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SELECTIVE CHANGES IN LOCOMOTOR ACTIVITY IN MICE DUE TO LOW-INTENSITY MICROWAVES AMPLITUDE MODULATED IN THE EEG SPECTRAL DOMAIN

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Abstract—Despite the numerous benefits of microwave applications in our daily life, microwaves were associated with diverse neurological complaints such as headaches and impaired sleep patterns, and changes in the electroencephalogram (EEG). To which extent microwaves influence the brain function remains unclear. This exploratory study assessed the behavior and neurochemistry in mice immediately or 4 weeks after a 6-day exposure to low-intensity 10-GHz microwaves with an amplitude modulation (AM) of 2 or 8 Hz. These modulation frequencies of 2 and 8 Hz are situated within the delta and theta-alpha frequency bands in the EEG spectrum and are associated with sleep and active behavior, respectively. During these experiments, the specific absorbance rate was 0.3 W/kg increasing the brain temperature with 0.23 °C. For the first time, exposing mice to 8-Hz AM significantly reduced locomotor activity in an open field immediately after exposure which normalized after 4 weeks. This in contrast to 2-Hz AM which didn't induce significant changes in locomotor activity immediately and 4 weeks after exposure. Despite this difference in motor behavior, no significant changes in striatal dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels and DOPAC/DA turnover nor in cortical glutamate (GLU) concentrations were detected. In all cases, no effects on motor coordination on a rotarod, spatial working memory, anxiety nor depressive-like behavior were observed. The outcome of this study indicates that exposing mice to low-intensity 8-Hz AM microwaves can alter the locomotor activity in contrast to 2-Hz AM which did not affect the tested behaviors. **© 2017 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: microwave exposure, active behavior, dopamine, glutamate.

INTRODUCTION

With the developments of numerous wireless applications, microwaves become more prominent in our daily lives (Panagopoulos, 2014). These microwaves are non-ionizing electromagnetic (EM) waves with frequencies between 300 MHz and 300 GHz and are currently exploited for a diverse set of applications ranging from heating food to radar and medical appliances. Despite of the benefits of these microwave applications, questions arose about the safety of this technology which resulted in numerous epidemiological studies (Ruediger, 2009). A variety of neurological effects were observed due to radiofrequency and microwave exposure including headaches, impaired sleep patterns and modification of the electroencephalogram (EEG) (Bise, 1977; Frey, 1998; Wagner et al., 1998).

Animal studies already demonstrated changes in behavior and cognitive function (memory and learning) in mice due to microwave exposure (D'Andrea et al., 2003; Deshmukh et al., 2015). The majority of these studies are situated in the frequency range of mobile phone communication which is between 900 MHz and 2.45 GHz. With the introduction of 5G technology in the near future, the used frequency bands are expected to evolve toward the higher GHz range (Radio Spectrum Policy Group, 2016). Other devices for civil and military purposes such as radars, aircraft and weather forecast systems commonly use frequencies within the X-band (8–12 GHz) of which the biological effects are not well known yet.

Since the brain generates electrical waves which can be detected using EEG, external EM signals could influence these brain signals affecting the normal brain function. Within the EEG spectrum, specific frequency bands (e.g. alpha waves, beta waves, etc.) are associated with certain behaviors. This poses the question if amplitude modulated (AM) microwaves by

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Abbreviations: AM, amplitude modulation; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; EEG, electroencephalogram; EM, electromagnetic; FST, forced swim test; GLU, glutamate; OFT, open-field test; SAR, specific absorbance rate; SEM, standard error of the mean; ø, diameter.

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which the modulation frequency falls within the specified frequency bands in the EEG spectrum, can specifically influence the brain activity and consequently behavior. Therefore, we evaluated the behavior and neurochemistry in mice after exposure to low-intensity microwaves of 10 GHz with an AM of 2 or 8 Hz. The modulation frequency of 2 Hz is situated within the delta frequency band and is associated with sleep, while 8 Hz falls within the theta-alpha frequency band and corresponds with active behavior (Vyazovskiy et al., 2006). Behavioral and neurochemical measurements were performed at two different time points, i.e. immediately following the 6-day microwave exposure and 4 weeks after the 6-day exposure. These experiments were executed in different batches of animals to exclude a learning or habituation effect in the behavioral tests.

For the behavioral tests, we opted to study changes in locomotor activity in an open field and on the rotarod, anxiety-like behavior by measuring the time avoiding the central zone of an open field, depressive-like behavior as determined in the forced swim test (FST), and possible deficits in spatial working memory via spontaneous alternation in a Y-maze. For the neurochemical analysis of the homogenates, we studied possible changes in dopamine (DA) and glutamate (GLU) content in relevant brain areas. Changes in dopaminergic neurotransmission are well-known to be implicated in both locomotor activity and depression in view of the clinically used dopaminergic drugs for treating parkinsonian patients and bupropion for major depression, respectively, but DA is also involved in anxiety (De Bundel et al., 2016) and spatial working memory (Werlen and Jones, 2015). It is common knowledge that glutamatergic projections to the motor cortex are deeply involved in motor regulation. Moreover, alterations in glutamatergic transmission have been implicated in anxiety (Murrough et al., 2015), depression (Murrough et al., 2017) and spatial working memory (Robbins and Murphy, 2006) as well.

