UNIWERSYTET MIKOŁAJA KOPERNIKA W TORUNIU
WYDZIAŁ NAUK BIOLOGICZNYCH I WETERYNARYJNYCH

Angelika Klimek

Wpływ pola elektromagnetycznego o niskiej częstotliwości (50 Hz) na status oksydacyjny i reakcje stresowe u szczura – efekt hormezy

Rozprawa na stopień naukowy doktora

Promotor:
prof. dr hab. Justyna Rogalska

Promotor pomocniczy:
dr hab. Łukasz Pepłowski, prof. UMK

Toruń 2024

Składam serdeczne podziękowania Pani prof. dr hab. Justynie Rogalskiej

za nieocenioną pomoc w realizacji niniejszej pracy, cenne wskazówki, poświęcony czas, cierpliwość, wsparcie, zarówno merytoryczne jak i duchowe oraz okazaną życzliwość.

Pragnę podziękować Panu dr hab. Łukaszowi Pepłowskiemu, prof. UMK

za pomoc w tworzeniu tej rozprawy, cenne sugestie oraz przekazaną wiedzę.

Dziękuję również wszystkim koleżankom i kolegom, pracownikom Katedry Fizjologii Zwierząt i Neurobiologii

za wszelką pomoc oraz przyjazną atmosferę w trakcie realizacji pracy doktorskiej.

Badania będące podstawą niniejszej rozprawy doktorskiej zostały sfinansowane z grantu

Narodowego Centrum Nauki:

“Nowe spojrzenie na wpływ pola elektromagnetycznego niskiej częstotliwości (50 Hz) na reakcje stresowe i plastyczność mózgową – efekt hormezy” (OPUS nr 2017/25/B/NZ7/00638)

oraz projektu:

Universitas Copernicana Thoruniensis In Futuro- modernizacja Uniwersytetu Mikołaja Kopernika w ramach Zintegrowanego Programu Uczelni. (Nr projektu: POWR.03.05.00-00-Z302/17-00)

Spis treści

Wykaz publikacji wchodzących w skład rozprawy doktorskiej

Praca przeglądowa:

Praca 1

A. Klimek, J. Rogalska (2021) Extremely Low-Frequency Magnetic Field as a Stress Factor—Really Detrimental?—Insight into Literature from the Last Decade. Brain Sciences, 2021, 11(2):174

IF: 3,13 (2021)

MNiSW: 100

Prace eksperymentalne:

Praca 2

A. Klimek, A. Nowakowska, H. Kletkiewicz, J. Wyszkowska, J. Maliszewska, M. Jankowska, L. Peplowski, J. Rogalska (2022) Bidirectional Effect of Repeated Exposure to Extremely Low-Frequency Electromagnetic Field (50 Hz) of 1 and 7 mT on Oxidative/Antioxidative Status in Rat's Brain: The Prediction for the Vulnerability to Diseases, Oxidative Medicine and Cellular Longevity, 2022(2):1-14

IF: 7,31 (2022)

MNiSW: 100

Praca 3

A. Klimek, H. Kletkiewicz, A. Siejka, J. Wyszkowska, J. Maliszewska, M. Klimiuk, M. Jankowska, J. Seckl, J. Rogalska (2022) New view on the impact of the low-frequency electromagnetic field (50 Hz) on stress responses – hormesis effect, Neuroendocrinology, 2023;113(4):423-441

IF: 4,1 (2023)

MNiSW: 100

Praca 4

A. Klimek, H. Kletkiewicz, A. Siejka, J. Wyszkowska, J. Maliszewska, M. Klimiuk, M. Jankowska, J. Rogalska, The electromagnetic field (50 Hz) can establish a new "set-point" for the activity of the locus coeruleus–noradrenergic (LC-NA) system in rat.

Praca zostanie złożona do czasopisma Neuroscience

IF: 3,3 (2023)

MNiSW: 140

Wprowadzenie

1. Charakterystyka pola elektromagnetycznego

W naukach biologicznych oraz medycznych od wielu lat bada się wpływ obecności pola elektromagnetycznego na organizmy żywe [1-5]. Pole elektromagnetyczne powstaje na skutek nałożenia na siebie dwóch pól: pola elektrycznego oraz pola magnetycznego. Oba te pola są polami wektorowymi. Pole elektryczne wytwarzane jest przez ładunki elektryczne (dodatnie lub ujemne) [6, 7]. Pole magnetyczne wytwarzane jest poprzez poruszające się ładunki elektryczne lub w magnesach trwałych. W szczególności pole magnetyczne może być wytwarzane poprzez prąd płynący w przewodniku, ponieważ prąd elektryczny jest uporządkowanym ruchem ładunków elektrycznych. Jeśli natężenie prądu płynącego w przewodniku będzie stałe wytworzone zostanie stałe pole magnetyczne, natomiast zmieniając natężenie płynącego prądu w przewodniku można zmieniać natężenie pola magnetycznego, nawet otrzymując pole magnetyczne o wymaganej częstotliwości [6, 7]. Pole magnetyczne można opisać przy pomocy wektora indukcji magnetycznej: B wyrażonej w teslach [T] [8]. Jest to całkowita miara linii pola przechodząca przez daną powierzchnię.

Oba pola, elektryczne i magnetyczne mogą istnieć niezależnie, jednakże w naukach biologicznych w większości prac naukowych niezależnie czy bada się wpływ pola elektrycznego czy magnetycznego czy obu na raz przyjęło się stosować pojęcie pola elektromagnetycznego.W przedstawionych badaniach weryfikowano wpływ pola magnetycznego na układ nerwowy, jednak według obowiązującej nomenklatury w publikacjach naukowych stosowane będzie pojęcie pola elektromagnetycznego [9-11].

Pole elektromagnetyczne ekstremalnie niskiej częstotliwości (ang. *extremely low-frequency electromagnetic field*; EMF) jest generowane przez mi.in. urządzenia elektryczne używane powszechnie w życiu codziennym. Najczęściej występującą częstotliwością omawianego przeze mnie EMF jest 50 Hz oraz 60 Hz (odpowiednio dla Europy/Azji oraz Stanów Zjednoczonych) [12]. Ciągły rozwój nowoczesnych technologii, znajdujących zastosowanie w wielu dziedzinach życia sprawia, że ludzie są wystawieni na działanie EMF niemal nieustannie. Do innych źródeł EMF pochodzenia antropogenicznego należą wszystkie przewodniki prądowe, np. linie wysokiego napięcia oraz przewody zasilające urządzenia elektryczne. Wobec wzrastającej liczby źródeł EMF zasadnym staje się pytanie o możliwe skutki oddziaływania tego rodzaju pola dla ludzkiego zdrowia i życia.

2. Mechanizm oddziaływania pola elektromagnetycznego na organizm

Powszechność występowania źródeł EMF w środowisku skłania do poddania analizie jego oddziaływania na organizmy. Dotychczas przeprowadzono szereg badań eksperymentalnych dotyczących różnych efektów biologicznych pola elektromagnetycznego w zakresie najniższych częstotliwości (1-300 Hz).

W pracy przeglądowej, włączonej do cyklu prac rozprawy doktorskiej (*praca 1*), przedstawiłam przegląd doniesień literaturowych (opublikowanych w latach 2010-2020) na temat skutków biologicznych EMF na różnych poziomach organizacji organizmu (wymienione w Tabeli 1, która stanowi suplement do pracy przeglądowej) oraz ich konsekwencji dla zdrowia człowieka.

Szczególną uwagę przywiązuje się do negatywnego oddziaływania zarówno niskich, jak i wysokich częstotliwości pól elektromagnetycznych ze względu na liczne możliwe efekty patologiczne oraz liczne raporty dotyczące kancerogenności wywołanej przez EMF [13]. Udowodniono, że EMF jest czynnikiem stresowym, co może prowadzić do morfologicznych i fizjologicznych zmian w układach związanych ze stresem [9]. Ekspozycja na EMF "włącza" różne wewnątrzkomórkowe mechanizmy - kompensacyjne lub szkodliwe - oraz modyfikuje funkcje związane ze stresem układu nerwowego, hormonalnego i immunologicznego. Wśród zaobserwowanych reakcji na EMF odnotowano zmiany w poziomie hormonów i neuroprzekaźników oraz modyfikacje ekspresji ich receptorów, mających kluczowe znaczenie dla odpowiedzi na stres [9, 10, 14-17]. Ekspozycja na EMF może wywoływać odpowiedzi zarówno komórkowe jak i na poziomie całego organizmu, charakterystyczne dla ogólnej reakcji stresowej.

Zintegrowana reakcja na bodźce stresowe jest kluczowym elementem procesów adaptacyjnych, niezbędnych dla przeżycia organizmu. Nieprawidłowy przebieg odpowiedzi na stres jest uważany za jedną z głównych neuropatologicznych przyczyn zaburzeń związanych ze stresem. Zdrowy organizm potrafi efektywnie aktywować lub wygaszać fizjologiczne i psychologiczne odpowiedzi na bodźce, jednakże konsekwencją nieadekwatnej reakcji układów stresu – zbyt powolnej lub zbyt intensywnej – może być zwiększona podatność na choroby związane ze stresem. Adaptacja do powtarzającego się stresu związana jest z aktywacją różnorodnych systemów molekularnych, neuronalnych i neurochemicznych, odpowiadających za zachowania i reakcje fizjologiczne [18].

Modyfikacje mechanizmów molekularnych pod wpływem EMF obejmują: zmiany w produkcji wolnych rodników oraz ochronie antyoksydacyjnej [11, 19]; zmiany w homeostazie wapnia [20, 21]; zmiany w poziomie ekspresji czynników neurotroficznych, takich jak BDNF (ang. *brain-derivedneurotrophicfactor*) [22, 23]; wpływ na poziom białek związanych z

plastycznością mózgową, neurogenezą, proliferacją, różnicowaniem komórek, neuroprotekcją oraz prawidłowym funkcjonowaniem mózgu [24-29]. Wymienione mechanizmy mają swoje następstwa w postaci zmienionych wzorców aktywności neuroprzekaźników, wydzielania hormonów oraz metabolizmu mózgu.

3. Stres oksydacyjny – jako efekt odziaływania pola elektromagnetycznego

Ważnym zjawiskiem zaobserwowanym jako odpowiedź organizmu na ekspozycję na EMF są wspomniane wcześniej zmiany w równowadze oksydacyjno-antyoksydacyjnej. Równoważenie puli reaktywnych form tlenu (ROS) przez antyoksydanty jest kluczowe dla utrzymania homeostazy organizmu. ROS takie jak anionorodnik ponadtlenkowy ($O2^{\bullet -}$), nadtlenek wodoru (H_2O_2), rodnik hyroksylowy ($HO^{\bullet}$) czy tlen singletowy ($^1O_2$) odgrywają ważne role fizjologiczne m.in. podczas sygnalizacji komórkowej. Są one produktami ubocznymi metabolizmu organizmów żywych, jednak w pewnych sytuacjach następuje ich wzmożona produkcja (narażenie na zanieczyszczenia, metale ciężkie, promienie UV lub promieniowanie jonizujące). Wśród antyoksydantów wyróżnić można białka wiążące jony metali, np. metalotioneiny, witaminy E i C, karotenoidy, polifenole oraz enzymy antyoksydacyjne. Do tych ostatnich zalicza się m.in. dysmutazę ponadtlenkową (SOD), katalazę (CAT) czy peroksydazę glutationową (GPX) [30]. Jeżeli system antyoksydacyjny nie jest wydajny na tyle, aby zrównoważyć nadmiar ROS, organizm doświadcza stresu oksydacyjnego. Następstwem jest uszkodzenie komórek i tkanek, a w skrajnej postaci rozwój niektórych chorób (cukrzyca i inne zaburzenia metaboliczne, miażdżyca i choroby układu krążenia, nowotwory, choroby neurodegeneracyjne czy psychiczne) [31, 32]. Należy zauważyć, iż mózg jest organem niezwykle wrażliwym na stres oksydacyjny ze względu na wysoką zawartość nienasyconych lipidów, metabolizm neuroprzekaźników generujący nadtlenek wodoru (H_2O_2), zdolność neuroprzekaźników do autooksydacji oraz zaangażowanie reaktywnych form tlenu w sygnalizację wapniową [32].

Wyniki badań dotyczących wpływu EMF (50/60 Hz) na stres oksydacyjny i obronę antyoksydacyjną często są sprzeczne lub niewystarczające do sformułowania definitywnych wniosków. Zatem ustalenie czy EMF ma szkodliwy, czy korzystny wpływ na organizm jest kwestią wymagającą pogłębionych badań oraz wieloaspektowego podejścia. Możliwość indukcji stresu oksydacyjnego jest głównym negatywnym skutkiem narażenia na EMF [33-35]. W kilku badaniach u osób i zwierząt narażonych na EMF zaobserwowano wzrost stężenia malondialdehydu (MDA) – jednego z najczęściej oznaczanych markerów peroksydacji lipidów [33, 34]. Ponadto indukowane przez EMF uszkodzenia oksydacyjne często są związane z obniżeniem obrony antyoksydacyjnej [35-37]. Z drugiej strony istnieją doniesienia o

przeciwstawnym działaniu EMF, np. o aktywacji systemów antyoksydacyjnych [11, 37, 38]. W badaniach na liniach komórkowych i zwierzętach wykazano, że EMF może zwiększać ekspresję enzymów antyoksydacyjnych, w tym peroksydazy glutationowej (GPX), S-transferazy glutationowej (GST), katalazy (CAT), dysmutazy ponadtlenkowej (SOD) [37, 39, 40]. Ekspozycja na EMF może również wpłynąć na redukcję ROS oraz markerów stresu oksydacyjnego [41, 42]. Te właściwości protekcyjne EMF są również wykorzystywane w medycynie, np. w leczeniu uszkodzeń mózgu [11].

Wiele wskazuje na to, że ostateczny efekt EMF w kierunku pobudzenia lub zahamowania obrony antyoksydacyjnej zależy od stanu w jakim znajduje się komórka/organizm (fizjologiczny/patologiczny) oraz od czynników zewnętrznych (dodatek różnych substancji chemicznych, np. leków). Istnieją dowody na to, że prekondycjonowanie komórek poprzez ekspozycję na EMF może zabezpieczać przed stresem oksydacyjnym wywołanym przez czynnik prooksydacyjny, jakim jest H_2O_2. Komórki traktowane H_2O_2 po uprzedniej ekspozycji na EMF wykazywały większą przeżywalność oraz brak znaczącego wzrostu w poziomie ROS. Po inkubacji z H_2O_2 w komórkach prekondycjonowanychEMF nastąpił również wzrost aktywności dysmutazy ponadtlenkowej zależnej od manganu -MnSOD [43].

4. EMF jako czynnik stresowy

Ekspozycja na EMF może być traktowana jako sytuacja stresowa [9, 15-17], która jest w stanie aktywować szerokie spektrum procesów wewnątrzkomórkowych, które z kolei determinują fizjologiczne i behawioralne odpowiedzi na stres. Jako łagodny stresor EMF staje się sygnałem, który aktywuje neurony podwzgórza. Pierwszym etapem odpowiedzi organizmu na stres jest pobudzenie układu sympatyczno-nadnerczowego (ang. *sympatho-adrenomedullary system*; SAM). Mobilizacja układu autonomicznego skutkuje uwolnieniem noradrenaliny oraz adrenaliny z komórek rdzenia nadnerczy do krwioobiegu. Hormony, które docierają w ten sposób do narządów obwodowych mogą w krótkim czasie regulować ich funkcje [44]. Noradrenergiczna odpowiedź na bodziec stresowy jest kontrolowana głównie przez neurony jądra sinawego (ang. *locus coeruelus*; LC), które jest głównym miejscem syntezy noradrenaliny (NA). System noradrenergiczny miejsca sinawego (LC-NA) wywiera szeroki wpływ na obwody neuronalne. Liczne projekcje wychodzące z LC unerwiają różne struktury mózgowe m.in. hipokamp i podwzgórze. Dysregulacja systemu LC-NA może być związana z licznymi zaburzeniami poznawczymi i afektywnymi, w tym zaburzeniami związanymi ze stresem [45, 46]. Noradrenalina uwalniana w warunkach stresu działa poprzez specyficzne receptory adrenergiczne, prowadząc do zwiększonego stanu pobudzenia, co jest

kluczowe dla adaptacyjnych odpowiedzi na stres [47]. Głównym metabolitem pochodzącym z degradacji NA jest 3-metoksy-4-hydroksyfenyloglikol (MHPG). Indeks utylizacyjny noradrenaliny (MHPG/NA) jest ważnym markerem aktywności noradrenergicznej [48].

Po zainicjowaniu odpowiedzi stresowej, jako druga zostaje aktywowana oś HPA. Pobudzenie szlaku noradrenergicznego jest niezbędne do aktywacji osi HPA [49]. Dochodzi wtedy do wydzielenia glikokortykoidów (GC) (kortykosteronu u szczurów, CORT) z kory nadnerczy [44]. Odpowiedź osi HPA jest zapoczątkowana poprzez wydzielanie kortykoliberyny (ang. *corticotropin-releasing hormone*; CRH) przez neurony jądra przykomorowego (ang. *paraventricular nucleus*; PVN) w podwzgórzu. CRH trafia poprzez przysadkowy splot krążenia wrotnego do przedniego płata przysadki mózgowej [50]. W tej strukturze CRH wiąże się do receptora CRH-R1 powodując produkcję i wydzielanie hormonu adrenokortykotropowego (ang. *adrenocorticotropic hormone*; ACTH) [51]. ACTH z krwią trafia do kory nadnerczy, gdzie działa na receptor melanokortynowy 2 (MC2R) indukując w ten sposób syntezę i wydzielanie GC [50]. Uwolniony kortykosteron jest ligandem dla receptorów mineralokortykoidowych (MR) o wysokim powinowactwie i glikokortykoidowych (GR) o niskim powinowactwie do hormonu. Receptory te występują w dużym stężeniu w hipokampie, dzięki czemu hipokamp ma duży wpływ na aktywność osi HPA [52, 53]. Oś HPA działa na zasadzie ujemnego sprzężenia zwrotnego, gdzie GC wraz z receptorami MR i GR są zaangażowane w regulację odpowiedzi na stres zarówno na poziomie przysadki, podwzgórza jak i hipokampu. Równowaga pomiędzy aktywacją obu typów receptorów MR/GR ma kluczowe znaczenie dla prawidłowego przebiegu reakcji stresowej [54]. Chroniczny stres prowadzący do utrzymywania się wysokiego stężenia GC we krwi zaburza funkcjonowanie komórek, a nawet może prowadzić do rozwoju chorób, w których etiologii udowodniono udział glikokortykoidów [55].

Wpływ EMF na aktywność osi HPA i układu noradrenergicznego został potwierdzony w wielu badaniach [9, 17, 56-58]. Stwierdzono, że długotrwała ekspozycja na EMF (0,5 mT) wywołuje pewne objawy stresu lub aktywacji osi HPA: podwyższony poziom glukozy we krwi i zwiększenie poziomu mRNA proopiomelanokortyny – prekursora ACTH w przysadce mózgowej, bez zmian w poziomie stężenia ACTH i kortykosteronu [9]. Badania Mahdavi'ego i wsp. (2014) wykazały, że długotrwała ekspozycja na EMF (0,1 mT) podnosi poziom ACTH u szczurów przy jednoczesnym spadku wydzielania CORT. W innym badaniu zauważono wzrost stężenia ACTH i adrenaliny po rozpoczęciu ekspozycji na EMF, następnie nastąpiło stopniowe obniżenie poziomu hormonów aż do osiągnięcia wartości kontrolnej [17]. Badania na modelu mysim wykazały, że stosunkowo niski poziom EMF (12 nT) może prowadzić do wzrostu poziomu kortykosteronu [59]. Dowiedziono, że ekspozycja na EMF (10 Hz; 1,8–3,8 mT) wiąże się ze zmianami reaktywności receptorów układów monoaminergicznych, a także ma wpływ na pewne zachowania, które znajdują się pod kontrolą tych układów [14]. Ponadto, EMF (2 mT) powoduje spadek poziomu noradrenaliny w strukturach mózgu takich jak: prążkowie,

wzgórze, móżdżek i hipokamp [60]. Istnieją również badania wykazujące brak jakichkolwiek zmian w poziomach hormonów stresu u zwierząt i ludzi narażonych na EMF [61].

