brain inflammation

The larger GFAP increase in brain with SRS reflects the higher average SAR of 4.7 W kg^{-1} allowed by a continuous emission of SRS pulses instead of spaced 10 s pulses with average SAR of 0.34 W kg^{-1} in the SRX. These results are consistent with those previously found with a much lighter modulation of microwaves $1/8^{th}$ of the time, such as the one produced by GSM mobile phones [21]. In comparison, the modulation of HPM is 1 ns at 100 pps, i.e. a ratio of 10^{-7}. Alterations in the glial cell marker GFAP could represent a marker of a long-term risk in rats, but this has yet not been shown. Mainly known as a marker of traumatic injury, GFAP has been considered by previous studies as non- specific, therefore compromising its prognostic power [22].

Lifespan, lethal tumours and anatomopathology

More importantly, an increased and early rate of sarcomas and fibrosarcomas and higher associated mortality were observed in animals exposed to repetitive sessions at an average SAR of 0.8 W kg^{-1}, five times below the thermal threshold (Table 1). The spontaneous rate of fibrosarcomas

found in old Sprague Dawley rats at termination of two-year studies is usually around 1 to 3%, and tumours are rarely reported as cause of life shortening [23]. Several studies have tested the impact of HPM, but chronic exposure was only performed with continuous waves, radar-type microwave pulses of the order of microseconds [11,24,25], or mobile phone-type exposures [26]. Effects on cancer and lifespan were reported with SARs close to or above the thermal level [24,27,28]. Only Chou et al reported an increase in primary malignancies, without life shortening, with pulsed waves at low SAR levels (0.15-0.4 W kg^{-1}) and exposures lasting 21.5 h/day for 25 months [25]. Recently, a NIEHS study of the National Toxicological Program reported an increased incidence of heart schwannoma and glioma in whole-body exposed rats to phone-type microwaves at much higher SARs than those used in humans [26]. Although experiments with newer extremely short pulses (a few ns long) have been performed, HPM had only been used in acute experiments, and most studies reporting an effect looked at physiological reactions, without addressing genotoxicity or carcinogenicity endpoints. This work therefore corresponds to the first report with in vivo exposure to extremely short duration peak pulses, with a high repetition rate, and with a design of repeated exposure for eight weeks.

Tinkey showed that very high doses of X-rays (> 46 Gy) were needed to induce sarcomas in Sprague-Dawley rats [29]. then the low 0.8 Gy residual X-ray level of this study cannot explain the observed early tumour increase. Therefore, this study shows that the observed tumours and decreased lifespans were due to repeated exposures at a SAR below the known health threshold of 4 W kg^{-1} (given that the peak SAR was of the order of 3.3 MW kg^{-1}; E-field above 0.5 MV m^{-1}).

Conversely, some studies would support a protective effect of HPM against cancer. Devyatkov found a decrease in cell proliferation in vitro and an increase in survival time of rats implanted

with a liver carcinosarcoma and exposed to 10 ns pulses at 9 GHz, which paradoxically was beneficial [10]. The peak power was 100 MW, but the electric field or SAR was not specified. More recently, after 16 – 1000 ns ultra-wide band pulses (UWB) with a frequency of 0.6 – 1.0 GHz, a duration of 4 – 25 nanosecond, an amplitude of 0.1 – 36 kV cm^{-1}, and a pulse repetition rate of 13 pulses per second (pps), Zharkova also found an inhibition of mitochondrial activity which has been interpreted rather as an anti-tumoral activity [30].

Other studies bring some mechanistic explanation that would support a cancerogenic effect. Dorsey found an increase in mitogenic activity of mouse hepatocytes [12]. Natarajan published genotoxic effects [31] and Shckorbatov showed some changes in chromatin [32], which studies bring arguments rather in favour of a carcinogenic effect.

Observed tumours were mostly subcutaneous, but were also ubiquitous, which is not indicative of a specific mechanism or sensitivity of a given tissue or organ. This means that inflammatory processes or genotoxic effects should be investigated in the different target tissues where tumours appeared: connective tissue, muscle, fat, vessels, pituitary gland, lymph nodes, etc. To check if this is a general phenomenon or a strain/specie specific effect, this experiment should be repeated with other rat strains and different animal species (e.g. mice, and/or rabbits) which are usual models for human toxicology.