EXPERIMENTAL PROCEDURES

Animals

In this study, 60 male NMRI mice (Charles River Laboratories, France) were utilized of which three of them were used for brain temperature measurements, while the rest of the animals were subjected to one of the four experimental groups (Fig. 1). These conditions included a 6-day exposure (24 h/day) to microwaves of 10 GHz at a 2- or 8-Hz AM followed by a battery of behavioral tests and post-mortem neurotransmitter measurements in brain homogenates, immediately after microwave exposure or 4 weeks after exposure (Fig. 2). For each exposure condition, seven mice were used (exceptionally one group of eight mice) and compared to their controls (i.e. sham exposed). Control mice were not subjected to any radiation. Influences due to age differences during the behavioral experiments immediately or 4 weeks after exposure, were managed using respectively 8.5- and 4.5-week-old mice at the start of the exposure. Before the experiments started, all mice were group housed for 1 week in the animal facilities of the Vrije Universiteit Brussel in standard laboratory conditions (average room temperature 23 °C and a 12-/12-h light/dark cycle (lights on 7:00 a.m., lights off 7:00 p.m.)) with *ad libitum* access to food and water. All experimental procedures were approved by the Ethics Committee on Animal Experimentation of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel.

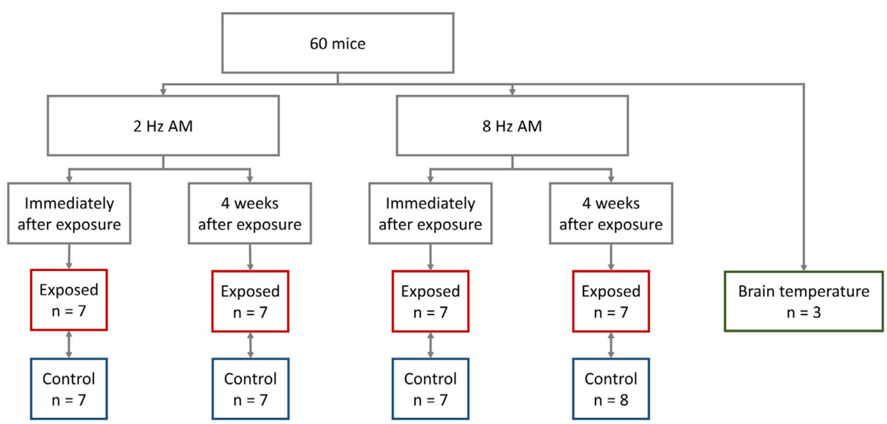

Fig. 1. Representation of all 10-GHz microwave exposure sessions. In total 60 mice were used, three of them to evaluate the brain temperature, while the rest of the animals were divided over four experimental groups. These include the 2- and 8-Hz amplitude modulation (AM) by which the effects on behavior and neurotransmitter levels were evaluated both immediately following exposure or 4 weeks after exposure and compared to respective control mice.

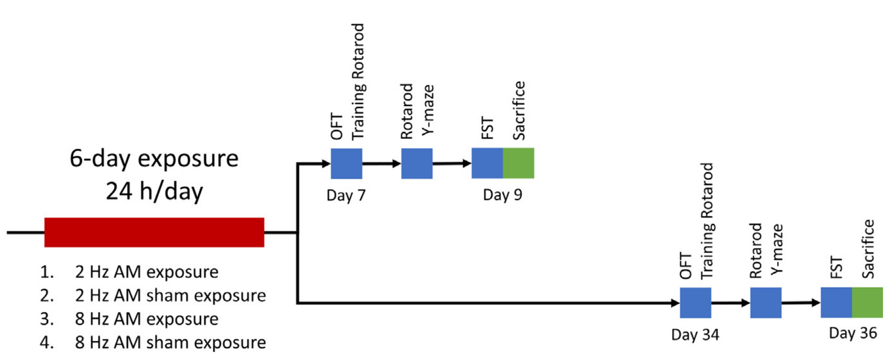

Fig. 2. Flow chart of the experimental protocol. During six consecutive days, mice were subjected 24 h/day to one of the four exposure conditions followed by a battery of behavioral tests (open-field test (OFT), rotarod and forced swim test (FST)) and sacrifice for post-mortem neurotransmitter analysis in brain homogenates, immediately after microwave exposure (day 7–9) or 4 weeks after exposure (day 34–36).

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