Zmiany poziomu hormonów stresu indukują określone zaburzenia behawioru. GC wpływając na fizjologię synaps i regulując obwody neuronalne układów stresu mobilizują zasoby energetyczne, które mają zapewnić organizmowi adaptację behawioralną do stresu. Jednak, gdy mechanizmy adaptacyjne stają się niewydolne wzrasta ryzyko chorób związanych z podwyższonym poziomem stresu i lęku [62]. Istnieją badania wskazujące na efekt anksjogeniczny EMF oraz możliwość pojawienia się zachowań depresyjno-podobnych u gryzoni, co skorelowane jest z wystąpieniem stresu oksydacyjnego lub podwyższeniem stężeń hormonów stresu [9, 17, 63]. Na modelu zwierzęcym wykazano, że ekspozycja na EMF spowodowała wzrost wydzielania kortykosteronu z jednoczesnym nasileniem zachowań lękowych i depresyjnych [16, 64]. U osób narażonych zawodowo na działanie EMF zaobserwowano zmieniony wzorzec wydzielania kortyzolu oraz pogorszoną jakość snu i zwiększony poziom stresu i lęku, a także zaburzeń depresyjnych [65, 66].

5. Wpływ pola elektromagnetycznego na plastyczność mózgową

Niektóre badania sugerują pozytywny wpływ EMF na funkcje poznawcze [26, 67]. W przypadku zwierzęcego modelu uszkodzenia mózgu obserwuje się wzmocnienie procesów uczenia się i formowania pamięci przestrzennej [26]. Natomiast u pacjentów po przebytym udarze niedokrwiennym terapia z zastosowaniem EMF przyczynia się do redukcji stresu oksydacyjnego i równolegle do poprawy statusu mentalnego oraz ogólnego funkcjonowania [11]. Podłożem korzystnego wpływu EMF na procesy poznawcze może być wzmacnianie plastyczności mózgowej. Jak wykazują badania mechanizm odpowiedzialny za neuroplastyczny efekt EMF może obejmować aktywację receptorów w układach stresu: mineralokortykoidowych, glikokortykoidowych oraz noradrenergicznych [44, 52]. Relacje pomiędzy układami noradrenergicznym i kortykoidowym a plastycznością mózgową zostały potwierdzone w badaniach eksperymentalnych [68, 69]. Dlatego też można przypuszczać, że zmiany na którymkolwiek etapie reakcji stresowej mogą skutkować modyfikacjami plastyczności mózgowej.

Stwierdzono, że EMF wpływa szczególnie na funkcje hipokampu– obszaru mózgu, w którym u dorosłych osobników procesy plastyczności mózgowej przebiegają najintensywniej [70, 71]. Kortykosteroidy oddziałując ze swoimi receptorami wpływają na procesy plastyczności w hipokampie. Aktywacja poszczególnych typów receptorów, a co za tym idzie rodzaj odpowiedzi organizmu, zależy od poziomu GC. Wykazano, że niskie poziomy

kortykosteronu aktywują receptory MR, natomiast do aktywacji GR konieczne są ich wyższe stężenia [54]. W przypadku związania się GC z MR indukowane zostają procesy neuroadaptacyjne warunkujące przeżywalność neuronów, natomiast aktywacja GR prowadzi do podniesienia wrażliwości na ekscytotoksyczność, a w konsekwencji do neurodegeneracji [52]. Kortykosteron wywiera więc dwukierunkowy wpływ – zależny od dawki - na funkcje hipokampu. Taki efekt może być uznany za hormezopodobny [52, 54].

Noradrenalina moduluje procesy plastyczności w hipokampie, działając poprzez receptory β2-adrenergiczne (β2-AR) [68, 69]. Stres stymuluje uwalnianie NA z LC, która wywiera silny efekt na funkcję hipokampu i poprzez receptory β2-AR reguluje transkrypcję genów związanych z odpowiedzią na stres [68]. Dzięki temu mechanizmowi noradrenalina wspiera procesy neuroplastyczności w hipokampie [68]. Aktywacja neuronów noradrenergicznych przyczynia się do aktywacji endogennej neurotrofiny BDNF i jej receptora TrkB [69]. Uwalnianie noradrenaliny z miejsca sinawego uwrażliwia neurony hipokampu, a stan ten może utrzymywać się 24 h, co sugeruje, że NA uczestniczy w metaplastycznej regulacji kodowania informacji w hipokampie [72]. Dynamika oddziaływania noradrenaliny z receptorami β2-AR może być uznana za wyznacznik tego czy dane doświadczenie będzie skutkowało uruchomieniem mechanizmów neuroplastycznych w hipokampie oraz decyduje o kierunku zmian siły synaptycznej [73]. Udowodniono, że receptory β2-AR biorą udział w nasilaniu ekspresji BDNF w hipokampie podczas terapii lekami antydepresyjnymi [74]. Te odkrycia wskazują, że zwiększona neurotransmisja noradrenaliny wywiera wpływ na syntezę BDNF [73].

Szereg badań potwierdza, że ekspozycja na EMF może modulować procesy plastyczności synaptycznej, takie jak długotrwałe wzmocnienie synaptyczne (ang. *long-term potentiation*; LTP) oraz długotrwałe osłabienie synaptyczne (ang. *long-term depression*; LTD) [75]. Zjawiska te, choć mają odmienny wpływ na aktywność synaps, są kluczowe dla uczenia się oraz procesów pamięciowych. Wykazano również, że wpływ EMF na LTP jest zależny od dawki tego bodźca (częstotliwości, wartości indukcji magnetycznej oraz czasu ekspozycji) [76, 77].

Badano również wpływ EMF na neurogenezę. W przypadku udaru niedokrwiennego wykazano, że ekspozycja na EMF (1 mT) powoduje wzrost liczby nowych neuronów. Ponadto, efekt ten był skorelowany z nasileniem ekspresji białek promujących proliferację oraz różnicowanie komórek macierzystych [25]. Stymulujące właściwości EMF są także coraz częściej wykorzystywane w medycynie, mi.in. u osób z uszkodzeniami mózgu, u których pobudzenie procesów neuroplastyczności jest kluczowym elementem przywrócenia sprawności i poprawy jakości życia. Cichoń i wsp. przeprowadzili serię eksperymentów w grupie pacjentów po przebytym udarze [11, 23, 78]. W badaniach tych zaobserwowano, że

ekspozycja na EMF ma wpływ na wzrost stężenia czynników wzrostu, takich jak BDNF oraz innych mediatorów regulujących procesy neuroplastyczności. W badaniach na zwierzętach również wykazano podwyższony poziom czynników wzrostu takich jak BDNF i GDNF (ang. *glial cell line-derived neurotrophic factor*) w odpowiedzi na działanie EMF [42, 79].

6. Hipoteza o dwukierunkowym działaniu EMF – efekt hormezy

Bazując na dotychczas opublikowanych wynikach badań dotyczących wpływu EMF na organizmy żywe założono, że oddziaływanie tego rodzaju bodźca fizycznego ma charakter hormetyczny. Hormeza jest zjawiskiem dwukierunkowej reakcji organizmu polegającej na tym, że czynnik, który w dużej dawce jest szkodliwy dla organizmu, w małej dawce działa stymulująco wywołując odpowiedź adaptacyjną [80, 81]. W farmakologii oraz toksykologii powszechnie obserwuje się efekt zależny od dawki substancji. W biologii i medycynie, hormeza jest definiowana jako odpowiedź adaptacyjna komórek i organizmu na umiarkowany (przerywany) stres [82]. Indukowane polem elektromagnetycznym zmiany reakcji stresowej na skutek aktywacji odpowiednich receptorów zwrotnie modulują aktywność układów stresu – ustalają nowy stan równowagi, a zatem efekty każdej następnej ekspozycji będą się nakładały na zmiany wywołane podczas pierwszej ekspozycji. Ta teoria jest zgodna z założeniami hormezy, która zakłada kluczowe znaczenie komponenty czasowej w tym procesie.

Hormeza może być uznana za przystosowanie ewolucyjne, które definiuje granice plastyczności biologicznej w odpowiedzi na stres [80]. Tym samym słabe pole elektromagnetyczne (o niskiej wartości indukcji magnetycznej) mogłoby pobudzać mechanizmy antyoksydacyjne organizmu i indukować szlaki komórkowe zaangażowane w neuroplastyczność, co ma fundamentalne znaczenie dla zachowania homeostazy w trudnych warunkach. Wymaga to ekspresji genów zapewniających komórkom odporność na stres i syntezy białek o funkcji ochronnej, do których należą: białka z rodziny HSP, chaperony i enzymy antyoksydacyjne [83, 84]. Z drugiej strony silne pole (o wysokiej wartości indukcji magnetycznej)będzie bodźcem przekraczającym limit plastyczności biologicznej i wywołującym uszkodzenia komórek/tkanek przy braku wydajnych mechanizmów ochronnych.

Wiele czynników środowiskowych oraz eksperymentalnych ma udokumentowany wpływ na organizm [54, 72, 85]. Zmieniają one fenotypową odpowiedź organizmu, co sprawia, że punkt nastawczy aktywności układów stresu zmienia się i organizm reaguje inaczej na kolejne czynniki stresowe [52, 54, 72, 86]. Z badań, które zostały opisane powyżej wynika, że EMF może zmieniać stężenia markerów stresu. Można więc przypuszczać, że zmiany te

indukują przesunięcie punktu nastawczego układów endokrynologicznych, co modyfikuje odpowiedź na przyszłe wydarzenia stresowe [15].

Tzw. „two-hit model" jest ciekawym konceptem wyjaśniającym etiologię wielu zaburzeń związanych z układem nerwowym. Według tego modelu czynniki środowiskowe („first hit") mają możliwość modulowania funkcji układu nerwowego w trwały sposób. W konsekwencji następuje zmiana odpowiedzi na kolejny czynnik stresowy („second hit"), co prowadzić może do pogłębionych zaburzeń homeostazy. Jest to stan prowadzący często do rozwoju chorób [87]. Ponadto, udokumentowana plastyczność mózgowa pozwala przypuszczać, że może również dochodzić do uruchomienia mechanizmów kompensacyjnych w odpowiedzi na stres, co sugerowałoby neuroadaptację do ekspozycji na inne rodzaje stresu.

Indukowane małą dawką zaburzenie równowagi wywołuje reakcję adaptacyjną, a organizm mobilizuje swe siły obronne w pewnym nadmiarze, przygotowując się do kolejnego zagrożenia, natomiast w przypadku dużych dawek zaburzenie homeostazy jest tak duże, że uruchomienie powyższego mechanizmu jest niemożliwe.

7. Uzasadnienie podjęcia badań

Przegląd literatury z zakresu działania EMF na układ nerwowy pozwala zauważyć, że wyniki dotychczasowych badań są bardzo zróżnicowane. Badania wskazują na dwie możliwe reakcje organizmu w odpowiedzi na ekspozycję na EMF: 1) negatywną – obejmującą zakłócenia w funkcjonowaniu organizmu oraz 2) pozytywną – poprawę różnych parametrów fizjologicznych oraz ogólnych czynności organizmu, co może być podstawą dla wykorzystania EMF w terapii. Wielkość wywoływanego efektu również różni się w poszczególnych badaniach.

Rozbieżności w wynikach badań nad wpływem EMF na żywe komórki, zwierzęta i ludzi mogą być związane z zastosowaniem pola o różnych częstotliwościach, wartościach indukcji magnetycznej oraz odmiennego czasu ekspozycji. Warto również zwrócić uwagę na fakt, iż ekspozycji poddawane są komórki znajdujące się w różnych fazach cyklu komórkowego, jak również różne typy komórek: macierzyste czy nowotworowe. Doświadczenia na zwierzętach wykonywane są na osobnikach zdrowych jak i na modelach chorób neurodegeneracyjnych. U osób po przebytych urazach mózgu ekspozycja terapeutyczna przynosi rezultaty w postaci poprawy funkcjonowania i przyspieszenia rekonwalescencji. Jednakże osoby pozostające w stałym narażeniu na EMF ze względu na miejsce zamieszkania lub wykonywany zawód wykazują negatywne objawy powiązane z działaniem tego czynnika, takie jak obniżenie nastroju, zaburzenia snu czy depresja. Ze względu na powszechność występowania źródeł EMF w środowisku, koniecznym jest ustalenie możliwych efektów biologicznych pola oraz poznanie dokładnego mechanizmu jego działania w organizmach żywych, a także ustalenie

bezpiecznych limitów dotyczących natężenia pola oraz czasu narażenia na ten czynnik. Ponadto dalsze badania umożliwią pełne poznanie właściwości terapeutycznych EMF jako alternatywy lub uzupełnienia dla dotychczas znanych metod leczenia, które nie zawsze przynoszą oczekiwane rezultaty.

W wielu wypadkach negatywny efekt EMF jest skorelowany z zastosowaniem pola o bardzo dużej indukcji magnetycznej, powyżej 7 mT [16, 64, 88, 89]. Natomiast pole o niższych wartościach indukcji magnetycznej wywołuje słabszy efekt [9, 15, 17]. Co więcej, w wielu badaniach wykazano, że pozytywne działanie EMF (np. wzrost przekaźnictwa synaptycznego w hipokampie dzięki wzmocnieniu LTP) występuje zwłaszcza w przypadku zastosowania wartości indukcji magnetycznej na poziomie 1 mT i niższym [70, 71].

Według dostępnej wiedzy, hormeza EMF (o częstotliwości 50 Hz) została wykazana tylko na modelu zwierzęcym owada (*Drosophilamelanogaster*) [90]. Zatem przeprowadzone badania mają charakter pionierski, ponieważ po raz pierwszy zaplanowano zweryfikować dwukierunkowy (hormetyczny) mechanizm działania EMF u zwierząt kręgowych (na modelu szczura).

Cele i hipotezy

Aktualny stan wiedzy pozwolił na postawienie hipotezy, że EMF wykazuje hormezę, tzn. dwukierunkowe działanie uzależnione od natężenia pola elektromagnetycznego (indukcji magnetycznej).

Według powyższej hipotezy okresowa, powtarzana ekspozycja na EMF zmienia “punkt nastawczy” (set-point) aktywności układów stresu, a dynamika tego procesu oraz kierunek zmian zależą od siły tego pola (indukcji magnetycznej). A zatem EMF zmienia wrażliwość organizmu na kolejne czynniki stresowe, a tym samym na choroby, szczególnie układu nerwowego. Przedstawiona w pracy doktorskiej propozycja mechanizmu EMF obejmuje zmiany w statusie oksydacyjno-antyoksydacyjnym mózgu oraz w funkcjonowaniu układów stresu – osi podwzgórze-przysadka-nadnercza (HPA) i układu noradrenergicznego (LC-NA), czego konsekwencją będą zmiany w poziomie hormonów stresu i ich receptorów. Wydzielane w zmienionych stężeniach hormony stresu, głównie kortykosteron i noradrenalina, mogą wpływać na funkcjonowanie hipokampu i modulować procesy neuroplastyczności w tej strukturze [91, 92]. Procesy te odpowiadają z kolei za indukowaną przez EMF hormezę komórkową czyli przeciwstawną reakcję wewnątrzkomórkowych szlaków sygnałowych w zależności od natężenia pola: 1) o charakterze kompensacyjnym, która uruchamia

mechanizmy adaptacyjne do kolejnych zdarzeń stresowych lub 2) uwrażliwienie układów stresu na kolejne czynniki stresowe z powodu niezdolności do przywrócenia homeostazy wewnątrzkomórkowej.

W badaniach zweryfikowano cztery szczegółowe hipotezy robocze:

1. U podłoża dwukierunkowego działania EMF leżą zmiany statusu oksydacyjno-antyoksydacyjnego mózgowia (hipoteza I)
2. EMF inicjuje zmiany w odpowiedzi stresowej, których kierunek i nasilenie zależą od natężenia pola elektromagnetycznego (hipoteza II)
3. EMF trwale modyfikuje status oksydacyjno-antyoksydacyjny i poziom aktywności układów stresu i tym samym zmienia odpowiedź na kolejne czynniki stresogenne (hipoteza III)
4. Zmiany w odpowiedzi stresowej indukowanej przez EMF modulują plastyczność mózgową (hipoteza IV)

Celem proponowanych badań było 1) określenie czy pole elektromagnetyczne o niskiej częstotliwości (50 Hz) wykazuje hormezę, tzn. dwukierunkowe działanie uzależnione od natężenia tego pola (indukcji magnetycznej, wyrażanej w Teslach) i 2) wyjaśnienie mechanizmu tego zjawiska na modelu zwierzęcym.

Cele szczegółowe niniejszej pracy obejmowały weryfikację postawionych hipotez:

1. Określenie wielkości i kierunku zmian w stężeniach markerów stresu oksydacyjnego i poziomie obrony antyoksydacyjnej w korze przedczołowej u szczurów poddanych trzykrotnej ekspozycji na zmienne EMF o częstotliwości 50 Hz (w skrócie: 50 Hz EMF) oraz o dwóch wartościach indukcji magnetycznej – 1 mT i 7 mT (***Praca 2***)

2. Określenie wielkości i kierunku zmian w stężeniach hormonów osi HPA i układu LC-NA oraz ekspresji ich receptorówu szczurów poddanych trzykrotnej ekspozycji na 50 Hz EMF o dwóch wartościach indukcji magnetycznej – 1mT i 7 mT (***Prace 3; 4***)

3. Określenie wpływu ekspozycji na EMF o dwóch wartościach indukcji magnetycznej – 1mT i 7 mT na wielkość zmian w stężeniach hormonów osi HPA i układu LC-NA i ekspresji ich receptorów oraz markerów stresu oksydacyjnego i obrony antyoksydacyjnej indukowanych ekspozycją na kolejny czynnik stresowy (***Prace 2; 3; 4***)

4. Określenie poziomu ekspresji receptorów mineralokortykoidowych (MR) a także receptorów adrenergicznych β2 (β2-AR) w hipokampie u szczurów poddanych trzykrotnej ekspozycji na 50 Hz EMF o dwóch wartościach indukcji magnetycznej – 1mTi 7 mT (***Prace 3; 4***)

Weryfikacja stawianych hipotez ma istotne znaczenie dla określenia wpływu pola elektromagnetycznego na organizm. Poznanie i zrozumienie tego zjawiska pozwoli na określenie ewentualnych zagrożeń związanych z ekspozycją na EMF. Może mieć również znaczenie aplikacyjne i posłużyć do rozwinięcia metod terapeutycznych w różnego typu zaburzeniach funkcji układu nerwowego.

Metody badawcze

Na badania przeprowadzone w ramach niniejszej pracy uzyskano zgodę Lokalnej Komisji Etycznej w Bydgoszczy (nr zezwolenia 3/2018).

1. Zwierzęta doświadczalne

Do badań zostały wybrane dorosłe (trzymiesięczne) samce szczurów rasy Wistar Han o wadze pomiędzy 300 a 350 g. Jest to uniwersalny model zwierzęcy wykorzystywany w badaniach z zakresu fizjologii, w tym także reakcji stresowej. Zatem gatunek ten jest dobrze poznany pod względem wszystkich elementów odpowiedzi na stres od poziomu molekularnego, poprzez fizjologię, kończąc na behawiorze. Całkowita liczba zwierząt przeznaczonych do doświadczeń wynosiła 180. Zwierzęta zostały podzielone na 6 grup:

1) EMF/1 mT: zwierzęta poddane ekspozycji na EMF (50 Hz, 1 mT)

2) EMF/BT/1 mT: zwierzęta poddane ekspozycji na EMF (50 Hz, 1 mT), a następnie testom behawioralnym - otwartego pola (OF) oraz podwyższonego labiryntu krzyżowego (EPM)

3) EMF/7 mT: zwierzęta poddane ekspozycji na EMF (50 Hz, 7 mT)

4) EMF/BT/7 mT: zwierzęta poddane ekspozycji na EMF (50 Hz, 7 mT), a następnie testom behawioralnym - otwartego pola (OF) oraz podwyższonego labiryntu krzyżowego (EPM)

5) C: zwierzęta kontrolne, poddawane tym samym procedurom co zwierzęta eksperymentalne z grup 1 i 3, z wyjątkiem ekspozycji na EMF

6) C/BT: zwierzęta kontrolne, poddawane tym samym procedurom co zwierzęta eksperymentalne z grup 2 i 4, z wyjątkiem ekspozycji na EMF

2. Aparatura

Pole elektromagnetyczne z przewagą składowej magnetycznej zostało wytworzone przy użyciu cewek o średnicy 20 cm (Elektronika i Elektromedycyna Sp. J., Otwock, Polska). Każda z cewek składała się z 282 zwojów drutu miedzianego. Wytwarzane przez cewki i zasilacz Variac pole elektromagnetyczne było jednorodne i miało przebieg sinusoidalny naprzemienny o częstotliwości 50 Hz i zakresie indukcji magnetycznej 0,1 - 8 mT. Wykorzystane w doświadczeniach wartości indukcji magnetycznej to 1 mT i 7 mT. Przed każdą ekspozycją dokonywano pomiarów wartości indukcji magnetycznej przy użyciu miernika gaussa (model GM2, AlphaLab, Inc, USA). Niejednorodność pola elektromagnetycznego w obszarze, w którym znajdowała się klatka ze zwierzęciem wynosiła 10%. Zwierzęta z grup kontrolnych poddano tej samej procedurze eksperymentalnej (ekspozycja symulowana) bez udziału pola elektromagnetycznego (<10 μT). Temperatura w cewkach była monitorowana w trakcie każdej ekspozycji za pomocą termopar.