The actual corner stones of guidelines for RF exposures consider behavioral effects as the most sensitive biological endpoint that had yet been observed as a deleterious effect on health. Up to date, this decreased behavioral performance is today attributed to the temperature elevation produced in rodents or primates, consecutive to the above-mentioned dielectric absorption, only linked to the average absorbed power (rms SAR). This study shows that extremely high intensity

microwave pulses, around one million volts per meter (1 MV.m-1), comparable to those that have in part been used in the Gulf War, produce a clear increased incidence of cancer in exposed animals. Furthermore, it tells that even an aggressive damage such as cancer can occur without so much decreased cognitive performance, even at a level below the known thermal threshold of whole-body SAR (4W/kg). Then the peak SAR should be re-considered in the definition of guidelines.

The original hypothesis was: is there an effect of high-power microwaves? In which conditions? If yes, does it obey to a classical thermal mechanism or a mechanism other than thermal? This study showed: i) few behavioural effects from either acute or repeated exposure; ii) an inflammatory effect of acute exposure to HPM; and iii) a surprising increase of lethal cutaneous or subcutaneous tumour incidence of sarcoma or fibrosarcoma type, in the repetitively exposed group (46% versus 8% in the sham-control group). This increased cancer incidence was associated with decreased lifespan in rats exposed to HPM with an average SAR level below the thermal threshold of 4 W kg^{-1}. Furthermore, this effect was not associated with clear effects on behaviour, as could have been expected from previous knowledge. The underlying mechanisms are likely to be different from thermal effects and need to be further explored. Also, the thresholds or dose-responses in SAR level, duration and number of exposure sessions need to be defined.

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286 References

287 1. D'Andrea J (1999) Behavioral evaluation of microwave irradiation. Bioelectromagnetics Suppl
288 4: 64-74.

289 2. Lu S, Brown D, Johnson C, Mathur S, Elson E (1992) Abnormal cardiovascular responses
290 induced by localized high power microwave exposure. IEEE Trans Biomed Eng 39: 484-
291 492.

292 3. Pakhomov A, Mathur S, Doyle J, Stuck B, Kiel J, et al. (2000) Comparative effects of extremely
293 high power microwave pulses and a brief CW irradiation on pacemaker function in isolated
294 frog heart slices. Bioelectromagnetics 21: 245-254.

295 4. Elder J (2003) Ocular Effects of Radiofrequency Energy. Bioelectromagnetics Supplement 6:
296 S148-S161.

297 5. Elder J, Chou C (2003) Auditory Response to Pulsed Radiofrequency Energy.
298 Bioelectromagnetics Supplement 6: S162-S173.

299 6. Lin J, Lin M (1980) Studies on microwave and blood-brain barrier interaction.
300 Bioelectromagnetics 1: 313-323.

301 7. Goldman H, Lin JC, Murphy S, Lin MF (1984) Cerebrovascular permeability to 86Rb in the rat
302 after exposure to pulsed microwaves. Bioelectromagnetics 5: 323-330.

303 8. Lai H, Singh N (1997) Melatonin and a spin-trap compound block radiofrequency
304 electromagnetic radiation-induced DNA strand breaks in rat brain cells.
305 Bioelectromagnetics 18: 445-454.

306 9. Chemeris N, Gapeyev A, Sirota N, Gudkova O, Kornienko N, et al. (2004) DNA damage in frog
307 erythrocytes after in vitro exposure to a high peak-power pulsed electromagnetic field.
308 Mutat Res 558: 163-176.

309 10. Devyatkov N, Pletnyov S, Betskii O, Faikin V (2001) Effect of low-energy and high-peak-
310 power nanosecond pulses of microwave radiation on malignant tumors. Crit Rev Biomed
311 Eng 29: 98-110.

312 11. Elder J (2003) Survival and Cancer in Laboratory Mammals Exposed to Radiofrequency
313 Energy. Bioelectromagnetics Supplement 6: S101-S106.