W badaniach zdecydowano wykorzystać pole elektromagnetyczne o dwóch wartościach indukcji magnetycznej: 1 mT i 7 mT. Według dyrektywy unijnej 2013/35/EU [93] wartości pola od 1 mT – 6 mT wyznaczają zakres wartości pola elektromagnetycznego, w którym stwierdza się zmiany aktywności mózgu, jednak bez ryzyka trwałych zaburzeń. Niespecyficzne efekty pola magnetycznego w żywych tkankach są możliwe tylko przy wartościach powyżej 1 mT, która jest wartością progową do wytwarzania prądów elektrycznych w ludzkim mózgu, natomiast 6 mT jest górną wartością progową dla minimalnych przejściowych zmian w niektórych funkcjach mózgu, a wartości indukcji magnetycznej poniżej tej wartości uznawane są za bezpieczne dla ludzi.

3. Procedura doświadczalna

Tuż przed ekspozycją na pole elektromagnetyczne lub warunki kontrolne szczury były umieszczane pojedynczo w plastikowych klatkach (12 cm × 20 cm × 14 cm) z otworami zapewniającymi dostęp powietrza. Następnie klatki wkładano do cewek. Ekspozycje (1h ekspozycji dziennie przez 7 kolejnych dni) były powtarzane 3-krotnie w 3-tygodniowych

odstępach czasu. Po każdym z 3 okresów ekspozycji część zwierząt z grup EMF/1mT i EMF/7mT była dekapitowana i pobierano tkanki do oznaczeń. Część zwierząt z grup EMF/BT/1mT i EMF/BT/7mT po każdej ekspozycji na pole elektromagnetyczne była poddana testom behawioralnym (otwarte pole i podwyższony labirynt krzyżowy) i następnie dekapitowana w celu pobrania tkanek do oznaczeń. Szczury kontrolne były poddawane identycznym procedurom jak odpowiadające im zwierzęta z grup eksponowanych na EMF, z wykluczeniem ekspozycji na EMF.

4. Przygotowanie prób

W celu wykonania oznaczeń z mózgu wyizolowano hipokamp, podwzgórze, przysadkę, miejsce sinawe i korę przedczołową. Pobrano także nadnercza oraz krew z serca. Struktury mózgowe i nadnercza zostały zważone, a następnie zanurzone w ciekłym azocie i przechowywane w temperaturze -80ºC do chwili wykonania oznaczeń. Krew pobrano do probówek zawierających roztwór soli disodowej kwasu etylenodiaminotetraoctowego (Na_2EDTA, Sigma-Aldrich) i odwirowano. Otrzymane osocze przechowywano w temperaturze -20ºC do chwili oznaczeń. Przed wykonaniem oznaczeń struktury mózgowe oraz nadnercza zostały zhomogenizowane odpowiednio w buforze PBS lub buforze fosforanowym, tak aby otrzymać dziesięciokrotne rozcieńczenie. Próby odwirowano i zebrano supernatant do oznaczeń.

5. Oznaczanie markerów stresu oksydacyjnego (*Praca 2*)

Aby oznaczyć stężenie grup karbonylowych białek (CP) użyto kitu Protein Carbonyl Content Assay Kit (Sigma-Aldrich, No MAK094, USA). Zgodnie z instrukcją producenta przeprowadzono reakcję z 2,4-dinitrofenylohydrazyną (DNPH). Końcowy produkt tej reakcji to addukty hydrazonudinitrofenylu (DNP) oznaczane spektrofotometrycznie przy długości fali 375 nm. Ich stężenie jest proporcjonalne do stężenia CP. Otrzymane stężenia zostały przeliczone na 1 mg białka.

Stężenie 8-izoprostanów (8-epi PGF2 α) – końcowego produktu utleniania kwasu arachidonowego, określono przy pomocy testu kompetecyjnego ELISA (8-Isoprostane ELISA Kit; CaymanChemical, No 516351, USA). Test opiera się na konkurencji pomiędzy 8-izoprostanem i koniugatem 8-izoprostanu-acetylocholinoesterazy (AChE) (znacznik 8-prostanAChE) o miejsca wiązania przeciwciał króliczych specyficznych dla 8-izoprostanu.

Zgodnie z metodą stężenie znacznika 8-izoprostanu-AChE utrzymuje się na stałym poziomie, natomiast stężenie 8-izoprostanu w próbach się zmienia. Zatem ilość znacznika 8-izoprostanu-AChE, która jest w stanie związać się z przeciwciałem króliczym jest odwrotnie proporcjonalna do stężenia 8-izoprostanu w próbie. Następnie kompleks królicze przeciwciało-8-izoprostan (wolny lub znacznikowy) wiąże się z króliczym mysim przeciwciałem monoklonalnym IgG. Ostatecznie po dodaniu odczynnika Ellmana (zawierającego substrat do AChE) powstaje produkt o żółtej barwie, której intensywność odczytywana jest spektrofotometrycznie przy λ = 412 nm. Stężenie wolnego 8-izoprostanu w próbie jest odwrotnie proporcjonalne do intensywności zabawienia. Stężenie 8-epi PGF2α wyrażono w pikogramach na mililitr próbki.

6. Oznaczanie całkowitej pojemności antyoksydacyjnej (TAC) (*Praca 2*)

Całkowita pojemność antyoksydacyjna próby odnosi się do ilości antyoksydantów małocząsteczkowych oraz białkowych (z wyłączeniem układów enzymatycznych) i reprezentuje zdolność organizmu do przeciwdziałania uszkodzeniom powodowanym przez wolne rodniki. Całkowita ilość antyoksydantów nieenzymatycznych została określona przy pomocy Total Antioxidant Capacity Assay Kit (Sigma-Aldrich, No MAK187, USA). Do przeprowadzenia reakcji użyto roztworu zawierającego jony Cu^{2+}, które w obecności antyoksydantów są redukowane do Cu^{+} tworząc chelaty. Absorbancję prób odczytano przy λ = 570 nm. Do stworzenia krzywej standardowej użyto rozpuszczalnego w wodzie analogu witaminy E (Trolox). Wyniki wyrażono w nanomolach na mililitr próby.

7. Oznaczanie stężeń hormonów osi HPA (CRH, ACTH, CORT) (*Praca 3*) oraz układu noradrenergicznego (NA, MHPG) (*Praca 4*)

Do wykonania oznaczeń stężeń hormonów oraz metabolitu MHPG zastosowano test ELISA. Użyto przy tym komercyjnie dostępnych kitów. Wszystkie procedury zostały wykonane zgodnie z instrukcjami dołączonymi przez producentów. Każda z prób oznaczana była w dwóch powtórzeniach. Do pomiaru zmian kolorymetrycznych użyto wielotrybowego czytnika mikropłytek Epoch 2 (BioTek Instruments, Inc., Winooski, UT, USA). Pomiaru absorbancji prób dokonywano przy długości fali 450 nm. Krzywe standardowe zostały wygenerowane przy użyciu oprogramowania Gen5 Software stosując dopasowanie do czteroparametrowej krzywej logistycznej.

8. Oznaczanie ekspresji receptorów GR, MR, CRH-R1, MC2R, (*Praca 3*) oraz β2-AR (*Praca 4*)

Aby oznaczyć ekspresję badanych receptorów wykorzystano metodę RT-qPCR (ang. *quantitativereversetranscription PCR*). Używając kitów wyekstrahowano RNA (receptory hormonów osi HPA: HP RNA tissue kit RNA Roche Cat. No. 11828665001; β-AR: EXTRACT ME RNA & DNA KIT CytogenCat. No. EM15). Następnie dokonano pomiaru stężeń (ng/µl) przy użyciu spektrofotometru NanoDrop One (Thermo Fisher Scientific). Przy pomocy EvoScript Universal cDNA Master (Roche nr kat. 07 912 455 001)przeprowadzono odwrotną transkrypcję wykorzystując 1 µg RNA. Następnie użyto po 10 ngcDNA, specyficzne primery (Sigma-Aldrich) i SYBR Green PCR Master Mix (Roche nr kat. 12239264001) w systemie LightCycler® 96 (Roche) do przeprowadzenia qPCR. *ACT* (β-aktyna) i *GAPDH* zostały użyte jako geny referencyjne. Do obliczenia względnej ilości transkryptu mRNA badanych genów zastosowano metodę zaproponowaną przez Pfaffla i wsp. [94] z wykorzystaniem oprogramowania REST.

9. Testy behawioralne (*Prace 2; 3; 4*)

Test otwartego pola (OF) jest standardową procedurą pozwalającą na określenie stopnia aktywności lokomotorycznej oraz eksploracyjnej gryzonia. Pozwala także na analizę zachowań lękowych w nieznanym środowisku, takich jak tigmotaksja czy zmiana dynamiki ruchu [95]. Do badań wykorzystano skrzynię o wymiarach 100 cm x 100 cm x 50 cm (wysokość ścian). Nad skrzynią umieszczono źródło światła (żarówka halogenowa o mocy 100 W) oraz kamerę. W obszarze skrzyni zostały wyznaczone strefy: centrum (20 cm x 20 cm), strefa wewnętrzna, strefa zewnętrzna wraz z narożnikami. Zwierzęta były umieszczane w centrum, zawsze skierowane w tę samą stronę. Czas testu wynosił 5 minut. W celu dezynfekcji skrzynia była przemywana preparatem Mediseptol H Neutral (Alpinus Chemia, Polska) każdorazowo po wyjęciu zwierzęcia. Analizie poddano następujące parametry: przebyty dystans (cm), średnia prędkość (cm/s), czas spędzony w ruchu (s), czas do wykonania pierwszego ruchu (s), czas spędzony w strefie zewnętrznej (s), czas spędzony w narożnikach (s), czas spędzony w centrum (s) oraz liczba wejść do centrum.

Podwyższony labirynt krzyżowy (EPM) służy do obserwacji zachowań lękowych u gryzoni. Wykorzystuje się w nim skłonność zwierząt do unikania oświetlonych i otwartych przestrzeni oraz preferencji zamkniętych i zacienionych obszarów [96]. Labirynt w kształcie krzyża, którego ramiona miały długość 40 cm i szerokość 10 cm, został umieszczony 50 cm nad podłożem. Dwa przeciwległe ramiona były ograniczone ścianą o wysokości 40 cm (ramiona zamknięte), pozostałe dwa ramiona zostały zabudowane przezroczystą płytą Plexiglas

(ramiona otwarte), aby uniemożliwić zwierzęciu zeskoczenie z platformy. Nad labiryntem umieszczono źródło światła. Czas testu wynosił 5 minut. Labirynt był przemywany preparatem Mediseptol H Neutral każdorazowo po wyjęciu zwierzęcia w celu dezynfekcji. Następujące parametry zostały poddane analizie: przebyty dystans (cm), średnia prędkość (cm/s), czas spędzony w ruchu (s), czas spędzony w ramionach zamkniętych (s), liczba wejść w ramiona otwarte. Behawior zwierząt był analizowany przy użyciu oprogramowania EthoVision 11 (Noldus, Wageningen, Holandia).

10. Analiza statystyczna

Do analizy uzyskanych danych zastosowano Ogólny Model Liniowy (ang. *general linear model*; GLM), który pozwolił na określenie wpływu EMF o różnych wartościach indukcji magnetycznej oraz ilości przeprowadzonych ekspozycji na równowagę oksydacyjno – antyoksydacyjną mózgu oraz zmiany parametrów reakcji stresowej. Ocena zmian behawioralnych była przeprowadzona przy użyciu wieloczynnikowej analizy wariancji (MANOVA). Następnie wykonano testy post – hoc z poprawką Bonferroniego. Dane, które nie spełniały kryteriów dla GLM oraz MANOVA zostały poddane transformacji (logarytm naturalny, logarytm dziesiętny, pierwiastek kwadratowy). W przypadku, gdy transformacja danych nie pozwoliła na zastosowanie testów parametrycznych przeprowadzono analizę nieparametryczną (test Kruskala-Wallisa z poprawką Bonferroniego). Wszystkie analizy statystyczne zostały wykonane przy pomocy programu komputerowego SPSS 25.0 (IBM Inc.).

Omówienie wyników

1. U podłoża dwukierunkowego działania EMF leżą zmiany statusu oksydacyjno-antyoksydacyjnego mózgowia (weryfikacja hipotezy 1, praca 2)

Wyniki tej części badań wykazały, że powtarzające się narażenie na pole elektromagnetyczne (EMF) o niskiej częstotliwości (50 Hz) zmienia status oksydacyjno-antyoksydacyjny kory przedczołowej szczurów w sposób zależny od intensywności EMF i liczby ekspozycji.

Poziom wskaźników stresu oksydacyjnego i antyoksydantów u szczurów narażonych na EMF o indukcji 1 mT nie różnił się znacząco od wartości kontrolnych. U szczurów z tej grupy

zaobserwowano istotny wzrost poziomu grup karbonylowych białek (CP) dopiero po trzeciej ekspozycji na EMF. Mechanizmy kompensacyjne uruchamiane w odpowiedzi na EMF 1 mT są w stanie utrzymać równowagę oksydacyjną nawet po dwóch ekspozycjach na EMF; dopiero po trzeciej ekspozycji był widoczny skumulowany efekt wszystkich ekspozycji w postaci wzrostu poziomu CP. Ekspozycja na EMF 1 mT nie spowodowała istotnych zmian w poziomie 8-izoprostanów (8-epi PGF2α), jednak zauważono tendencję do zmniejszania się ich poziomu z każdą kolejną ekspozycją. Ponadto, w tej grupie nie stwierdzono wyraźnych zmian w poziomie całkowitego potencjału antyoksydacyjnego (TAC). Poziom TAC był nieznacznie wyższy niż w grupie kontrolnej po pierwszej i drugiej ekspozycji na EMF 1 mT.

Powtarzana ekspozycja na EMF 7 mT powoduje zaburzenie równowagi oksydacyjnej w kierunku wyższego poziomu stresu oksydacyjnego. Stwierdzono wyraźny wzrost poziomu CP w korze przedczołowej, widoczny wcześniej niż w grupie narażonej na EMF 1 mT - już po drugiej ekspozycji i pozostał wysoki również po trzeciej ekspozycji. Ponadto zauważono tendencję do wzrostu poziomu 8-epi PGF2α z każdą kolejną ekspozycją, a po ostatniej trzeciej ekspozycji poziom 8-epi PGF2α był istotnie statystycznie wyższy niż w grupie kontrolnej. U zwierząt eksponowanych na EMF 7 mT stwierdzono tendencję do obniżania się poziomu TAC po każdej kolejnej ekspozycji. Uzyskane wyniki wskazują, że w tej grupie wzrost poziomu stresu oksydacyjnego był przynajmniej w pewnym zakresie skorelowany z obniżeniem poziomu TAC.

Uzyskane wyniki pozwalają stwierdzić, że EMF o wartości indukcji magnetycznej równej 1 mT jest czynnikiem, który nie zaburza znacząco równowagi oksydacyjno-antyoksydacyjnej. Natomiast silniejsze pole (7 mT) indukuje uszkodzenia oksydacyjne białek oraz lipidów, a także ma potencjał do osłabiania obrony antyoksydacyjnej mózgu. Mózg, a szczególnie kora przedczołowa, jest szczególnie wrażliwy na działanie wolnych rodników [97]. Udowodniono, że doświadczenie stresu środowiskowego jest skorelowane ze stanem stresu oksydacyjnego w mózgu [98]. Ponadto, chroniczny stres upośledza funkcje kory przedczołowej i tym samym przyczynia się do rozwoju wielu zaburzeń neuropsychiatrycznych [99]. Warto również dodać, że wolne rodniki mogą uszkadzać receptory glikokortykoidowe, co prowadzi do wzmożonej sekrecji CRH i zaburzeń w funkcjonowaniu osi HPA [63, 97]. Z drugiej strony, podwyższone stężenie kortykosteroidów przyczynia się do zwiększonej produkcji wolnych rodników [100].

2. EMF inicjuje zmiany w odpowiedzi stresowej, których kierunek i nasilenie zależą od natężenia pola elektromagnetycznego (weryfikacja hipotezy 2, prace 3; 4)

Przeprowadzone badania wykazały, że wpływ powtarzanej ekspozycji na EMF na aktywność układów stresu: osi HPA (*praca 3*) i układu LC-NA (*praca 4*) również zależy od intensywności EMF i liczby ekspozycji. Dynamika odpowiedzi stresowej jest zależna od poziomu uwalniania hormonów i poziomu neuroprzekaźników, jak również aktywności ich receptorów [52].

Układy stresu odpowiadają na każdą ekspozycję na stres podczas powtarzających się ekspozycji, co prowadzi do obserwowanego efektu kumulacyjnego [101]. Ekspozycja szczurów na EMF o niskiej lub wysokiej indukcji magnetycznej (1 mT lub 7 mT) w prezentowanych badaniach powtarzana była trzykrotnie, ponieważ założono, że hormetyczna zależność dawka-odpowiedź może wystąpić po początkowym zaburzeniu homeostazy podczas pierwszej ekspozycji. Następnie kierunek i dynamika zmian po kolejnych ekspozycjach będą konsekwencją poprzednich. Dynamika zmian aktywności układu LC-NA indukowana ekspozycją na EMF była ściśle skorelowana ze zmianami w odpowiedzi osi HPA.

Wzrost stężeń hormonów osi HPA u szczurów eksponowanych na EMF 1 mT był maksymalny po pierwszej ekspozycji na EMF, następnie wraz z każdą kolejną ekspozycją obserwowano spadek ich poziomu. Początkowy wzrost CRH spowodował zmniejszenie ekspresji receptorów CRH-R1 po każdej kolejnej ekspozycji na EMF. Może to odzwierciedlać proces adaptacyjny mający na celu zmniejszenie aktywności osi HPA w odpowiedzi na umiarkowany stres [102]. Na kolejnym poziomie osi HPA w przysadce mózgowej, EMF o wartości 1 mT początkowo spowodowało wzrost stężenia ACTH, jednak ilość tego hormonu obniżyła się po trzeciej ekspozycji. Wzrostowi wydzielania ACTH towarzyszyła redukcja ekspresji MC2R tylko po pierwszej ekspozycji. Profil uwalniania kortykosteronu z nadnerczy u zwierząt z grupy eksponowanej na EMF 1 mT był sumarycznym efektem procesów na wyższych poziomach osi HPA: stwierdzono początkowy wzrost, a następnie spadek stężenia CORT po kolejnych ekspozycjach. Niemniej jednak należy podkreślić, że stężenia CORT w tej grupie były wyższe niż u zwierząt kontrolnych po każdej kolejnej ekspozycji.

Oprócz ujemnego sprzężenia zwrotnego między strukturami osi HPA, istotną rolę w regulacji jej aktywności odgrywają receptory GR i MR w hipokampie. MR są kluczowe dla uruchomienia reakcji na stres [53], GR utrzymują tę zainicjowaną reakcję stresową, a następnie tłumią pobudzenie neuronów, normalizując aktywność mózgu [103]. Ekspozycja na EMF 1 mT spowodowała przejściowy wzrost w ekspresji MR w hipokampie (*praca 3*). Aktywacja receptorów MR w hipokampie, który pełni ważną rolę w mechanizmie ujemnego

sprzężenia zwrotnego osi HPA w trakcie stresu może być ważnym mechanizmem, który pomaga minimalizować ekspozycję tkanek docelowych na kortykosteroidy, zwłaszcza w warunkach powtarzającego się stresu [54, 101, 104]. Wzrost ekspresji MR może więc prowadzić do zahamowania aktywności osi HPA [105, 106], obserwowanej w omawianych eksperymentach po kolejnych ekspozycjach na EMF 1 mT.

Stężenie noradrenaliny oraz poziomy MHPG i stosunek MHPG/NA u szczurów po ekspozycji na EMF o intensywności 1 mT nie różniły się istotnie od wartości kontrolnych (*praca 4*). Znaczący wzrost poziomu NA zaobserwowano tylko po pierwszej ekspozycji na EMF w podwzgórzu - głównej strukturze koordynującej reakcję na stres. W grupie EMF 1 mT redukcja ekspresji β2-AR w hipokampie była niewielka.

W grupie eksponowanej na EMF o wysokiej intensywności (7 mT), stężenia hormonów osi HPA były znacząco wyższe niż wartości zarejestrowane zarówno w grupie kontrolnej, jak i w grupie eksponowanej na niższą dawkę EMF (1 mT). Nie stwierdzono również habituacji do powtarzanej ekspozycji na tego rodzaju bodziec. Badania wykazały, że znaczny wzrost stężenia CRH świadczy o zwiększonej reaktywności na stres [107], zjawisko to może wyjaśniać wysoką aktywność osi HPA w grupie eksponowanej na EMF o wartości indukcji magnetycznej 7 mT, gdzie stężenie CRH po każdej ekspozycji na EMF jest wyższe niż w grupie kontrolnej. Brak regulacji w dół receptorów CRH-R1 w tej grupie może być wynikiem stymulującej roli znacznie wyższego stężenia CORT u szczurów eksponowanych na EMF 7 mT niż u tych eksponowanych na 1 mT, który ma również wpływ na ekspresję tych receptorów [108]. Odnotowano 4-krotny wzrost stężenia ACTH w przysadce mózgowej po pierwszej ekspozycji na EMF 7 mT, a następnie coraz wyższy poziom tego hormonu po każdej kolejnej ekspozycji. Podobne zmiany w profilu uwalniania ACTH stwierdzono w osoczu. W grupie eksponowanej na EMF 7 mT wzrost stężenia ACTH był znacznie bardziej wyraźny niż w grupie EMF 1 mT. Chociaż zaobserwowano spadek ekspresji MC2R po pierwszej i drugiej ekspozycji na EMF 7 mT, uwalnianie CORT w nadnerczach nie zostało zahamowane. Po każdej kolejnej ekspozycji poziom CORT był coraz wyższy. Podobny wzorzec zmian stężeń kortykosteronu był widoczny w osoczu.

Ekspresja receptorów GR i MR w hipokampie obniżyła się drastycznie po ekspozycji na EMF 7 mT. Jak wykazano obniżenie ekspresji MR i GR w tym obszarze może być skorelowane z nadmiernym uwalnianiem CORT [109, 110]. A zatem wzrost poziomu CORT po każdej kolejnej ekspozycji na EMF 7 mT może również być związany z osłabieniem hamującego wpływu hipokampu na aktywność osi HPA, wynikającym z obniżenia ekspresji GR i jeszcze bardziej znaczącego obniżenia poziomu MR. Zmiany w równowadze między receptorami MR i GR zostały również stwierdzone u pacjentów z zaburzeniami psychicznymi

[67]. Te wyniki sugerują wzrost ryzyka zachorowania na tego typu choroby po ekspozycji na wysokie dawki EMF.

W grupie eksponowanej na EMF 7 mT, stężenia NA były coraz wyższe po każdej kolejnej ekspozycji, analogicznie do wzorca uwalniania kortykosteronu, co sugeruje reakcję uwrażliwienia na ten rodzaj homotypowego silnego czynnika stresogennego. Co istotne, znaczący wzrost poziomu hormonu uwalniającego kortykotropinę (CRH) w podwzgórzu w tej grupie może przyczyniać się do stymulacji aktywności miejsca sinawego w warunkach stresu [45, 46]. W podwzgórzu występuje stosunkowo gęste unerwienie noradrenergiczne, a więc LC może również zwrotnie modyfikować stan aktywności neuronów HPT [111, 112]. To może z kolei tłumaczyć najbardziej wyraźną odpowiedź noradrenergiczną na EMF 7 mT w podwzgórzu. Spadek poziomu MHPG u szczurów eksponowanych na EMF o wartości 7 mT, był zdecydowanie bardziej widoczny niż w grupie EMF 1 mT. Adekwatnie do poziomu NA i MHPG w LC i osoczu, poziom indeksu utylizacji noradrenaliny MHPG/NA w grupie 7 mT był niższy niż w innych grupach bezpośrednio po pierwszej ekspozycji, a kolejne ekspozycje pogłębiały ten efekt. Niski indeks MHPG/NA w grupie EMF 7 mT sugeruje zaburzoną szybkość przemiany NA. Istnieje wiele dowodów na to, że poziom MHPG jest obniżony w depresji [113, 114] i chorobie Parkinsona [115]. Dane te wskazują, że niskie poziomy MHPG w grupie EMF o wartości 7 mT mogą stanowić biomarker dysfunkcji układu nerwowego.

W grupie eksponowanej na EMF 7 mT zaobserwowano zdecydowany spadek ekspresji β2-AR w hipokampie po każdej kolejnej ekspozycji. Może to odzwierciedlać dobrze znany proces adaptacyjny mający na celu utrzymanie odpowiedniej aktywności układu noradrenergicznego w odpowiedzi na umiarkowany stres [116]. Niemniej jednak, kiedy organizm jest narażony na tak silny bodziec stresowy, ta fizjologiczna odpowiedź adaptacyjna może być niewystarczająca, co prowadzi do coraz wyższego poziomu uwalniania noradrenaliny w odpowiedzi na kolejne homotypowe bodźce. To zjawisko może tłumaczyć zaburzoną odpowiedź adrenergiczną na ekspozycję na EMF o wartości indukcji magnetycznej 7 mT.

Przedstawione wyniki sugerują, że nawet ekspozycja na EMF o niskiej intensywności (1 mT) stanowi wyzwanie dla organizmu i uruchamia reakcję stresową, chociaż raczej niewielką i tymczasową. Oznacza to, że słabe pole (1 mT), poprzez aktywację układów stresu jest sygnałem pobudzającym mechanizmy kompensacyjne w organizmie, co skutkuje adaptacją do powtarzających się ekspozycji na ten sam rodzaj stresora. Badania eksperymentalne wykazały, że powtarzająca się ekspozycja na pewne czynniki stresogenne osłabia reaktywność układów stresu i tym samym uwalnianie kortykosteronu i noradrenaliny w

odpowiedzi na ten sam (homotypowy) bodziec [117, 118]. Tym samym można uznać, że EMF o indukcji magnetycznej 1 mT jest wartością bezpieczną dla układu nerwowego.

EMF o wartości 7 mT jest natomiast silnym stresorem, który wywiera znaczny wpływ na układy stresu i zaburza ich funkcjonowanie. Zaobserwowano zwiększoną reaktywność układów stresu po każdej kolejnej ekspozycji na ten czynnik stresowy. Wydaje się, że w tak silnej sytuacji stresowej, mechanizmy regulacyjne stały się niewydolne i powtarzana ekspozycja na silny stres doprowadziła do kumulacji efektów poszczególnych ekspozycji widocznej jako wzmacnianie reakcji stresowej po każdej kolejnej ekspozycji. A zatem wyniki przeprowadzonych badań sugerują, że silne pole elektromagnetyczne (7 mT) może zaburzać homeostazę odpowiedzi stresowej i tym samym może być uznane za szkodliwe dla układu nerwowego.

3. EMF trwale modyfikuje status oksydacyjno-antyoksydacyjny i poziom aktywności układów stresu i tym samym zmienia odpowiedź na kolejne czynniki stresogenne (weryfikacja hipotezy 3, prace 2; 3; 4)

Uzyskane wyniki pozwoliły nam stwierdzić, że powtarzana ekspozycja na pole elektromagnetyczne ma dwukierunkowy, zależny od dawki wpływ na poziom stresu oksydacyjnego i aktywność układów stresu. W związku z tym zasadne było zweryfikowanie czy ta zmiana punktu nastawczego aktywności układów oksydacyjno-antyoksydacyjnych i układów stresu może wpływać na reakcję na kolejny heterotypowy stres. Wiele badań wskazuje, że stres powoduje zakłócenie homeostazy [119, 120], co prowokuje odpowiedź kompensacyjną w celu przywrócenia równowagi. Ten proces może prowadzić do ustanowienia nowego punktu nastawczego indukowania odpowiedzi na kolejne stresory.

Ekspozycja szczurów na pole elektromagnetyczne o wartości indukcji magnetycznej 1 mT miała wpływ na status oksydacyjno-antyoksydacyjny indukowany w odpowiedzi na kolejny czynnik stresowy - test otwartego pola (OF). W grupie eksponowanej na EMF 1 mT stwierdzono niewielkie zwiększenie stężenia CP wywołane testem otwartego pola tylko po pierwszej ekspozycji na EMF, po każdej kolejnej ekspozycji indukowany stresem otwartego pola poziom tego wskaźnika ulegał obniżeniu. Poziom 8-izoprostanów po OF był obniżony po dwóch pierwszych ekspozycjach na EMF, a po trzeciej ekspozycji powrócił do wartości odnotowanej w tej grupie, ale nieeksponowanej na test OF. Wzmocnienie obrony antyoksydacyjnej w odpowiedzi na nowy czynnik stresowy u szczurów z grupy EMF 1 mT, było widoczne po pierwszej ekspozycji na EMF, a następnie zaobserwowano adaptację, tj. spadek poziomu TAC.

Uzyskane wyniki pozwoliły nam stwierdzić, że subtelne zmiany w poziomie statusu oksydacyjnego wywołane ekspozycją na pole elektromagnetyczne o intensywności 1 mT (pierwszy czynnik stresowy) były wystarczające, żeby zmienić profil procesów oksydacyjnych po ekspozycji na inny rodzaj czynnika stresowego – test otwartego pola. Sugeruje to, że może to być rodzaj habituacji, kiedy jeden czynnik stresowy zmniejsza reakcję na drugi [10].

Charakterystyka zmian stężeń hormonów stresu indukowanych testem otwartego pola lub ekspozycją na podwyższony labirynt krzyżowy oraz ekspresja receptorów była zbliżona do ich poziomu podstawowego w poszczególnych grupach, obserwowanych bezpośrednio po kolejnych ekspozycjach na pole elektromagnetyczne lub warunki kontrolne. Natomiast analiza procentowych zmian w stężeniach hormonów wykazała, że u zwierząt eksponowanych na EMF 1 mT odpowiedź układów stresu na kolejny czynnik stresowy była osłabiona, co sugeruje adaptację do innych heterotypowych czynników stresowych. Co ważne było to skorelowane z przywróceniem kontrolnego poziomu ekspresji receptora GR, który był obniżony po ekspozycji na EMF. Ponadto, indukowany OF wzrost ekspresji receptora MR w hipokampie stwierdzono nie tylko po pierwszej ekspozycji na EMF, ale nawet wyższy jego poziom odnotowano również po drugiej ekspozycji. U zwierząt eksponowanych na EMF 1 mT test podwyższonego labiryntu krzyżowego spowodował znaczący spadek stężenia NA i wzrost poziomu MHPG oraz wskaźnika MHPG/NA tylko w miejscu sinawym. Natomiast stwierdzono wzrost ekspresji receptorów β2-AR w hipokampie, ale tylko po pierwszej ekspozycji na EMF 1 mT. Ten efekt wydaje się być raczej konsekwencją samej ekspozycji na kolejny czynnik stresowy, ponieważ analogiczne zmiany zaobserwowano w grupie kontrolnej.

Przedstawione wyniki sugerują, że ekspozycja na pole elektromagnetyczne o wartości indukcji magnetycznej 1 mT działa jako prekondycjonowanie, które ułatwia adaptacyjną odpowiedź na kolejne zdarzenia stresowe. Jest to zgodne z założeniami hormezy, że ekspozycja na niskie poziomy jednego rodzaju czynnika stresowego (pole elektromagnetyczne) może indukować szlaki wewnątrzkomórkowe, chroniące komórki przed konsekwencjami kolejnego innego typu stresora [80, 121].

Analizie poddano również behawior zwierząt w teście otwartego pola i podwyższonego labiryntu krzyżowego, który częściowo może wynikać ze zmian aktywności układów stresu [122]. Nie stwierdzono różnic w zachowaniu u zwierząt wcześniej eksponowanych na EMF 1 mT ani w teście otwartego pola, ani w teście podwyższonego labiryntu krzyżowego w porównaniu do zwierząt kontrolnych. Sugeruje to, że reakcja układów stresu wywołana

niską dawką czynnika stresogennego - EMF 1 mT nie była wystarczająco silna, aby zmienić ich zachowanie.

Profil zmian indukowanych ekspozycją na kolejny czynnik stresowy w grupie eksponowanej na pole elektromagnetyczne o intensywności 7 mT był odmienny. Stwierdzono zakłócenie równowagi oksydacyjno-antyoksydacyjnej indukowanej ekspozycją na kolejny czynnik stresowy. Wzorzec i wielkość zmian poziomu 8-epi PGF2α po kolejnych ekspozycjach na OF, u szczurów wcześniej narażonych na EMF 7 mT były zbliżone do zmian w poziomie podstawowym w tej grupie, z kolei indukowany OF poziom CP wzrastał z każdą kolejną ekspozycją na EMF. Natomiast, indukowany OF poziom TAC po pierwszej ekspozycji na EMF 7 mT uległ obniżeniu, a następnie wzrósł do wartości nie różniącej się od tej w grupie kontrolnej.

Wydaje się zatem, że ekspozycja na jeden silny czynnik stresowy (EMF 7mT) czyni organizm bardziej wrażliwym na drugi czynnik stresowy (test otwartego pola), co prowadzi do szybszego wystąpienia stresu oksydacyjnego i jego wyższego poziomu u zwierząt narażonych na tego rodzaju bodziec w porównaniu do wartości obserwowanych u szczurów z grupy EMF 1 mT. Uzyskane wyniki sugerują, że wysokie wartości indukcji magnetycznej (7 mT) pola elektromagnetycznego są w stanie zakłócić status oksydacyjno-antyoksydacyjny mózgu, co skutkuje nasileniem procesów oksydacyjnych w odpowiedzi na kolejne zdarzenia stresowe.

W grupie narażonej na EMF 7 mT stężenia wszystkich hormonów osi HPA indukowane testem otwartego pola były kilkukrotnie niższe niż ich odpowiednie stężenia podstawowe. Podobnie poziom NA po teście w podwyższonym labiryncie krzyżowym w grupie eksponowanej EMF 7 mT było niższe w LC i osoczu, bez zmiany poziomu MHPG, co skutkowało zwiększeniem indeksu MPHG/NA. Należy zwrócić uwagę, że podstawowe stężenia hormonów stresu były kilkukrotnie wyższe niż wartość obserwowana u zwierząt kontrolnych. A zatem jest prawdopodobne, że niższy poziom hormonów stresu po ekspozycji na kolejny czynnik stresowy jest wynikiem tzw. "efektu sufitowego". To znaczy, że dalszy wzrost stężeń kortykosteronu czy noradrenaliny już kilkukrotnie podwyższonych po ekspozycji na EMF, w odpowiedzi na kolejny czynnik stresowy był niemożliwy, z powodu niewydolności procesów syntezy tych hormonów. Co istotne, po narażeniu na kolejny czynnik stresogenny poziom stężenia kortykosteronu i NA pomimo obniżenia w stosunku do poziomu podstawowego w tej grupie, był znacząco wyższy niż w grupach kontrolnej i eksponowanej na EMF 1 mT. Ponadto u szczurów narażonych na EMF 7 mT, zaobserwowano znaczące zmniejszenie poziomu ekspresji β2-AR w hipokampie. Oznacza to, że zmiany w aktywności osi HPA i układu noradrenergicznego wywołane ekspozycją

na pole elektromagnetyczne determinują endokrynologiczną odpowiedź organizmu na kolejne czynniki stresowe.

Szczury eksponowane na EMF 7 mT w teście otwartego pola wykazywały mniej zachowań związanych z lękiem niż zwierzęta kontrolne i eksponowane na EMF 1 mT. Odmienny profil zachowania zaobserwowano w tej grupie zwierząt w teście podwyższonego labiryntu krzyżowego – niewielkie nasilenie zachowań lękowych. W przeprowadzonej procedurze badawczej zastosowano rekomendację wykonania testu otwartego pola przed testem w podwyższonym labiryncie krzyżowym w celu zwiększenia aktywności eksploracyjnej i uzyskać bardziej zróżnicowane wyniki w parametrach związanych z lękiem [96]. W naszych eksperymentach nie zaobserwowano takiego efektu.

Uzyskane wyniki nie dały jednoznacznej odpowiedzi na pytanie dotyczącej charakteru zmian fenotypu behawioralnego zwierząt po ekspozycji na wysokie dawki EMF. Natomiast zdecydowanie potwierdziły, że zarówno aktywność układów stresu jak i zachowanie w odpowiedzi na stres są zaburzone u zwierząt eksponowanych na EMF o indukcji magnetycznej 7 mT. A zatem ekspozycja na EMF o takiej wartości uwrażliwia organizm na kolejne stresory i w ten sposób staje się czynnikiem ryzyka rozwoju zaburzeń układu nerwowego [55].

4. Zmiany w odpowiedzi stresowej indukowanej przez EMF modulują plastyczność mózgową (weryfikacja hipotezy 4, prace 3; 4)

Efekty ekspozycji na EMF w hipokampie były również uzależnione od natężenia pola elektromagnetycznego. Szereg badań potwierdziło, że zarówno noradrenalina, jak również glikokortykoidy wpływają na przeżycie neuronów hipokampu i neuroplastyczność poprzez aktywację odpowiednich receptorów: β-adrenergicznych (β2-AR) i mineralokortykoidowych (MR) [44, 52, 73, 104, 123]. Odkrycie, że EMF o indukcji magnetycznej 1 mT specyficznie stymuluje ekspresję MR w hipokampie, jest zgodne z wcześniejszymi ustaleniami, że subtelne czynniki stresowe mogą zwiększać ekspresję MR w neuronach i stanowić mechanizm kompensacyjny mający na celu indukcję plastyczności neuronalnej. A zatem, dwukierunkowy wpływ EMF o niskiej i wysokiej indukcji magnetycznej może być związany z różną ekspresją MR, ponieważ te receptory nie tylko determinują aktywność osi HPA, ale również mają wartość neuroprotekcyjną. Wyniki te mogą stanowić wyjaśnienie efektu terapeutycznego EMF o niskiej intensywności, potwierdzonego w wielu badaniach [11, 23, 25, 67, 78, 124].

W hipokampie również aktywność układu noradrenergicznego odgrywa ważną rolę w indukowaniu procesów plastyczności mózgowej [125]. Spadek ekspresji receptorów β2-AR w hipokampie wywołany przez EMF o indukcji 7 mT może mieć głębokie konsekwencje. W warunkach, w których następuje indukowany stresem wzrost uwalniania noradrenaliny z LC dochodzi do pobudzenia aktywności neuronów hipokampu poprzez aktywację receptorów β2-AR. Jednym z efektów tego procesu jest ekspresja genów związanych z plastycznością [68, 72]. Aktywacja receptorów β2-AR może być zatem decydująca dla siły połączeń synaptycznych oraz trwałości plastyczności synaptycznej [126, 127]. Istotna rola β2-AR w procesach plastyczności pozwala wnioskować, że ekspozycja na EMF 7 mT, która znacząco obniża ekspresję tych receptorów, może silnie hamować procesy neuroplastyczne w hipokampie, pomimo wysokiego stężenia noradrenaliny. Zaburzenia w ekspresji β2-AR są powiązane z wystąpieniem neuropatologii takich jak depresja [128]. A zatem ekspozycja na EMF 7 mT ze względu na indukowanie zaburzeń funkcji hipokampu może stanowić czynnik ryzyka dla tego typu chorób.

5. Podsumowanie

Przeprowadzone badania pozwalają na stwierdzenie, że natężenia pola elektromagnetycznego o niskiej częstotliwości (50 Hz) jest kluczowym czynnikiem determinującym jego dwukierunkowy wpływ na mózg: pozytywny - promujący plastyczność mózgu, niwelujący zaburzenia równowagi oksydacyjnej i poprawiający neuroadaptację do kolejnych bodźców stresowych; lub negatywny - odpowiedzialny za zaburzenie reakcji na stres, osłabienie obrony antyoksydacyjnej oraz zwiększenie wrażliwości na kolejne czynniki stresowe.

Powtarzana ekspozycja na EMF 1 mT wywołuje reakcję stresową o niewielkiej intensywności, jednak nawet subtelne zmiany przez nią wywołane mogą zmieniać funkcjonowanie obwodów neuronalnych. Uzyskane wyniki potwierdziły, że ekspozycja na EMF 1 mT zmienia „punkt nastawczy” dla regulacji procesów oksydacyjnych i aktywności układów stresu i inicjuje adaptację komórkową do tego rodzaju stresu. W prezentowanych badaniach po raz pierwszy wykazano również indukcję ekspresji MR w neuronach hipokampu w odpowiedzi na EMF o intensywności 1 mT. Chociaż konsekwencje zwiększonej ekspresji MR nie są dokładnie poznane, to zjawisko może stanowić endogenną odpowiedź mającą na celu ochronę mózgu przed kolejnymi uszkodzeniami.