314 12. Dorsey WC, Ford BD, Roane L, Haynie DT, Tchounwou PB (2005) Induced mitogenic activity
315 in AML-12 mouse hepatocytes exposed to low-dose ultra-wideband electromagnetic
316 radiation. Int J Environ Res Public Health 2: 24-30.

13. Pakhomov A, Gajsek P, Allen L, Stuck B, Murphy M (2002) Comparison of dose dependences for bioeffects of continuous-wave and high-peak power microwave emissions using gel-suspended cell cultures. Bioelectromagnetics 23: 158-167.

14. Zhang M, Xiong ZA, Chen WJ, Yao CG, Zhao ZY, et al. (2013) Intense picosecond pulsed electric fields inhibit proliferation and induce apoptosis of HeLa cells. Mol Med Rep 7: 1938-1944.

15. Seaman RL (2007) Effects of exposure of animals to ultra-wideband pulses. Health Phys 92: 629-634.

16. Schunck T, Bieth F, Pinguet S, Delmote P (2016) Penetration and propagation into biological matter and biological effects of high-power ultra-wideband pulses: a review. Electromagn Biol Med 35: 84-101.

17. Gandhi OP (1975) Conditions of strongest electromagnetic power deposition in man and animals. IEEE Trans MTT 23: 1021-1029.

18. Durney CH, Massoudi H, Iskander MF (1986) In: Brooks Air Force Base TRRD, editor. Radiofrequency radiation dosimetry handbook. pp. 151-166.

19. ICNIRP (1998) Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300GHz). Health Phys 74 494-522.

20. Preece A, Iwi G, Davies-Smith A, Wesnes K, Butler S, et al. (1999) Effect of a 915-MHz simulated mobile phone signal on cognitive function in man. International Journal of Radiation Biology 75: 447-456.

21. Ammari M, Gamez C, Lecomte A, Sakly M, Abdelmelek H, et al. (2010) GFAP expression in the rat brain following sub-chronic exposure to a 900 MHz electromagnetic field signal. International Journal of Radiation Biology 86: 367-375.

22. Petzold A (2015) Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease. Brain Res 1600: 17-31.

23. He Y, Zhang S, Zhang H (2017) Pathologic changes of spontaneous tumors in Sprague-Dawley and Wistar rats. Zhonghua Bing Li Xue Za Zhi 46: 249-254.

24. Szmigielski S, Szudzinski A, Pietraszek A, Bielec M, Janiak M, et al. (1982) Accelerated Development of Spontaneous and Benzopyrene-Induced Skin Cancer in Mice Exposed to 2450-MHz Microwave Radiation. Bioelectromagnetics 3: 179-191.

25. Chou CK, Guy AW, Kunz LL, Johnson RB, Crowley JJ, et al. (1992) Long-term, low-level microwave irradiation of rats. Bioelectromagnetics 13: 469-496.

26. NTP (2018) Technical report on the toxicology and carcinogenesis studies in Hsd:Sprague Dawley SD rats exposed to whole-body radio frequency radiation at a frequency (900 MHz) and modulations (GSM and CDMA) used by cell phones - TR 595. NIEHS.

27. Prausnitz S, Susskind C (1962) Effects of chronic microwave irradiation on mice. IRE Trans Biomed Electron 9: 104-108.

28. Liddle C, Putnam J, Huey O (1994) Alteration of lifespan of mice chronically exposed to 2.45 GHz microwaves. Bioelectromagnetics 15: 177-181.

29. Tinkey PT, Lembo TM, Evans GR, Cundiff JH, Gray KN, et al. (1998) Postirradiation sarcomas in Sprague-Dawley rats. Radiat Res 149: 401-404.

30. Zharkova L, Romanchenko I, Bolshakov M, Rostov V (2017) Mitochondrial respiration inhibition after exposure to UWB pulses as a possible mechanism of antitumor action. In: Series JoPC, editor. International Conference on Recent Trends in Physics 2016: IOP Publishing. pp. 012010.

31. Natarajan M, Nayak BK, Galindo C, Mathur SP, Roldan FN, et al. (2006) Nuclear translocation and DNA-binding activity of NFKB (NF-kappaB) after exposure of human monocytes to pulsed ultra-wideband electromagnetic fields (1 kV/cm) fails to transactivate kappaB-dependent gene expression. Radiat Res 165: 645-654.