Uzyskane wyniki wskazują, że w sytuacji narażenia na stresory o dużej intensywności, jak powtarzana ekspozycja na EMF 7 mT, równowaga zostaje zaburzona w kierunku

zarówno wyższego poziomu stresu oksydacyjnego, jak również nasilonej reakcji stresowej. Sugeruje to że odpowiedź organizmu na tak silny rodzaj stresu wymaga większych nakładów energetycznych, którym organizm może nie sprostać [10]. W rezultacie dochodzi do kumulacji efektów powtarzanej ekspozycji na EMF 7 mT. Ten efekt może być trwały i mieć długoterminowy wpływ na funkcjonowanie organizmu - zmieniać podatność organizmu na kolejne czynniki stresogenne i tym samym zwiększać ryzyko wystąpienia chorób układu nerwowego.

Uzyskane wyniki po raz pierwszy dokumentują „hormetyczny mechanizm działania" EMF (50 Hz) u kręgowców. Stwarzają nowe perspektywy do wykorzystania EMF w celach terapeutycznych, jak również dostarczają nowych danych do oceny ryzyka narażenia na EMF.

Wnioski

1) Powtarzana ekspozycja na EMF o niskiej częstotliwości (50Hz) zmienia status oksydacyjno-antyoksydacyjny i aktywność układów stresu w sposób zależny od wartości indukcji magnetycznej EMF i liczby ekspozycji.

2) Ekspozycja na EMF o indukcji magnetycznej 1 mT jest czynnikiem, który nie zaburza znacząco równowagi oksydacyjno-antyoksydacyjnej w korze przedczołowej szczura, natomiast silniejsze EMF (7 mT) indukuje uszkodzenia oksydacyjne białek oraz lipidów, a także ma potencjał do osłabiania obrony antyoksydacyjnej mózgu.

3) Ekspozycja na EMF o niskiej intensywności (1 mT) stanowi umiarkowane wyzwanie dla organizmu i aktywacja układów stresu (oś HPA i układ LC-NA) w odpowiedzi na ten bodziec jest niewielka i ma charakter przejściowy. Oznacza to, EMF 1 mT jest sygnałem pobudzającym mechanizmy kompensacyjne w organizmie, co skutkuje adaptacją do powtarzających się ekspozycji na ten sam rodzaj stresora.

4) EMF o wartości 7 mT jest silnym stresorem i zwiększa reaktywność układów stresu po każdej kolejnej ekspozycji na ten czynnik stresowy. Sugeruje to niewydolność mechanizmów regulacyjnych, których efektem jest kumulacja efektów poszczególnych ekspozycji widoczna jako wzmacnianie reakcji stresowej po każdej kolejnej ekspozycji na EMF.

5) EMF modyfikuje status oksydacyjno-antyoksydacyjny i zmienia punkt nastawczy dla aktywności układów odpowiedzi stresowej w sposób trwały, i tym samym modyfikuje odpowiedź na kolejny czynnik stresowy.

6) Niewielkie zmiany w poziomie statusu oksydacyjnego i reakcji stresowej wywołane ekspozycją na pole elektromagnetyczne o indukcji 1 mT są wystarczające aby wywołać efekt prekondycjonowania, który ułatwia adaptacyjną odpowiedź na kolejne zdarzenia stresowe, natomiast konsekwencją narażenia na EMF 7 mT może być pogłębienie zaburzeń równowagi oksydacyjno-antyoksydacyjnej w mózgu szczura w odpowiedzi na kolejne zdarzenia stresowe.

7) EMF o niskiej wartości indukcji magnetycznej (1 mT) przesuwa aktywność osi HPA oraz układu LC-NA w kierunku adaptacji do warunków stresogennych, natomiast

wysoka wartość indukcji magnetycznej EMF (7 mT) uwrażliwia organizm na kolejne zdarzenie stresowe.

8) Aktywacja układów stresu wywołana niską dawką czynnika stresogennego - EMF 1 mT nie była wystarczająco silna, aby zmienić ich zachowanie w odpowiedzi na kolejny czynnik stresowy, natomiast ekspozycja na EMF o wartości indukcji magnetycznej 7 mT powoduje zaburzenia behawioru.

9) EMF o indukcji magnetycznej 1 mT specyficznie wywołuje ekspresję receptorów mineralokortykoidowych w hipokampie, co sugeruje wpływ tego rodzaju stymulacji na indukcję plastyczności neuronalnej, z kolei EMF 7 mT, znacząco obniża ekspresję receptorów β-adrenergicznych i w ten sposób może hamować procesy neuroplastyczne w hipokampie.

10) EMF jest czynnikiem, którego działania wykazuje efekt hormezy, ponieważ kierunek i intensywność odpowiedzi organizmu na ten rodzaj stresu są zależne od jego siły, tzn. wartości natężenia pola magnetycznego: słabe EMF (1 mT) uruchamia w organizmie mechanizmy kompensacyjne, które zapewniają ochronę przed negatywnymi skutkami narażenia na ten stres, natomiast silne EMF (7 mT) jest bodźcem przekraczającym możliwości adaptacyjne organizmu, a w konsekwencji prowadzącym do zaburzeń homeostazy.

11) EMF o niskiej indukcji magnetycznej (1 mT) dzięki stymulacji mechanizmów kompensacyjnych i plastyczności mózgowej może znaleźć zastosowanie jako metoda terapeutyczna w leczeniu niektórych schorzeń.

12) EMF o wysokiej indukcji magnetycznej (7 mT), może nasilać podatność organizmu na kolejne czynniki stresogenne i tym samym zwiększać ryzyko wystąpienia chorób układu nerwowego.

Literatura

1. Ubeda A, Díaz-Enriquez M, Martínez-Pascual MA, Parreño A. Hematological changes in rats exposed to weak electromagnetic fields. Life Sci. 1997;61(17):1651-6. doi: 10.1016/s0024-3205(97)00770-4.
2. Yokus B, Cakir DU, Akdag MZ, Sert C, Mete N. Oxidative DNA damage in rats exposed to extremely low frequency electromagnetic fields. Free Radic Res. 2005 Mar;39(3):317-23. doi: 10.1080/10715760500043603.
3. Zhang Y, Liu X, Zhang J, Li N. Short-term effects of extremely low frequency electromagnetic fields exposure on Alzheimer's disease in rats. Int J Radiat Biol. 2015 Jan;91(1):28-34. doi: 10.3109/09553002.2014.954058.
4. Bagheri Hosseinabadi M, Khanjani N, Ebrahimi MH, Mousavi SH, Nazarkhani F. Investigating the effects of exposure to extremely low frequency electromagnetic fields on job burnout syndrome and the severity of depression; the role of oxidative stress. J Occup Health. 2020 Jan;62(1):e12136. doi: 10.1002/1348-9585.12136.
5. Abkhezr H, Mohaddes G, Nikniaz Z, Farhangi MA, Heydari H, Nikniaz L, The effect of Extremely Low Frequency Electromagnetic Field on spatial memory of mice and rats: A systematic review. Learning and Motivation. 2023 Feb;81: 101873. doi: 10.1016/j.lmot.2023.101873
6. Halliday D, Resnick R i Walker J. 2003. Podstawy Fizyki, t. 3. Warszawa: PWN SA
7. Hewitt P.G. 2006. „Elektryczność i Magnetyzm" W Fizyka wokół nas. 372-436. Warszawa: PWN SA
8. West G.F, Macnae J.C, 1991. "PHYSICS OF THE ELECTROMAGNETIC INDUCTION EXPLORATION METHOD", Electromagnetic Methods in Applied Geophysics: Volume 2, Application, Parts A and B, Misac N. Nabighian
9. Szemerszky R, Zelena D, Barna I, Bárdos G. Stress-related endocrinological and psychopathological effects of short- and long-term 50Hz electromagnetic field exposure in rats. Brain Res Bull. 2010 Jan 15;81(1):92-9. doi: 10.1016/j.brainresbull.2009.10.015.
10. Mahdavi SM, Sahraei H, Yaghmaei P, Tavakoli H. Effects of electromagnetic radiation exposure on stress-related behaviors and stress hormones in male wistar rats. BiomolTher (Seoul). 2014 Nov;22(6):570-6. doi: 10.4062/biomolther.2014.054.
11. Cichoń, N., Bijak, M., Miller, E., Saluk, J. Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients. Bioelectromagnetics, 38(5), pp. 386-396, 2017, doi: 10.1002/bem.22055
12. Garg, R., Varna, A.L., Hajj-Ahmad, A., Wu, M., "Seeing" ENF: Power-Signature-Based Timestamp for Digital Multimedia via Optical Sensing and Signal Processing, IEEE Transactions on Information Forensics and Security 8(9), pp. 1417-1432, 2013.
13. Baan R, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Islami F, Galichet L, Straif K; WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of radiofrequency electromagnetic fields. Lancet Oncol. 2011 Jul;12(7):624-6. doi: 10.1016/s1470-2045(11)70147-4. Erratum in: Lancet Oncol. 2015 Aug;16(8):e379.
14. Sieroń A, Labus Ł, Nowak P, Cieślar G, Brus H, Durczok A, Zagził T, Kostrzewa RM, Brus R. Alternating extremely low frequency magnetic field increases turnover of dopamine and serotonin in rat frontal cortex. Bioelectromagnetics. 2004 Sep;25(6):426-30. doi: 10.1002/bem.20011.
15. Sedghi H, Zare S, Hayatgeib, H, Alivandi S, Ebadi, AG, Effects of 50 HZ Magnetic Field on Some Factors of Immune System in the Male Guinea Pigs. American Journal of Immunology, 2005, 1(1):37-41. doi: 10.3844/ajisp.2005.37.41

16. Kitaoka K, Kitamura M, Aoi S, Shimizu N, Yoshizaki K. Chronic exposure to an extremely low-frequency magnetic field induces depression-like behavior and corticosterone secretion without enhancement of the hypothalamic-pituitary-adrenal axis in mice. Bioelectromagnetics. 2013 Jan;34(1):43-51. doi: 10.1002/bem.21743.
17. Mahdavi SM, Rezaei-Tavirani M, Nikzamir A, Ardeshirylajimi A, 12 Hz electromagnetic field changes stress-related hormones of rat. Archives of Advances in Biosciences, 2014, 5(4). doi: 10.22037/jps.v5i4.7811
18. Hajós-Korcsok E, Robinson DD, Yu JH, Fitch CS, Walker E, Merchant KM. Rapid habituation of hippocampal serotonin and norepinephrine release and anxiety-related behaviors, but not plasma corticosterone levels, to repeated footshock stress in rats. PharmacolBiochemBehav. 2003 Feb;74(3):609-16. doi: 10.1016/s0091-3057(02)01047-x.
19. Panagopoulos, D.J., Karabarbounis, A., Yakymenko, I., Chrousos, G.P. Human-made electromagnetic fields: Ion forced-oscillation and voltage-gated ion channel dysfunction, oxidative stress and DNA damage (Review). Int J Oncol., 59(5):92, 2021, doi: 10.3892/ijo.2021.5272
20. Grassi C, D'Ascenzo M, Torsello A, Martinotti G, Wolf F, Cittadini A, Azzena GB. Effects of 50 Hz electromagnetic fields on voltage-gated Ca2+ channels and their role in modulation of neuroendocrine cell proliferation and death. Cell Calcium. 2004 Apr;35(4):307-15. doi: 10.1016/j.ceca.2003.09.001.
21. Luo FL, Yang N, He C, Li HL, Li C, Chen F, Xiong JX, Hu ZA, Zhang J. Exposure to extremely low frequency electromagnetic fields alters the calcium dynamics of cultured entorhinal cortex neurons. Environ Res. 2014 Nov;135:236-46. doi: 10.1016/j.envres.2014.09.023.
22. Li Y, Yan X, Liu J, Li L, Hu X, Sun H, Tian J. Pulsed electromagnetic field enhances brain-derived neurotrophic factor expression through L-type voltage-gated calcium channel- and Erk-dependent signaling pathways in neonatal rat dorsal root ganglion neurons. NeurochemInt. 2014 Sep;75:96-104. doi: 10.1016/j.neuint.2014.06.004.
23. Cichoń N, Bijak M, Czarny P, Miller E, Synowiec E, Sliwinski T, Saluk-Bijak J. Increase in Blood Levels of Growth Factors Involved in the Neuroplasticity Process by Using an Extremely Low Frequency Electromagnetic Field in Post-stroke Patients. Front AgingNeurosci. 2018 Sep 26;10:294. doi: 10.3389/fnagi.2018.00294.
24. Cheng, Y., Dai, Y., Zhu, X., Xu, H., Cai, P., Xia, R., et al. Extremely low-frequency electromagnetic fields enhance the proliferation and differentiation of neural progenitor cells cultured from ischemic brains. Neuroreport. 21;26(15), pp. 896-902, 2015, doi: 10.1097/WNR.0000000000000450
25. Gao Q, Leung A, Yang YH, Lau BW, Wang Q, Liao LY, Xie YJ, He CQ. Extremely low frequency electromagnetic fields promote cognitive function and hippocampal neurogenesis of rats with cerebral ischemia. Neural Regen Res. 2021 Jul;16(7):1252-1257. doi: 10.4103/1673-5374.301020.
26. Sakhaie MH, Soleimani M, Pourheydar B, Majd Z, Atefimanesh P, Asl SS, Mehdizadeh M. Effects of Extremely Low-Frequency Electromagnetic Fields on Neurogenesis and Cognitive Behavior in an Experimental Model of Hippocampal Injury. BehavNeurol. 2017;2017:9194261. doi: 10.1155/2017/9194261.
27. Wyszkowska J, Shepherd S, Sharkh S, Jackson CW, Newland PL. Exposure to extremely low frequency electromagnetic fields alters the behaviour, physiology and stress protein levels of desert locusts. Sci Rep. 2016 Nov 3;6:36413. doi: 10.1038/srep36413.
28. Rezaei-Tavirani, M., Hasanzadeh, H., Seyyedi, S., Ghoujeghi, F., Semnani, V., Zali, H. Proteomic Analysis of Extremely Low-Frequency ElectroMagnetic Field (ELF-EMF) With Different Intensities in Rats Hippocampus. ArchNeurosci., 5(1):e62954, 2018, doi: 10.5812/archneurosci.62954

29. Martínez-Sámano J, Flores-Poblano A, Verdugo-Díaz L, Juárez-Oropeza MA, Torres-Durán PV. Extremely low frequency electromagnetic field exposure and restraint stress induce changes on the brain lipid profile of Wistar rats. BMC Neurosci. 2018 May 21;19(1):31. doi: 10.1186/s12868-018-0432-1.
30. Halliwell B. Antioxidants in human health and disease. Annu Rev Nutr. 1996;16:33-50. doi: 10.1146/annurev.nu.16.070196.000341.
31. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative Stress: Harms and Benefits for Human Health. OxidMed Cell Longev. 2017;2017:8416763. doi: 10.1155/2017/8416763.
32. Cobley JN, Fiorello ML, Bailey DM. 13 reasons why the brain is susceptible to oxidative stress. Redox Biol. 2018 May;15:490-503. doi: 10.1016/j.redox.2018.01.008.
33. El-Helaly, M., Abu-Hashem, E. Oxidative stress, melatonin level, and sleep insufficiency among electronic equipment repairers. Indian J. Occup. Environ. Med., 14, 66–70, 2010, doi:10.4103/0019-5278.75692
34. Luo X, Chen M, Duan Y, Duan W, Zhang H, He Y, Yin C, Sun G, Sun X. Chemoprotective action of lotus seedpod procyanidins on oxidative stress in mice induced by extremely low-frequency electromagnetic field exposure. BiomedPharmacother. 2016 Aug;82:640-8. doi: 10.1016/j.biopha.2016.06.005.
35. Sun Y, Shi Z, Wang Y, Tang C, Liao Y, Yang C, Cai P. Coupling of oxidative stress responses to tricarboxylic acid cycle and prostaglandin E2 alterations in Caenorhabditis elegans under extremely low-frequency electromagnetic field. Int J Radiat Biol. 2018 Dec;94(12):1159-1166. doi: 10.1080/09553002.2019.1524943.
36. Goraca A, Ciejka E, Piechota A. Effects of extremely low frequency magnetic field on the parameters of oxidative stress in heart. J PhysiolPharmacol. 2010 Jun;61(3):333-8. PMID: 20610864.
37. Morabito, C., Rovetta, F., Bizzarri, M., Mazzoleni, G., Fanò, G., Mariggiò, M.A. Modulation of redox status and calcium handling by extremely low frequency electromagnetic fields in C2C12 muscle cells: A real-time, single-cell approach. FreeRadicBiol Med. 48(4), pp. 579-589, 2010, doi: 10.1016/j.freeradbiomed.2009.12.005
38. Patruno A, Tabrez S, Pesce M, Shakil S, Kamal MA, Reale M. Effects of extremely low frequency electromagnetic field (ELF-EMF) on catalase, cytochrome P450 and nitric oxide synthase in erythro-leukemic cells. Life Sci. 2015 Jan 15;121:117-23. doi: 10.1016/j.lfs.2014.12.003.
39. Falone, S., Grossi, M.R., Cinque, B., D'Angelo, B., Tettamanti, E., Cimini, A., et al. Fifty hertz extremely low-frequency electromagnetic field causes changes in redox and differentiative status in neuroblastoma cells. Int J Biochem Cell Biol., 39(11), pp. 2093-2106, 2007, doi: 10.1016/j.biocel.2007.06.001
40. Emre, M., Cetiner, S., Zencir, S., Unlukurt, I., Kahraman, I., Topcu, Z. Oxidative stress and apoptosis in relation to exposure to magnetic field. Cell Biochem. Biophys., 59, pp. 71–77, 2011, doi:10.1007/s12013-010-9113-0
41. Ehnert, S., Fentz, A.K., Schreiner, A., Birk, J., Wilbrand, B., Ziegler, P. et al. Extremely low frequency pulsed electromagnetic fields cause antioxidative defense mechanisms in human osteoblasts via induction of •O2- and H2O2. Sci. Rep., 7, 14544, 2017, doi.org/10.1038/s41598-017-14983-9
42. Tasset, I., Medina, F.J., Jimena, I., Agüera, E., Gascón, F., Feijóo, M., et al. Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: Effects on neurotrophic factors and neuronal density. Neuroscience, 209, pp. 54–63, 2012, doi:10.1016/j.neuroscience.2012.02.034
43. Falone, S., Marchesi, N., Osera, C., Fassina, L., Comincini, S., Amadio, M., et al. Pulsed electromagnetic field (PEMF) prevents pro-oxidant effects of H2O2 in SK-N-BE(2) human neuroblastoma cells. Int J Radiat Biol., 92(5), pp. 281-286, 2016, doi: 10.3109/09553002.2016.1150619

44. Krugers HJ, Karst H, Joels M. Interactions between noradrenaline and corticosteroids in the brain: from electrical activity to cognitive performance. Front Cell Neurosci. 2012 Apr 9;6:15. doi: 10.3389/fncel.2012.00015.
45. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev. 2003 Apr;42(1):33-84. doi: 10.1016/s0165-0173(03)00143-7.
46. McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP, Bruchas MR. CRH Engagement of the Locus Coeruleus Noradrenergic System Mediates Stress-Induced Anxiety. Neuron. 2015 Aug 5;87(3):605-20. doi: 10.1016/j.neuron.2015.07.002.
47. Tafet GE, Bernardini R. Psychoneuroendocrinological links between chronic stress and depression. ProgNeuropsychopharmacolBiol Psychiatry. 2003 Sep;27(6):893-903. doi: 10.1016/S0278-5846(03)00162-3.
48. Grunstein HS, Gleeson RM, Smythe GA. Relationship between hypothalamic noradrenergic neuronal activity and serum 3-methoxy-4-hydroxyphenylethylene glycol in the rat. Life Sci. 1986 Jul 21;39(3):207-13. doi: 10.1016/0024-3205(86)90532-1.
49. Young EA, Abelson JL, Cameron OG. Interaction of brain noradrenergic system and the hypothalamic-pituitary-adrenal (HPA) axis in man. Psychoneuroendocrinology. 2005 Sep;30(8):807-14. doi: 10.1016/j.psyneuen.2005.03.009.
50. Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, Scheimann J, Myers B. Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. ComprPhysiol. 2016 Mar 15;6(2):603-21. doi: 10.1002/cphy.c150015.
51. Fischer TW, Bergmann A, Kruse N, Kleszczynski K, Skobowiat C, Slominski AT, Paus R. New effects of caffeine on corticotropin-releasing hormone (CRH)-induced stress along the intrafollicular classical hypothalamic-pituitary-adrenal (HPA) axis (CRH-R1/2, IP3 -R, ACTH, MC-R2) and the neurogenic non-HPA axis (substance P, p75NTR and TrkA) in ex vivo human male androgenetic scalp hair follicles. Br J Dermatol. 2021 Jan;184(1):96-110. doi: 10.1111/bjd.19115.
52. Rogalska J. Mineralocorticoid and glucocorticoid receptors in hippocampus: their impact on neurons survival and behavioral impairment after neonatal brain injury. Vitam Horm. 2010;82:391-419. doi: 10.1016/S0083-6729(10)82020-5.
53. Koning ACAM, Buurstede JC, van Weert LTCM, Meijer OC. Glucocorticoid and Mineralocorticoid Receptors in the Brain: A Transcriptional Perspective. J EndocrSoc. 2019 Jul 24;3(10):1917-1930. doi: 10.1210/js.2019-00158.
54. De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. EndocrRev. 1998 Jun;19(3):269-301. doi: 10.1210/edrv.19.3.0331.
55. Sheng JA, Bales NJ, Myers SA, Bautista AI, Roueinfar M, Hale TM, Handa RJ. The Hypothalamic-Pituitary-Adrenal Axis: Development, Programming Actions of Hormones, and Maternal-Fetal Interactions. Front BehavNeurosci. 2021 Jan 13;14:601939. doi: 10.3389/fnbeh.2020.601939.
56. Klimek A, Kletkiewicz H, Siejka A, Wyszkowska J, Maliszewska J, Klimiuk M, Jankowska M, Seckl J, Rogalska J. New View on the Impact of the Low-Frequency Electromagnetic Field (50 Hz) on Stress Responses: Hormesis Effect. Neuroendocrinology. 2023;113(4):423-441. doi: 10.1159/000527878.
57. Rajendra P, Sujatha H, Devendranath D, Gunasekaran B, Sashidhar R, Subramanyam C, Channakeshava. Biological effects of power frequency magnetic fields: Neurochemical and toxicological changes in developing chick embryos. Biomagn Res Technol. 2004 Jan 31;2(1):1. doi: 10.1186/1477-044X-2-1.

58. Laszlo AM, Ladanyi M, Boda K, Csicsman J, Bari F, Serester A, Molnar Z, Sepp K, Galfi M, Radacs M. Effects of extremely low frequency electromagnetic fields on turkeys. PoultSci. 2018 Feb 1;97(2):634-642. doi: 10.3382/ps/pex304.
59. Harakawa S, Hori T, Nedachi T, Suzuki H. Gender and Age Differences in the Suppressive Effect of a 50 Hz Electric Field on the Immobilization-Induced Increase of Plasma Glucocorticoid in Mice. Bioelectromagnetics. 2020 Feb;41(2):156-163. doi: 10.1002/bem.22238.
60. Chung YH, Lee YJ, Lee HS, Chung SJ, Lim CH, Oh KW, Sohn UD, Park ES, Jeong JH. Extremely low frequency magnetic field modulates the level of neurotransmitters. Korean J PhysiolPharmacol. 2015 Jan;19(1):15-20. doi: 10.4196/kjpp.2015.19.1.15.
61. Touitou, Y., Selmaoui, B. The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system. DialoguesClinNeurosci, 14(4), pp. 381-399, 2012, doi: 10.31887/DCNS.2012.14.4/ytouitou
62. Myers B, McKlveen JM, Herman JP. Glucocorticoid actions on synapses, circuits, and behavior: implications for the energetics of stress. Front Neuroendocrinol. 2014 Apr;35(2):180-196. doi: 10.1016/j.yfrne.2013.12.003.
63. Djordjevic, N.Z., Paunović, M.G., Peulić, A.S. Anxiety-like behavioural effects of extremely low-frequency electromagnetic field in rats. EnvironSciPollut Res Int., 24(27), pp. 21693-21699, 2017, doi: 10.1007/s11356-017-9710-1
64. Korpinar MA, Kalkan MT, Tuncel H. The 50 Hz (10 mT) sinusoidal magnetic field: effects on stress-related behavior of rats. Bratisl Lek Listy. 2012;113(9):521-4. doi: 10.4149/bll_2012_117.
65. Touitou Y, Selmaoui B, Lambrozo J. Assessment of cortisol secretory pattern in workers chronically exposed to ELF-EMF generated by high voltage transmission lines and substations. EnvironInt. 2022 Mar;161:107103. doi: 10.1016/j.envint.2022.107103.
66. Bagheri Hosseinabadi M, Khanjani N, Ebrahimi MH, Haji B, Abdolahfard M. The effect of chronic exposure to extremely low-frequency electromagnetic fields on sleep quality, stress, depression and anxiety. ElectromagnBiol Med. 2019;38(1):96-101. doi: 10.1080/15368378.2018.1545665.
67. Liu, X., Zuo, H., Wang, D., Peng, R., Song, T., Wang, S., et al. Improvement of spatial memory disorder and hippocampal damage by exposure to electromagnetic fields in an Alzheimer's disease rat model. PLoS One, 15;10(5):e0126963, 2015, doi: 10.1371/journal.pone.0126963
68. Roszkowski M, Manuella F, von Ziegler L, Durán-Pacheco G, Moreau JL, Mansuy IM, Bohacek J. Rapid stress-induced transcriptomic changes in the brain depend on beta-adrenergic signaling. Neuropharmacology. 2016 Aug;107:329-338. doi: 10.1016/j.neuropharm.2016.03.046.
69. Aloyz R, Fawcett JP, Kaplan DR, Murphy RA, Miller FD. Activity-dependent activation of TrkB neurotrophin receptors in the adult CNS. LearnMem. 1999 May-Jun;6(3):216-31. PMID: 10492004
70. Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. ExpNeurol. 2010 Nov;226(1):173-82. doi: 10.1016/j.expneurol.2010.08.022.
71. Komaki A, Khalili A, Salehi I, Shahidi S, Sarihi A. Effects of exposure to an extremely low frequency electromagnetic field on hippocampal long-term potentiation in rat. Brain Res. 2014 May 20;1564:1-8. doi: 10.1016/j.brainres.2014.03.041.
72. Marien MR, Colpaert FC, Rosenquist AC. Noradrenergic mechanisms in neurodegenerative diseases: a theory. Brain Res Brain Res Rev. 2004 Apr;45(1):38-78. doi: 10.1016/j.brainresrev.2004.02.002.
73. Hagena H, Hansen N, Manahan-Vaughan D. β-Adrenergic Control of Hippocampal Function: Subserving the Choreography of Synaptic Information Storage and Memory. CerebCortex. 2016 Apr;26(4):1349-64. doi: 10.1093/cercor/bhv330.

74. Ivy AS, Rodriguez FG, Garcia C, Chen MJ, Russo-Neustadt AA. Noradrenergic and serotonergic blockade inhibits BDNF mRNA activation following exercise and antidepressant. PharmacolBiochemBehav. 2003 Apr;75(1):81-8. doi: 10.1016/s0091-3057(03)00044-3.
75. Xia, P., Zheng, Y., Dong, L., Tian, C. Short-Term Extremely Low-Frequency Electromagnetic Field Inhibits Synaptic Plasticity of Schaffer Collateral-CA1 Synapses in Rat Hippocampus via the Ca2+/Calcineurin Pathway. ACS Chem. Neurosci. 12(19), pp. 3550–3557, 2021, doi: 10.1021/acschemneuro.1c00500
76. Zheng, Y., Cheng, J., Dong, L., Ma, X., Kong, Q. Effects of exposure to extremely low frequency electromagnetic fields on hippocampal long-term potentiation in hippocampal CA1 region. BiochemBiophys Res Commun. 24; 517(3), pp. 513-519, 2019, doi: 10.1016/j.bbrc.2019.07.085
77. Zheng, Y., Zhao, L., Dong, L., Tian, C., Xia, P., Jin, Z. The Time-Dependence of Three Different Modes of ELF-EMF Stimulation on LTP at Schaffer Collateral-CA1 Synapses. Bioelectromagnetics. 42(7), pp. 538-549, 2021, doi: 10.1002/bem.22369
78. Cichoń, N., Czarny, P., Bijak, M., Miller, E., Śliwiński, T., Szemraj, J., et al. Benign Effect of Extremely Low-Frequency Electromagnetic Field on Brain Plasticity Assessed by Nitric Oxide Metabolism during Poststroke Rehabilitation. OxidMed Cell Longev., 2017:2181942, 2017, doi: 10.1155/2017/2181942
79. Zarrin, F., Mahdavi, S.M., Modarresi Chahardehi, A., Razzaghi, Z., Ahmadi, N. The Effect of Time-Dependence of 10 Hz Electromagnetic Field on Spatial Learning and Memory in Rats. J LasersMedSci. 12;13:e64, 2022, doi: 10.34172/jlms.2022.64
80. Calabrese, E.J., Mattson, M.P. How does hormesis impact biology, toxicology, and medicine? NPJ Aging Mech Dis., 15;3:13, 2017, doi: 10.1038/s41514-017-0013-z
81. Mushak P. How prevalent is chemical hormesis in the natural and experimental worlds? Sci Total Environ. 2013 Jan 15;443:573-81. doi: 10.1016/j.scitotenv.2012.11.028.
82. Mattson MP. Hormesis defined. Ageing Res Rev. 2008 Jan;7(1):1-7. doi: 10.1016/j.arr.2007.08.007.
83. Wiegant FA, de Poot SA, Boers-Trilles VE, Schreij AM. Hormesis and Cellular Quality Control: A Possible Explanation for the Molecular Mechanisms that Underlie the Benefits of Mild Stress. DoseResponse. 2012 Nov 16;11(3):413-30. doi: 10.2203/dose-response.12-030.Wiegant.
84. Sidibé A, Charles MT, Lucier JF, Xu Y, Beaulieu C. Preharvest UV-C Hormesis Induces Key Genes Associated With Homeostasis, Growth and Defense in Lettuce Inoculated With Xanthomonas campestris pv. vitians. Front Plant Sci. 2022 Jan 17;12:793989. doi: 10.3389/fpls.2021.793989.
85. Rogalska J, Caputa M. Neonatal asphyxia under hyperthermic conditions alters HPA axis function in juvenile rats. NeurosciLett. 2010 Mar 12;472(1):68-72. doi: 10.1016/j.neulet.2010.01.060.
86. Seckl JR. Glucocorticoids and Fetal Programming; Necessary and Sufficient?. In: Seckl, J., Christen, Y. (eds) Hormones, Intrauterine Health and Programming. Research and Perspectives in Endocrine Interactions, 2014, vol 12. Springer, Cham. doi: 10.1007/978-3-319-02591-9_1
87. Maynard TM, Sikich L, Lieberman JA, LaMantia AS. Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia. Schizophr Bull. 2001;27(3):457-76. doi: 10.1093/oxfordjournals.schbul.a006887.
88. Jadidi M, Firoozabadi SM, Rashidy-Pour A, Sajadi AA, Sadeghi H, Taherian AA. Acute exposure to a 50 Hz magnetic field impairs consolidation of spatial memory in rats. NeurobiolLearnMem. 2007 Nov;88(4):387-92. doi: 10.1016/j.nlm.2007.07.010.
89. Foroozandeh E, Ahadi H, Askari P, Naeini MS. Effects of single, brief exposure to an 8 mT electromagnetic field on avoidance learning in male and female mice. Psychol Neurosci. 2011Jan;4(1):143–8. doi: 10.3922/j.psns.2011.1.016

90. Graham JH, Fletcher D, Tigue J, McDonald M. Growth and developmental stability of Drosophila melanogaster in low frequency magnetic fields. Bioelectromagnetics. 2000 Sep;21(6):465-72. PMID: 10972950.
91. Maggio N, Segal M. Corticosteroid regulation of synaptic plasticity in the hippocampus. ScientificWorldJournal. 2010 Mar 16;10:462-9. doi: 10.1100/tsw.2010.48.
92. Maity S, Rah S, Sonenberg N, Gkogkas CG, Nguyen PV. Norepinephrine triggers metaplasticity of LTP by increasing translation of specific mRNAs. Learn Mem. 2015 Sep 15;22(10):499-508. doi: 10.1101/lm.039222.115.
93. Directive 2013/35/EU of the European Parliament and of the Council of 26 June 2013 on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (electromagnetic fields) 2013.
94. Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. NucleicAcids Res. 2002 May 1;30(9):e36. doi: 10.1093/nar/30.9.e36.
95. Kraeuter, A.K., Guest, P.C., Sarnyai, Z. The Open Field Test for Measuring Locomotor Activity and Anxiety-Like Behavior. Methods Mol Biol., 1916, pp. 99-103, 2019, doi: 10.1007/978-1-4939-8994-2_9
96. Walf, A.A., Frye, C.A. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc., 2(2), pp. 322-328, 2007, doi: 10.1038/nprot.2007.44
97. Zlatković J, Todorović N, Bošković M, Pajović SB, Demajo M, Filipović D. Different susceptibility of prefrontal cortex and hippocampus to oxidative stress following chronic social isolation stress. Mol Cell Biochem. 2014 Aug;393(1-2):43-57. doi: 10.1007/s11010-014-2045-z.
98. Herbet M, Korga A, Gawrońska-Grzywacz M, Izdebska M, Piątkowska-Chmiel I, Poleszak E, Wróbel A, Matysiak W, Jodłowska-Jędrych B, Dudka J. Chronic Variable Stress Is Responsible for Lipid and DNA Oxidative Disorders and Activation of Oxidative Stress Response Genes in the Brain of Rats. OxidMed Cell Longev. 2017;2017:7313090. doi: 10.1155/2017/7313090.
99. McKlveen JM, Morano RL, Fitzgerald M, Zoubovsky S, Cassella SN, Scheimann JR, Ghosal S, Mahbod P, Packard BA, Myers B, Baccei ML, Herman JP. Chronic Stress Increases Prefrontal Inhibition: A Mechanism for Stress-Induced Prefrontal Dysfunction. Biol Psychiatry. 2016 Nov 15;80(10):754-764. doi: 10.1016/j.biopsych.2016.03.2101.
100. Spiers JG, Chen HJ, Sernia C, Lavidis NA. Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress. Front Neurosci. 2015 Jan 19;8:456. doi: 10.3389/fnins.2014.00456.
101. Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am J PhysiolEndocrinolMetab. 2006 Nov;291(5):E965-73. doi: 10.1152/ajpendo.00070.2006.
102. Chu, S.C., Yu, C.H., Chen, P.N., Hsieh, Y.S., Kuo, D.Y. Role of oxidative stress in disrupting the function of negative glucocorticoid response element in daily amphetamine-treated rats. Psychoneuroendocrinology. 71, pp. 1-11, 2016, doi: 10.1016/j.psyneuen.2016.04.025
103. Arnett MG, Muglia LM, Laryea G, Muglia LJ. Genetic Approaches to Hypothalamic-Pituitary-Adrenal Axis Regulation. Neuropsychopharmacology. 2016 Jan;41(1):245-60. doi: 10.1038/npp.2015.215.
104. Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, Bell ME, Bhatnagar S, Laugero KD, Manalo S. Chronic stress and obesity: a new view of "comfort food". Proc NatlAcadSci U S A. 2003 Sep 30;100(20):11696-701. doi: 10.1073/pnas.1934666100.
105. Cole MA, Kalman BA, Pace TW, Topczewski F, Lowrey MJ, Spencer RL. Selective blockade of the mineralocorticoid receptor impairs hypothalamic-pituitary-adrenal axis expression of habituation. J Neuroendocrinol. 2000 Oct;12(10):1034-42. doi: 10.1046/j.1365-2826.2000.00555.x.

106. Gądek-Michalska A, Spyrka J, Rachwalska P, Tadeusz J, Bugajski J. Influence of chronic stress on brain corticosteroid receptors and HPA axis activity. Pharmacol Rep. 2013;65(5):1163-75. doi: 10.1016/s1734-1140(13)71474-9.
107. Ochedalski T, Rabadan-Diehl C, Aguilera G. Interaction between glucocorticoids and corticotropin releasing hormone (CRH) in the regulation of the pituitary CRH receptor in vivo in the rat. J Neuroendocrinol. 1998 May;10(5):363-9. doi: 10.1046/j.1365-2826.1998.00212.x.
108. Aguilera G. Corticotropin releasing hormone, receptor regulation and the stress response. TrendsEndocrinolMetab. 1998 Oct 1;9(8):329-36. doi: 10.1016/s1043-2760(98)00079-4.
109. McCullers DL, Sullivan PG, Scheff SW, Herman JP. Traumatic brain injury regulates adrenocorticosteroid receptor mRNA levels in rat hippocampus. Brain Res. 2002 Aug 23;947(1):41-9. doi: 10.1016/s0006-8993(02)02904-9.
110. Rogalska J, Kang P, Wotherspoon W, Macleod MR, Lai M. Effect of hyperthermia and anoxia on glucocorticoid and mineralocorticoid receptor expression in neonatal rat hippocampus. NeurosciLett. 2009 Jan 30;450(2):196-200. doi: 10.1016/j.neulet.2008.11.033
111. Giorgi FS, Galgani A, Puglisi-Allegra S, Busceti CL, Fornai F. The connections of Locus Coeruleus with hypothalamus: potential involvement in Alzheimer's disease. J NeuralTransm (Vienna). 2021 May;128(5):589-613. doi: 10.1007/s00702-021-02338-8.
112. Holland N, Robbins TW, Rowe JB. The role of noradrenaline in cognition and cognitive disorders. Brain. 2021 Sep 4;144(8):2243-2256. doi: 10.1093/brain/awab111.
113. Garvey M, Hollon S, DeRubeis R. Is there a relationship between MHPG and anxiety in depressed patients? Psychiatry Res. 1991 Aug;38(2):187-95. doi: 10.1016/0165-1781(91)90043-o.
114. Kurita M, Nishino S, Numata Y, Okubo Y, Sato T. The noradrenaline metabolite MHPG is a candidate biomarker between the depressive, remission, and manic states in bipolar disorder I: two long-term naturalistic case reports. NeuropsychiatrDisTreat. 2015 Feb 11;11:353-8. doi: 10.2147/NDT.S74550.
115. van der Zee S, Vermeiren Y, Fransen E, Van Dam D, Aerts T, Gerritsen MJ, Spikman JM, van Laar T, De Deyn PP. Monoaminergic Markers Across the Cognitive Spectrum of Lewy Body Disease. J ParkinsonsDis. 2018;8(1):71-84. doi: 10.3233/JPD-171228.
116. Stone EA, Platt JE. Brain adrenergic receptors and resistance to stress. Brain Res. 1982 Apr 15;237(2):405-14. doi: 10.1016/0006-8993(82)90452-8.
117. Abercrombie ED, Jacobs BL. Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. II. Adaptation to chronically presented stressful stimuli. J Neurosci. 1987 Sep;7(9):2844-8. doi: 10.1523/JNEUROSCI.07-09-02844.1987.
118. Nisenbaum LK, Zigmond MJ, Sved AF, Abercrombie ED. Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. J Neurosci. 1991 May;11(5):1478-84. doi: 10.1523/JNEUROSCI.11-05-01478.1991.
119. Calabrese EJ, Mattson MP. Hormesis provides a generalized quantitative estimate of biological plasticity. J Cell CommunSignal. 2011 Mar;5(1):25-38. doi: 10.1007/s12079-011-0119-1.
120. Mushak P. Temporal stability of chemical hormesis (CH): Is CH just a temporary stop on the road to thresholds and toxic responses? Sci Total Environ. 2016 Nov 1;569-570:1446-1456. doi: 10.1016/j.scitotenv.2016.06.233.
121. Chauhan E, Bali A, Singh N, Jaggi AS. Cross stress adaptation: Phenomenon of interactions between homotypic and heterotypic stressors. Life Sci. 2015 Sep 15;137:98-104. doi: 10.1016/j.lfs.2015.07.018.
122. Cirulli F, Alleva E. The NGF saga: from animal models of psychosocial stress to stress-related psychopathology. Front Neuroendocrinol. 2009 Aug;30(3):379-95. doi: 10.1016/j.yfrne.2009.05.002.