32. Shckorbatov Y, Pasiuga V, Kolchigin N, Batrakov D, Kazansky O, et al. (2009) Changes in the human nuclear chromatin induced by ultra wideband pulse irradiation. Central European Journal of Biology 4: 97-106.

Supporting information includes:

- S1 Figure
- S1 to S5 Tables

Complete anatomopathological examinations are also available in French (83 and 93 pages, resp., for sham and exposed rats).

S1 Fig. Avoidance test during SRS exposure: time% spent in the shielding box in habituation and exposure periods.

S1 Table. Rotarod test data after SRS exposure: time spent on the rods at training and at test.

S2 Table. Avoidance test during SRS exposure: time and time% spent in the shielding box in habituation and exposure periods.

380 **S3 Table.** GFAP immunohistochemical labeling after SRX exposure - raw values. Magnification
381 x10.

382 **S4 Table.** GFAP immunohistochemical labeling after SRS exposure - raw values. Magnification
383 x10.

384 **S5 Table.** Synthesis of histological lesions in sham (Sh) and exposed (Ex) groups after repeated
385 SRS exposure.

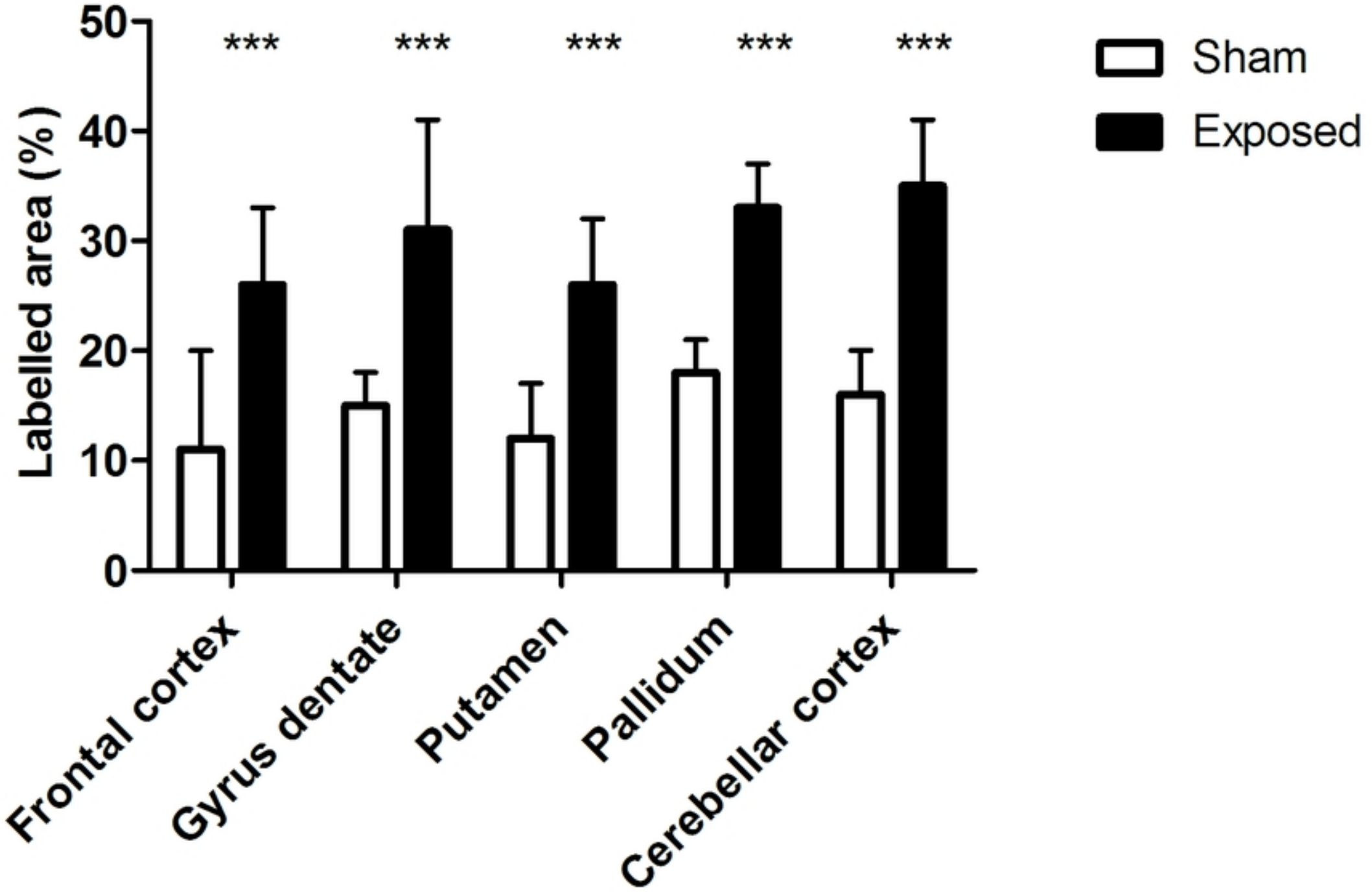
50
40
30
20
10
0
Labelled area (%)
***
***
***
***
***
Sham
Exposed
Frontal cortex
Gyrus dentate
Putamen
Pallidum
Cerebellar cortex

Figure 1

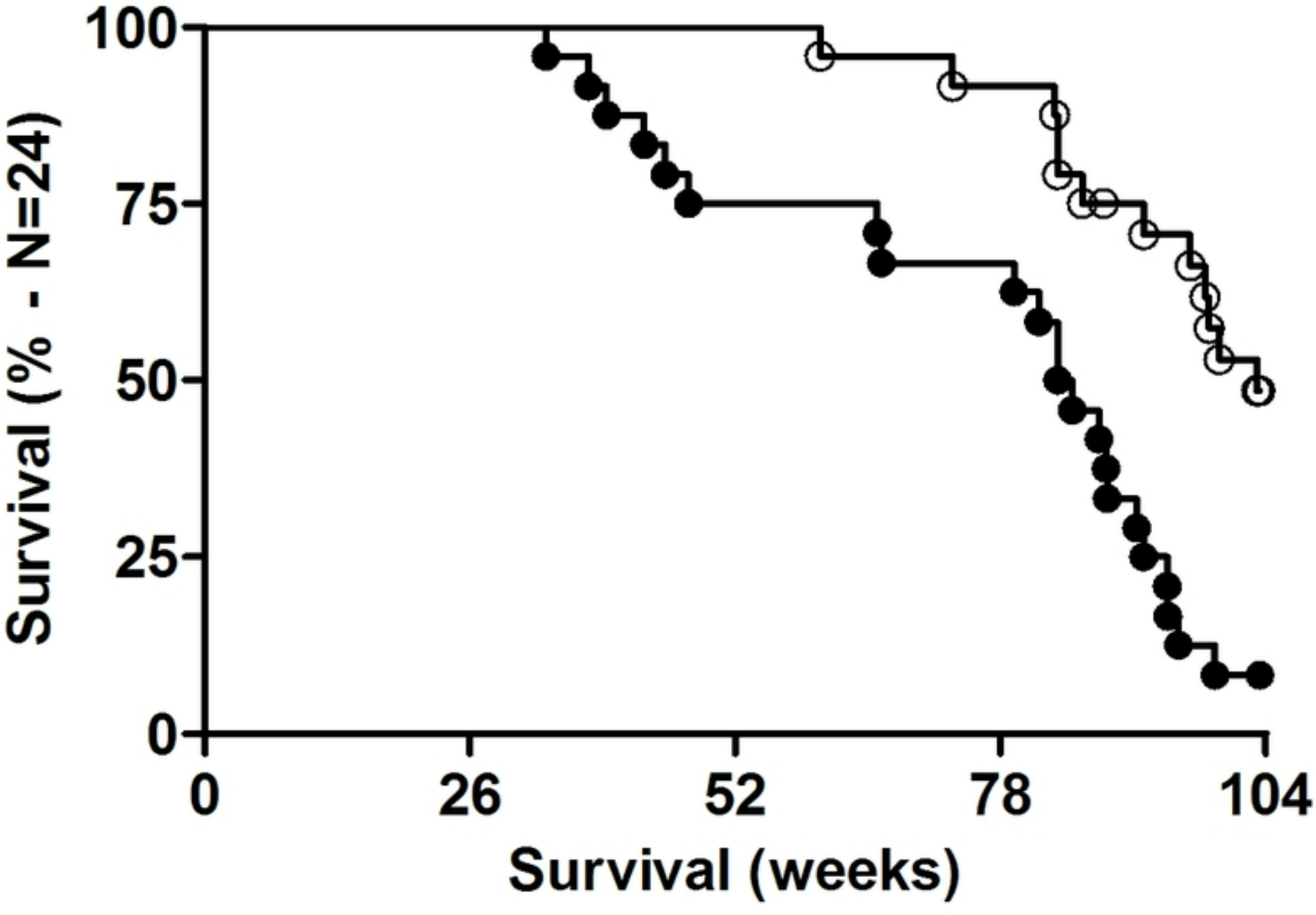