123. O'Dell TJ, Connor SA, Guglietta R, Nguyen PV. β-Adrenergic receptor signaling and modulation of long-term potentiation in the mammalian hippocampus. LearnMem. 2015 Aug 18;22(9):461-71. doi: 10.1101/lm.031088.113.
124. Guerriero F, Ricevuti G. Extremely low frequency electromagnetic fields stimulation modulates autoimmunity and immune responses: a possible immuno-modulatory therapeutic effect in neurodegenerative diseases. Neural Regen Res. 2016 Dec;11(12):1888-1895. doi: 10.4103/1673-5374.195277.
125. Bacon TJ, Pickering AE, Mellor JR. Noradrenaline Release from Locus Coeruleus Terminals in the Hippocampus Enhances Excitation-Spike Coupling in CA1 Pyramidal Neurons Via β-Adrenoceptors. CerebCortex. 2020 Nov 3;30(12):6135-6151. doi: 10.1093/cercor/bhaa159.
126. Roozendaal B, Okuda S, de Quervain DJ, McGaugh JL. Glucocorticoids interact with emotion-induced noradrenergic activation in influencing different memory functions. Neuroscience. 2006;138(3):901-10. doi: 10.1016/j.neuroscience.2005.07.049.
127. O'Dell TJ, Connor SA, Gelinas JN, Nguyen PV. Viagra for your synapses: Enhancement of hippocampal long-term potentiation by activation of beta-adrenergic receptors. Cell Signal. 2010 May;22(5):728-36. doi: 10.1016/j.cellsig.2009.12.004.
128. Zhang H, Cui M, Cao JL, Han MH. The Role of Beta-Adrenergic Receptors in Depression and Resilience. Biomedicines. 2022 Sep 23;10(10):2378. doi: 10.3390/biomedicines10102378.

Publikacje wchodzące w skład rozprawy doktorskiej

brain sciences

MDPI

Review

Extremely Low-Frequency Magnetic Field as a Stress Factor—Really Detrimental?—Insight into Literature from the Last Decade

Angelika Klimek and Justyna Rogalska *

Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University, 87-100 Torun, Poland; klimek@doktorant.umk.pl
* Correspondence: rogal@umk.pl; Tel.: +48-56-611-26-31

Abstract: Biological effects of extremely low-frequency magnetic field (ELF-MF) and its consequences on human health have become the subject of important and recurrent public debate. ELF-MF evokes cell/organism responses that are characteristic to a general stress reaction, thus it can be regarded as a stress factor. Exposure to ELF-MF "turns on" different intracellular mechanisms into both directions: compensatory or deleterious ones. ELF-MF can provoke morphological and physiological changes in stress-related systems, mainly nervous, hormonal, and immunological ones. This review summarizes the ELF-MF-mediated changes at various levels of the organism organization. Special attention is placed on the review of literature from the last decade. Most studies on ELF-MF effects concentrate on its negative influence, e.g., impairment of behavior towards depressive and anxiety disorders; however, in the last decade there was an increase in the number of research studies showing stimulating impact of ELF-MF on neuroplasticity and neurorehabilitation. In the face of numerous studies on the ELF-MF action, it is necessary to systematize the knowledge for a better understanding of the phenomenon, in order to reduce the risk associated with the exposure to this factor and to recognize the possibility of using it as a therapeutic agent.

Keywords: magnetic field; stress; HPA axis; catecholamines; cytokines; hormones; behavior; anxiety; neuroplasticity; cell survival

Citation: Klimek, A.; Rogalska, J. Extremely Low-Frequency Magnetic Field as a Stress Factor—Really Detrimental?—Insight into Literature from the Last Decade. *Brain Sci.* **2021**, *11*, 174. https://doi.org/10.3390/brainsci11020174

Academic Editor: Ulrich Palm
Received: 22 December 2020
Accepted: 27 January 2021
Published: 31 January 2021

1. Introduction

Many studies have suggested an association between extremely low-frequency magnetic field (ELF-MF) exposure and anxiety and/or depression. On the other hand, the ELF-MF-induced improvement of brain function has also been found. The mechanism of these effects is assumed to be a stress response induced by ELF-MF exposure. Extremely low-frequency MF is natural physical phenomenon in our environment. The rapid development of science and technology resulted in the introduction of many new devices and technologies in industry, agriculture, and everyday life. We are continuously exposed in our environment to ELF-MF (range of 0–300 Hz) [1]. MFs are either of natural origin (geomagnetic field, intense solar activity, thunderstorms) or human-made (factories, transmission lines, electric appliances at work and home, magnetic resonance imaging, medical treatment, etc.) [2]. Common used frequencies of electric and magnetic fields of the electric power supply and of electric and magnetic fields generated by electricity power lines and electric/electronic devices are 50 Hz in Europe and 60 Hz in North America [2]. Biological effects of ELF-MF and their consequences on human health have become the subject of important and recurrent public debate. Until now the reported studies are largely contradictory with regard to epidemiologic studies (some of the research studies found a relationship with development of diseases while the others failed to find any [3–8] (Table S1). Whether or not ELF-MF exposure is related to increased health risks, it has led many scientists to examine the potential mechanisms by which ELF-MF might affect human

health. Special attention is paid to the adverse impact of both low- and high-frequency MF (radio waves) due to many possible pathological effects and numerous reports on MF-induced carcinogenicity [9]. ELF-MF was proved to be a stress factor and as a consequence, it can provoke morphological and physiological changes in stress-related systems [10]. Some authors argue that ELF-MF evokes cell/organism responses that are characteristic to general stress reaction. ELF-MF exposure "turns on" different intracellular—compensatory or deleterious—mechanisms and modifies stress-related function of nervous, hormonal and immunological systems (Figure 1). ELF-MF influence on living matter can cause a detrimental increase in free radicals levels and radical-evoked damages in macromolecules [11]. Most studies on ELF-MF effects concentrate on its negative influence; however, in the last decade there was an increase in the number of research studies showing stimulating impact of ELF-MF on brain plasticity processes (the production of protective proteins (e.g., Hsp70 or BDNF) or an increase in the activity of antioxidant enzymes) [12]. Furthermore, long-term exposure to ELF-MF can cause permanent changes in behavior (towards depressive and anxiety disorders) that are related to exposure to chronic stress [10,13,14].

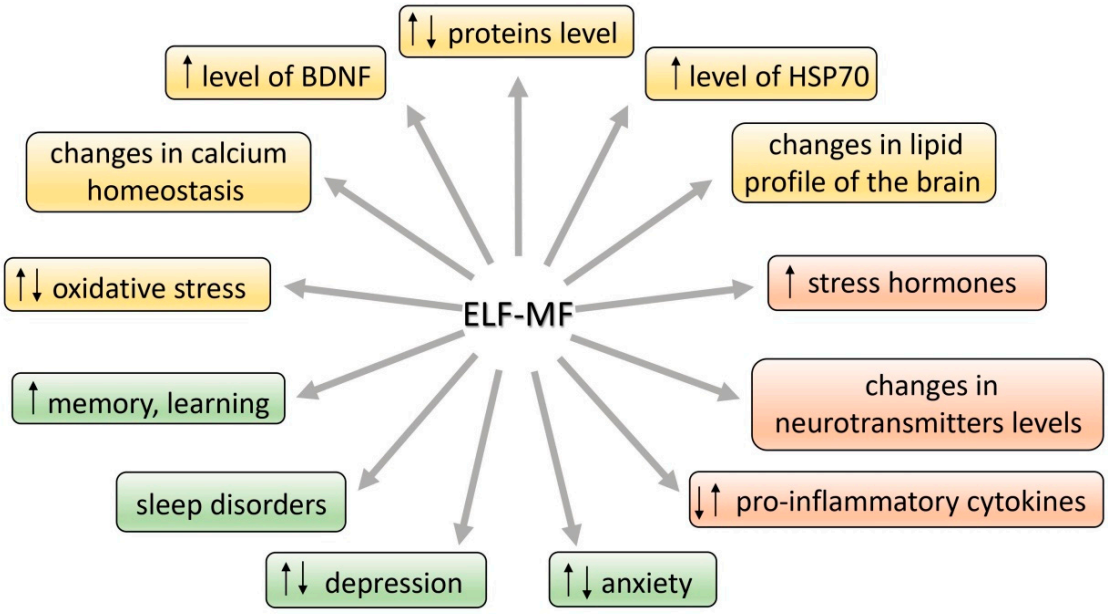


Figure 1. Effects of extremely low-frequency magnetic field (ELF-MF) action in the organism.

In the face of numerous studies on the effects of the ELF-MF, it is necessary to systematize the knowledge for a better understanding of this phenomenon, in order to reduce the risk associated with exposure to this factor, but also to recognize the possibility of using it as a therapeutic agent.

2. Stress—A Factor Determining the Function of Organism at All Levels of Organization

It is accepted that ELF-MF exposure may count as a mild stress situation [10,15–17] and it could activate a wide spectrum of interacting neuronal, molecular, and neurochemical systems that underpin behavioral and physiological responses. Chronic stress can promote and exacerbate pathophysiology leading to allostatic overload in human body [18]. The brain developed some adaptive mechanisms in the face of changing environments and stress factors imposed on the nervous system. Integrated response to stressful stimuli is an essential component of adaptive processes critical for survival of the organism. Failure of this stress adaptation is considered as one of the primary neuropathological causes of stress-related disorders. A healthy organism is able to turn on or off effectively physiological and psychological responses to stimuli; however, if the stress system response is not adequate—too slow or too high, its mediators will enhance vulnerability to stress-related

disease to which the individual is predisposed. Adaptation to repeated stress is associated with a complex cascade of molecular and cellular events, ranging from regulation of gene expression to release of neurotransmitters [19].

The definition of stress is not precise because the process is differently understood by people representing various fields of science. Stress can be discussed in the context of its influence on all levels of an organism's organization: molecular, cellular, physiological, and behavioral as well as psychological. The term "stress" was introduced by Hans Selye [20] and described as a result of disturbed homeostasis in the organism. Seyle [21] stated that stress is a "nonspecific response of the body to any demand". McEwen defined stress as "experiences that are challenging emotionally and physiologically" [18]. Other authors describe stress as a process involving perception, interpretation, response, and adaptation to harmful, threatening, or challenging events [22]. The reaction to a stress event is necessary for the organism to cope with danger [18]. Alarming signals include an internal, psychological, or environmental stimulus—such as ELF-MF. Some authors postulate that the changes turned on under the influence of exposure to ELF-MF are similar to those caused by other stress factors. The consequences of stress can be different and are dependent mainly on the strength of the stimulus. A low dose of stress can drive adaptive processes such as plasticity processes, e.g., the growth of postsynaptic (dendritic) spines, production of stress-resistant proteins, e.g., BDNF (brain-derived neurotrophic factor) and stimulation of neural stem cells to form new neurons that replace or cooperate with the existing ones [23], whereas even one high dose of a given factor may be harmful or even lethal [24].

In the neuroendocrine approach, stress is related to activation of the autonomic nervous system (SAM) and hypothalamo–pituitary–adrenal (HPA) axis. First, the autonomic nervous system is activated causing the release of noradrenaline and adrenaline from the adrenal medulla into the circulation, which—being a hormone—can rapidly regulate the function of peripheral organs [25] as well as the immunological response, which is supposed to adapt the organism to new, stressful conditions [26]. Acute activation of this system leads to release of noradrenaline from an extensive network of neurons throughout the brain, producing an enhanced state of arousal, which is critical for adaptive responses to stress [27]. Somewhat later, the HPA axis is activated, which causes the secretion of corticosteroid hormones from the adrenal cortex [25]. In response to a stressor, corticotropin-releasing hormone (CRH) is secreted in the hypothalamus. CRH is then driven with blood to anterior pituitary, where it causes adrenocorticotropic hormone (ACTH) release. In the next stage, ACTH reaches the adrenal glands and as a consequence glucocorticoids (cortisol and corticosterone) are secreted. The glucocorticoids cause increased arousal that ensures the organism's readiness for action. Thus, the HPA axis system regulates the intensity, dynamics, and termination of the stress response [28,29]. Hippocampus, which role is the inhibition the HPA axis via the negative feedback, is of crucial importance for the dynamic of stress response [30–32]. On the other hand, corticosteroids can also modulate hippocampal function in the opposite directions: causing neuron's dysfunction or plasticity and as a result, hippocampus-related behavioral changes can be observed. The hypothalamic–pituitary–adrenal (HPA) axis is sensitive to a broad spectrum of experimental and environmental events [30] that may result in physiological and behavioral changes in both directions: detrimental and compensatory ones. Sometimes these modifications are very subtle, but in some conditions they can even underlie the stress-related disorders or mediate the reversion of brain damage.

3. Molecular Stress Response to ELF-MF

Many studies show that stress induces the disruption in homeostasis [23,33] and as a consequence an overcompensation response is triggered to re-establish homeostasis. It needs gene expression and protein synthesis that progresses over time and leads to establish a new set-point for stress response systems. As ELF-MF is able to change the stress parameters, it is suggested that it can shift the set-point of endocrinological regulations

and determine the health status of the organism as a consequence [15]. The effects of exposure to ELF-MF are particularly prevalent in the hippocampal area of the brain [34,35]. As mentioned, the hippocampus is involved in regulating the HPA axis activity, but on the other hand, stress hormones (mainly corticosterone and noradrenaline) are known to modulate hippocampal function and they may determine the plasticity processes in this area; it means an adaptive response to ELF-MF's exposure. Targets for ELF-MF at molecular level include the cell membrane (e.g., its permeability, inorganic ion transport, receptor function), second messengers synthesis, chromosome structural changes and chemical changes in DNA structure, genes expression and protein synthesis (e.g., metabolism-related), free radicals, and neurotrophic factors. Such profound modifications have to be reflected in neurotransmitter activity, hormone release, and metabolism of the brain [36–44]. What is important, the effect of ELF-MF on molecular and/or cellular mechanisms is not obvious—it can be detrimental or protective. However, the research study on these mechanisms can shed some light on the possible metabolic pathway being possibly influenced by ELF-MF. ELF-MF-evoked cellular stress includes the modifications of key substances in cell metabolism—proteins and lipids. The mentioned alternations are mainly related to ELF-MF-induced oxidative stress. The consequence of these processes can be cell death such as apoptosis, necrosis, or autophagy [42,45–48].

3.1. Proteins and Lipids

As shown in in vivo research, ELF-MF can affect levels and function of proteins—crucial for maintenance of cell homeostasis, e.g., proteins anchored in lipid bilayer of the cell membrane functioning as ion channels, enzymes, and receptors, as well as the other proteins of key importance for the response to stress, regulation of apoptosis, and a number of metabolic processes [37,45,47,49,50]. Total protein level as well as its activity (e.g., alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, bilirubin) was augmented in rats exposed to 1.5 mT ELF-MF [45]. Exposure to both 0.5 and 1 mT ELF-MF altered protein pattern in rat's hippocampus. Gene ontology analysis showed that the most important function of the identified proteins altered after ELF-MF exposure is to ensure the functioning of the brain. Exposure to ELF-MF caused extreme downregulation of two proteins: Sptan1 and Dpysl2. The first is responsible for stabilization of cell membrane and organization of intracellular organelles. The second, Dpysl2, plays a key role in neuronal development and polarity, and additionally in neuron projection morphogenesis. Notably, the increased intensity of ELF-MF may be associated with more alteration in cell protein expression, and subsequent cell morphology and proliferation rate changes [47]. The chromogranin A (CgA) is another protein that should be mentioned as important in stress response and as a new target for electromagnetic radiation. It is a neuroendocrine secretory protein costored and coreleased with catecholamines from adrenal medulla, adrenergic nerve endings, and neuroendocrine cells. CgA is also a marker of sympathoadrenal activity, so its level gives information on the course of stress response [51]. The protein is also involved in maintaining calcium homeostasis in the cell [52]. What is important, its level increases during a depressive mood or stress situation [52]. The serum level of CgA in volunteer subjects chronically exposed to ELF-MF in the range 0.1–0.3 μT did not differ from the level in control group. However, a trend toward lower concentrations of CgA was observed in the group exposed to higher level of ELF-MF (>0.3 μT). Suppressive effects of ELF-MF on CgA level could be recognized as having inhibitory effects on the activity of the sympathetic nervous system [53].

In addition to proteins, the brain lipid profile is also influenced by ELF-MF exposure and taking into account multiple roles for lipids, they can be the medium for the ELF-MF action in the cell. Lipids are structural components of the cell membrane and they are involved in transfer of signals across membranes [17]. Apart from being structural elements, they are also required for axonal elongation and act as precursors for various secondary messengers, including arachidonic acid, docosahexaenoic acid, or 1,2-diacylglycerol [54]. Any changes in brain lipid metabolism lead to disturbances in homeostasis and are re-

sponsible for altered functioning at the cell and tissue levels. It was shown that 60 Hz 2.4 mT ELF-MF induces changes in the brain lipid profile and in corticosterone concentration. The level of these changes was similar to that in the positive control group of rats exposed to stress-RS (movement restraint). After 21 days of exposure to ELF-MF or RS or combined model (ELF-MF + RS), a general tendency to the decrease of total lipid level in brain structures was observed in each experimental group. Total cholesterol level was significantly increased in the cortex in the ELF-MF and RS + ELF-MF groups, and in subcortical structures in the RS + ELF-MF group. Inversely, polar lipids level in ELF-MF and RS + ELF-MF groups was decreased both in the cortex and in subcortical structures. Nonesterified fatty acid levels were found to be slightly higher in subcortical structures of the RS + ELF-MF group as compared to the control and RS groups. The analysis of fatty acid methyl esters revealed that the level of polyunsaturated fatty acids in cerebellum of ELF-MF-exposed rats was decreased, whereas their level in subcortical structures in the same group was increased. In addition to the changes in the amount of different kinds of lipids, the ELF-MF-induced lipid oxidative modifications were also noticed. The concentration of thiobarbituric acid reactive substances (TBARS, byproduct of lipid peroxidation) in lipids was higher, especially in the cortex and cerebellum of all treated groups [17]. Previous research has shown that immediate changes in lipid profile and TBARS levels after 2 h of singular exposure were visible only in the RS + ELF-MF group, whereas single exposure to ELF-MF or RS alone did not cause any changes in reduced glutathione and nitric oxide levels [55]. The increased level of lipid peroxidation was also noticed in rats exposed to ELF-MF (100 μT and 500 μT) [56]. The interesting research on ELF-MF-induced (50 Hz, 3 mT) changes in lipid profile (proteomic and transcriptomic profiling) in *Caenorhabditis elegans* was performed by Sun et al. [57]. In the glycerolipids (GLs) group, total triacylglycerols (TGs) content was increased while diacylglycerols (DGs) level was decreased. It should be also noted that among the most enriched proteins evaluated in this research, there were ones involved in lipid transport [57]. These studies indicate that ELF-MF affects the brain's lipid balance in a similar way to physiological stressors.

3.2. Oxidative Stress and Antioxidant Status

Stress can be a factor causing an increase of the level of oxidative stress parameters in the brain, including lipid peroxidation and on the other hand, it can activate antioxidant response [58,59]. Oxidative stress is the result of an imbalance between reactive oxygen species (ROS) and antioxidants [29]. Under normal conditions the synthesis of ROS is usually balanced, but when the production of ROS increases they become harmful for organism. The imbalance causes changes at the cellular level, which causes DNA, proteins, and lipids damage. ROS are involved in physiological processes, for instance, in cell signaling and respiratory chain and immune response, but some pathological factors can contribute to their increased level [60]. Overproduction of ROS occurs, inter alia, in response to stress (heat, anoxia, ultraviolet light, injury, environmental pollution, cigarette smoke, psychological trauma, and many others) [61]. It has also been reported that ROS levels increase after ELF-MF exposure and the reason for this phenomenon can be the failure of antioxidant defense.

The disturbance of oxidative homeostasis was proved in in vitro research. Exposure to ELF-MF of 1 mT resulted in free radical increase in mouse macrophages [62] and SH-SY5Y neuroblastoma cells [39]. ELF-MF-induced increased ROS production was also found in K562 human leukemia cell line (50 Hz, 0.025/0.05/0.1 mT) [63,64], and in human osteoarthritic chondrocytes (100 Hz) [65]. The viability decrease and morphological changes of rat hippocampal neurons concomitantly with the increase of MDA (malondialdehyde) and ROS levels and reduction of superoxide dismutase activity were noticed after exposure to ELF-MF (50 Hz, 8 mT) [42]. Similarly, exposure to ELF-MF (50 Hz, 25–200 μT) resulted in increased ROS production and diminished activity of antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR)) in the human keratinocyte cell line NCTC 2544 [66].