Figure 2

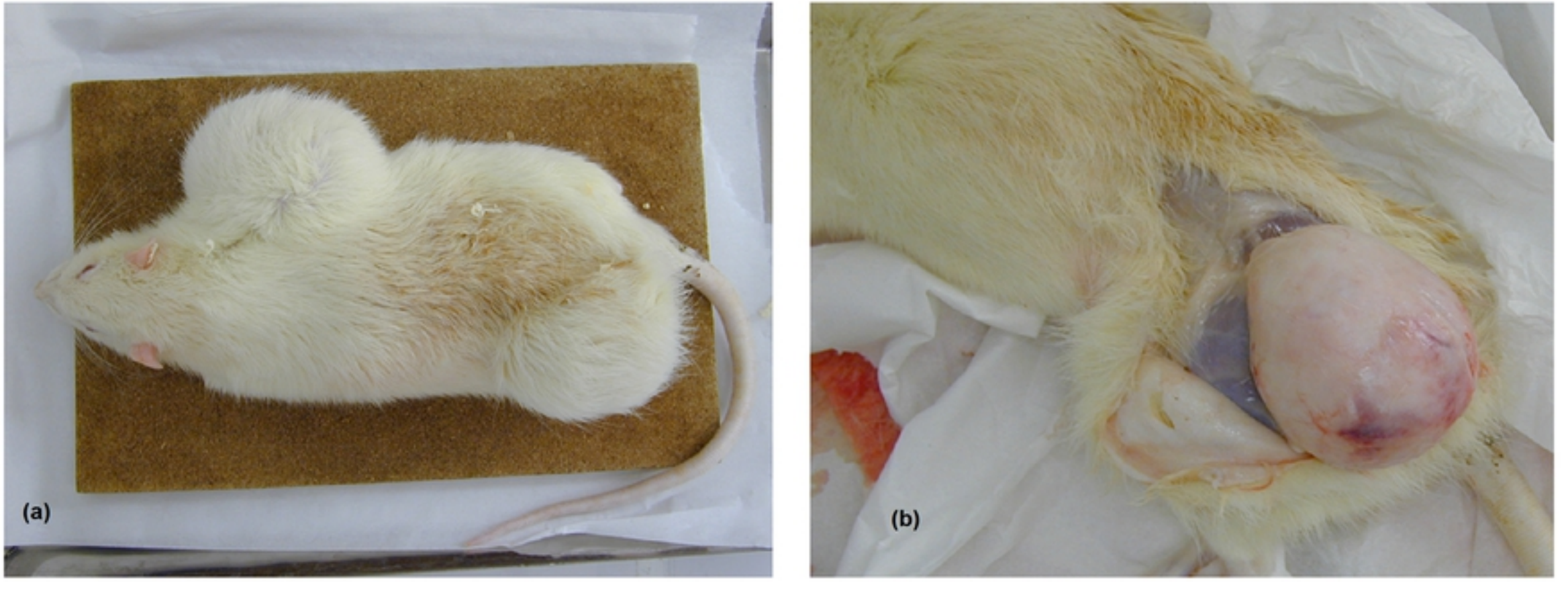

Figure 3