In vitro results have been confirmed in in vivo research. The shift into oxidative processes, presented as ROS-level elevation and significantly, the total antioxidative capacity (TAC) level decrease, were found in *Caenorhabditis elegans* exposed to ELF-MF (50 Hz, 3 mT) [67]. These results, proving the ELF-MF-induced impairment of antioxidant mechanisms in the organism, were also obtained from research using rodent models. The toxic, increasing oxidative stress level effect of ELF-MF was found mainly in the brain. Akdag et al. [68] demonstrated that the activity of antioxidant enzyme catalase (CAT) was decreased in ELF-MF-exposed animals regardless of ELF-MF intensity (100 and 500 μT). Moreover, in the group exposed to 500 μT, TAC was lower than in the 100 μT group. At the same time, in the 500 μT group the levels of oxidative stress markers, MDA and MPO (myeloperoxidase), and values of total oxidant status (TOS) and oxidative stress index (OSI) were significantly higher. TBARS concentrations increasing concomitantly with decreasing reduced glutathione (GSH), total free-SH group concentrations, and TAC levels were found in rats exposed to ELF-MF (40 Hz, 7 mT and 50 Hz, 12 and 18 kV/m) [69]. The activities of antioxidant enzymes in brain homogenates were also decreased in rats exposed to ELF-MF (50 Hz 10 kV/m, 4.3 pT) [70]. In addition, in mouse brain subjected to ELF-MF (50 Hz, 8 mT), the levels of MDA, ROS, nitric oxide (NO), and nitric oxide synthase (NOS) were increased, whereas activities of SOD, CAT, and GPx were decreased [71,72]. Free radical level (superoxide anion- $O_2\bullet^-$ and NO_2^-) was increased in the hypothalamus of rats exposed to ELF-MF (50 Hz, 10 mT) [73]. Acute exposure to ELF-MF (60 Hz 2.4 mT) resulted in the impairment of antioxidant mechanisms in the brain as well as in other tissues: heart, kidney, and plasma (decrease in SOD activity and reduced glutathione level) [55,74]. The disturbance of oxidative status was also found in testes of rats (diabetic model) exposed to ELF-MF (50 Hz, 8.2 mT): the increase in MDA and NO level, and diminished GSH level [75]. Many studies on the effects of ELF-MF have been conducted on people from risk groups, occupationally and residentially (living near high voltage lines) exposed to ELF-MF. El-Helaly and Abu-Hashem [76] carried out their research on a group of 50 electronic equipment installers and repairers. The serum malondialdehyde (MDA) level in the ELF-MF-exposed group was significantly higher than in control, and concomitantly the melatonin level (hormone supporting the antioxidant effect) in this group was lower. Similarly, the increment of oxidative stress and oxidative damage to DNA was also found in other research on power plant workers (occupational exposure, 110–420 kV and 4.09 V/m, 16.27 μT) [40,77,78]. The data suggest that exposure to ELF-MF could cause the failure of the antioxidant response and the collapse of homeostatic capability of the cell, leading to oxidative damage and functional impairment. However, the direct connection to the risk of disease development has not been unequivocally proved.

Subsequent studies shed light on the effects of ELF-MF on the antioxidant mechanisms that can underlie the protection against neurodegeneration. The ELF-MF-induced improvement of antioxidant protection has been evaluated in both in vitro and in vivo research. Exposure of C2C12 cells (myoblasts) to ELF-MF of 1 mT caused a drastic decrease in ROS level while total antioxidant status (TAS) and the activities of CAT and GPx were elevated [79]. Ehnert et al. [80] found ELF-MF-induced (16 Hz 6–282 μT) increase of SOD2, CAT, GPX3, and glutathione-disulfide reductase (GSR) activity concomitant with the reduction of ROS levels in human osteoblasts. Similarly, in the myelogenous leukemia cell line K562 exposed to ELF-MF (50 Hz, 1 mT) and in human blood platelets exposed to different sources of electromagnetic radiation (1 kHz, 0.5 mT; 50 Hz, 10 mT; or 1 kHz 220 V/m) CAT activity was increased [81,82]. Moreover, the exposure IMR-90 human lung fibroblasts for a total of 168 h to 6 mT ELF-MF contributed to decreased ROS level [83]. Exposure of human neuronal cell culture SH-SY5Y to 50 Hz ELF-MF with magnetic field intensity 1 mT resulted in elevated activity of NOS. This enzyme is controlled by proinflammatory cytokines that also activate ROS. After 1, 3, 6, and 24 h of exposure to ELF-MF, the activity of the enzyme was significantly increased. Moreover, the augmented production of O_2^- was also found. However, CAT activity increased as the exposure time increased, possibly indicating a gradual adaptation of cells to the conditions of oxidative stress. On the other

hand, these adaptive mechanisms turn out to be insufficient when ELF-MF exposure is combined with additional administration of H_2O_2—the oxidative effect is then exacerbated. These data suggest that ELF-MF may to some extent have neuroprotective effect. The combination of ELF-MF exposure and the stressor H_2O_2 prevents cells from being effectively defended against ROS [84]. However, when H_2O_2-treated cells were exposed to a higher value of ELF-MF (75 Hz, 2 mT), ROS level decreased and MnSOD activity increased [85]. It definitely suggests that the protective effect of ELF-MF depends on its intensity. The results of this in vitro research points out the beneficial effect of ELF-MF as an upregulation of antioxidant pathways, leading to protection against oxidative damage has been noted, reflecting an attempt to stimulate cellular response to neuronal damage.

In addition, ELF-MF as a mild stress factor activates an adaptive response that ensures the oxidative–antioxidant balance in rodent models as well as in humans. In a rat model of Huntington's disease, ELF-MF (60 Hz and 0.7 mT) was found to be able to reverse the process of neuronal degeneration and oxidative stress; it enhanced the antioxidant glutathione content and reduced the oxidative stress markers, 8-hydroxy-2′-deoxyguanosine and oxidized glutathione levels, in the whole-brain tissue [12]. Recent research evaluating the redox state in post-stroke patients demonstrated the beneficial effect of ELF-MF on oxidative status. High magnetic intensity, 5 or 7 mT of 40 Hz ELF-MF, significantly increased enzymatic antioxidant activity as compared to results obtained before treatment. The results were correlated with the improvement in functional and mental status of post-stroke patients [86,87]. These data show that ELF-MF is a factor that may both increase the production of ROS and activate organisms' antioxidant machinery in humans. In consequence, the electromagnetic radiation may drive the mechanisms underlying cell survival and plasticity.

3.3. Neuroprotective Proteins: Hsp70 and BDNF

Prosurvival responses include DNA repair processes and the increase in expression of chaperone protein—70-kDa heat shock proteins (Hsp70) and neurotrophin—brain-derived neurotrophic factor (BDNF) [88,89]. The expression of Hsp70 and BDNF appears to be a part of the general stress response and thus it is speculated to be associated with hormonal response to stress [89]. The increase of expression of these proteins would indicate the development of processes adapting neuronal networks in order to optimize circuits responding to the external environment and to integrate the response to challenges [90]. It was shown that stress hormones (mainly corticosterone and noradrenaline) influence via their receptors the plasticity processes in the hippocampus [31,91]. Noradrenaline can even dictate the direction of synaptic strength change in the hippocampus [91]. Under the influence of ELF-MF the expression of stress-response genes increases, resulting in higher levels of molecular chaperones such as Hsp70 [92–94]. The role of Hsp proteins is to stabilize polypeptide chains during their translocation across the cell membranes and to prevent aggregation of proteins with abnormal structure. Moreover, the antiapoptotic properties of Hsp70 and their role in appropriate folding and activation of proteins have also been proved [89,95]. As there are many pathways that could be affected to upregulate Hsp70 expression induced by stress, it is difficult to determine if any specific pathway may be affected.

The protective value of ELF-MF mediated by its influence on Hsp70 level was proved in in vitro research. Perez [96] showed that ELF-MF (50 MHz) leads to higher levels of Hsp70 in human T lymphocytes and fibroblast cell lines when subjected to stress, and that this response was of protective value. It seems to precondition and to enhance the cellular stress response when cells are provoked by toxic stimuli. Moreover, the cell protection was proportional to the levels of Hsp70. Exposure of human leukemia cell line K562 to ELF-MF (less than 0.1 and 1 mT) leads to increased Hsp70 levels [63,64]. More recent in vitro studies on ELF-MF with a density over 1 mT have shown marked effects, including an increase in Hsp70 transcription that results in protection against chronic hypoxia-induced injury [50].

Interesting data were also received in in vivo research on invertebrates. According to Gutzeit [97] exposure to 50 Hz ELF-MF with magnetic flux densities 50–150 μT enhances the response to thermal stress in *C. elegans*. ELF-MF-mediated specific genes activation could enhance transcription of an already activated set of heat shock genes by costressor (heat stress), thus providing an adequate and optimal defense response. Exposure to 60 Hz 8 μT ELF-MF caused regeneration of the heads and tails parts of the Planarian, *Dugesia dorotocethala*. This effect was accompanied by an increase in the level of Hsp70, which is triggered by extracellular signal-regulated kinase (ERK) cascade. It is known that ERK is activated as reaction to injury to promote regeneration [98].

In this approach, ELF-MF appears to be a mild stressor mobilizing the organism to cope with a dangerous situation [98]. The expression of the Hsp70 in response to stress serves to protect against the negative impact of stress. Hsp70 induction and stress systems function were shown to be two important inter-related mechanisms in maintaining the homeostasis under stress conditions [89]. According to the juxtaposition presented, some of beneficial effects of ELF-MF can be due to the protective role of Hsp70.

A substance of high importance for the nervous system is also brain-derived neurotrophic factor (BDNF). This neurotrophin is responsible for differentiation and survival of neurons during development, but it is also important for the adult brain, especially when subjected to stress conditions [90]. In a mature brain, BDNF ensures excitatory and inhibitory synaptic transmission and neuroplasticity [99]. The mechanism of neuroplasticity is crucial for learning and memory processes. It includes enhancement of the long-term potentiation (LTP), and stimulating and controlling neural growth. BDNF has a high affinity to full length tropomyosin receptor kinase B (TrkB) and its truncated isoform, p75 NTR. Through the activation of TrkB, the neurotrophin starts the cascade of signaling pathways, which results in neurogenesis, neuroplasticity, cell survival, and resistance to stress [100]. In vitro research proved that the exposure to pulsed ELF-MF (50 Hz; 1 mT for 2 h) increased the BDNF mRNA expression in cultured dorsal root ganglion neurons [101].

BDNF expression can be modulated by external, physiological, and pathological factors proven mainly in research on both rodents and humans. In the course of some diseases, such as Alzheimer disease, and during aging process or chronic stress, the inhibition of BDNF expression is noted, while exercise, enriched environment, and taking antidepressants are related to the intensified expression of BDNF [99]. ELF-MF is used in physical therapy due to its ability to stimulate BDNF synthesis. Several studies focused on this particular effect of ELF-MF on diseases and pathologies, like Huntington disease or stroke [12,102,103]. In post-stroke patients subjected to ten sessions of 15 min ELF-MF therapy (40 Hz 5 mT), plasma BDNF level was about 200% higher than before the treatment [102]. The study undertaken on a rat model of Huntington disease indicated that exposure to ELF-MF (60 Hz 0.7 mT 2 h in the morning and 2 h in the afternoon for 21 days) significantly elevated BDNF level in the rats with induced Huntington disease. Moreover, changes in the rats' behavior related to Huntington disease were neutralized by ELF-MF [12]. Urnukhsaikhan et al. [103] showed that expression of BDNF, TrkB, and phosphorylated protein kinase B was increased in ELF-MF-stimulated (60 Hz, 10 mT) ischemic mice. In vitro research proved that the exposure to pulsed EMF (50 Hz; 1 mT for 2 h) increased the BDNF mRNA expression in cultured dorsal root ganglion neurons [103]. Thus, there is evidence suggesting that the neuroprotective effect of the exposure to extremely low-frequency MFs may be due to, at least in part, the impact of the fields on neurotrophic factors levels, leading to an increase of cell survival.

3.4. Plasticity, Neurogenesis, Proliferation, and Differentiation

The level of activity of voltage-gated Ca^{2+} channels is an important factor determining the synaptic transmission and leading to stimulation of short-term synaptic plasticity [104]. Calcium ions are involved in secretion of neurotransmitters. The influx of Ca^{2+} through presynaptic voltage-gated Ca^{2+} (Cav) channels triggers the release of neurotransmitters from presynaptic part of synapses. Measurements at a large glutamatergic synapse in

the mammalian auditory brainstem—the calyx of Held—showed that vesicle endocytosis and synaptic transmission were enhanced in mice (8–10 postnatal days old) kept from birth under the influence of EMF (50 Hz EMF, 1 mT). Moreover, in mice exposed to EMF, the increase in expression of calcium channels at the presynaptic nerve terminal facilitating the influx of calcium was found. The observed mechanism is responsible for increasing endocytosis and synaptic plasticity [105]. In vitro research also evidenced the ELF-MF-induced increase in intracellular Ca^{2+} concentration. This effect was found in C2C12 cells (myoblasts) after 0.1 and 1 mT ELF-MF exposure [79], in human pluripotent stem cells (iPSCs) after 1.5 mT ELF-MF exposure [105], in dorsal root ganglion neurons after 0.1, 1, 10, and 100 mT ELF-MF application [101], and in rat hippocampal neurons exposed to 8 mT ELF-MF [42]. The influence of ELF-MF on proliferation and apoptosis and the participation of Ca^{2+} in these processes were also determined. In human neuroblastoma IMR32 and in rat pituitary GH3-cultured cells, the exposure to 1 mT 50 Hz ELF-MF caused the increased cell proliferation. At the same time, the increase of Ca^{2+} current density and of voltage-gated Ca^{2+} channel expression in the cell membrane was observed. In addition, blocking of Ca^{2+} channels by 15 μM Cd^{2+} alleviated the proliferative effect of ELF-MF. Apoptosis, induced by H_2O_2 or puromycin in IMR32 cells, was decreased after 72 h exposure to 1 mT 50 Hz ELF-MF. Blocking of L-type calcium channels by nifedipine also caused disappearance of the antiapoptotic effect of ELF-MF [106]. It has been also shown that ELF-MF influences calcium homeostasis in cultural entorhinal cortex neurons via calcium channel-independent mechanism. Twenty-four hour exposure to 1 or 3 mT ELF-MF does not affect voltage-gated calcium current and activity of calcium channels, but regulates intracellular calcium dynamics by decreasing the high-K^+-evoked intracellular calcium elevation [107]. In summary, this study suggests that the change in calcium currents through voltage-gated calcium channels is the mechanism responsible for the proliferation promotion and antiapoptotic effect of ELF-MF.

Measurements at a large glutamatergic synapse in the mammalian auditory brainstem—the calyx of Held—showed that vesicle endocytosis and synaptic transmission was enhanced in mice (8–10 postnatal days old) kept from birth under the influence of ELF-MF (50 Hz ELF-MF,1 mT). Moreover, in mice exposed to ELF-MF, the increase in expression of calcium channels at the presynaptic nerve terminal facilitating the influx of calcium was found. The observed mechanism is responsible for increasing endocytosis and synaptic plasticity [108].

Throughout the life course, new neurons are continuously formed in the hippocampus, which is therefore a major site of structural plasticity in the adult brain. The existence of a causal link between ELF-MF-enhanced synaptic plasticity and neurogenesis has been shown by a number of in vivo experimental studies. ELF-MF (60 Hz, 0.7 mT, applied over 21 days) improved neurological scores, enhanced neurotrophic factor levels, and reduced neuronal loss in a rat model of Huntington's disease [12]. In addition, prolonged exposure to ELF-MF (50 Hz, 100 μT; for 90 consecutive days; 2 h/day) increased LTP induction in rat's hippocampus [35]. In vivo exposure of adult mice to ELF-MF (50 Hz, 1 mT) produced a marked increase in the number of newly generated neurons in the granule cell layer of the dentate gyrus [34]. Although the ELF-MF of 1 mT (for 21 days) caused the decrease in the dendritic spine density of neurons in hippocampus after 7 and 10 days, the effects disappeared after 14 days [109]. Studies on the rat traumatic brain injury model [110] and on rat Alzheimer's disease model [49] have shown that ELF-MF reversed pathological brain damages and learning and memory abilities impairment. Similar effects were obtained after exposure of neurotoxin-injected mice to ELF-MF (50 Hz, 1 mT); the deficits such as neuronal maturation impairment, neurogenesis decrease, and memory disturbance decreased [111]. Studies on the beneficial effects of ELF-MF might yield fruitful insights related to clinical therapy of nervous-system-related diseases.

The cell differentiation at the expense of proliferation in different tissues and increased cell viability as an effect of exposure to low-frequency ELF-MFs is well evidenced in the literature. Collard et al. [112] reported an acceleration of proliferation and differentiation of

human epidermis cells after exposure to low frequency (40 Hz) and demonstrated that the processes were related to a significant modification of gene expression. Falone et al. [85] found that ELF-MF (75 Hz, 2 mT) alone did not affect the viability of the human neuroblastoma SH-SY5Y cell line and that ELF-MF exposure prevented reduced cell viability after H_2O_2 application. Vannoni et al. [65] concluded that ELF-MF (100 Hz) stimulation is a useful tool to induce more divisions and thus to enhance cell proliferation of human osteoarthritic chondrocytes. The treatment of HeLa cells IMR-90 fibroblasts with ELF-MF (60 Hz, 6 mT) increased cell viability and activated cell cycle progression. In addition, ELF-MF mitigated the antiproliferative effect of GOx (agent stimulating H_2O_2 production) [83]. Di Loreto et al. [113] found that ELF-MF (50 Hz, 0.1–1 mT) had a positive effect on cell viability in primary cultures of maturing rat cortical neurons. The research of Ardeshirylajimi and Soleimani [105] on human pluripotent stem cells (iPSCs) suggested that ELF-MF (50 Hz, 1.5 mT) increases cell viability, division, proliferation, and mineralization of extracellular matrix. These results indicated that ELF-MF would improve the viability, proliferation, and differentiation of cells, and may be beneficial for the development of novel therapeutic approaches in regenerative medicine. The papers pointing to detrimental effect of ELF-MF on viability, differentiation, and proliferation should also be mentioned. It is, however, important that this effect is caused by high values of ELF-MF induction. Yin et al. [42] showed that the number of rat hippocampal neurons in G0/G1 phase was decreased and cells in S phase were accumulated as the effect of exposure to ELF-MF (50 Hz, 8 mT). The exposure of mesenchymal stem cells (bone marrow or adipose tissue derived) to ELF-MF of 20 mT (50 Hz) resulted in decreased cell proliferation [44,114]. This effect appeared to be related to the diminished expression of genes responsible for pluripotency and neuronal differentiation [44].

4. ELF-MF-Induced Changes in Levels of Neurotransmitters, Hormones, and Cytokines

ELF-MF-induced molecular changes modify to a certain extent some crucial neuronal processes. As it is commonly known, the communication between main groups of signaling substances: neurotransmitters, cytokines, and hormones, is of high importance for the maintenance of health status of an individual. We have a lot of data confirming the effect of ELF-MF on functioning of nervous, immune, and endocrinological systems. However, the mechanisms by which the magnetic stimulation modulates the activity of these systems and the interplay between them are open to be identified. Up-to-date results concluded that the exposure of rats to ELF-MF may be sufficient to induce significant changes in the content of neurotransmitters. The levels of major inhibitory and excitatory amino acids and neurotransmitters: glutamate (Glu), glutamine (Gln), glycine (Gly), tyrosine (Tyr), and γ-aminobutyric acid (GABA), were elevated in the thalamus after five days of exposure to ELF-MF (60 Hz, 2 mT). In the striatum, higher levels of Gln, Gly and GABA were found as well, whereas their concentrations were decreased in cortex, cerebellum, and hippocampus. Dopamine level was increased in the thalamus [115]. Extremely low-frequency magnetic field (10 Hz; 1.8–3.8 mT) exposure was found to alter turnover and receptor reactivity of serotoninergic and dopaminergic systems and some behavioral disturbances induced by these systems [116]. The rats receiving chronic (10 days) repetitive transcranial magnetic stimulation (rTMS) treatment showed the symptoms of anxiety, and it was shown that the rTMS-induced anxiety might involve the serotonergic system [117]. The continuous exposure of rats to ELF-MF (50 Hz, 0.5 mT) affected cortical serotoninergic neurotransmission, and intensity of these changes depended on ELF-MF exposure duration [118]. The data may indicate the ability of ELF-MF to modify the function of main neurotransmitter systems and thus to modulation of some physiological processes, such as memory, emotionality, mood changes, sleep, alertness, or stress response. The response of individual brain tissues to exposure was varied; the level of one neurotransmitter increased in a given tissue appeared to be decreased in another, suggesting that the radiation can induce varying responses in the nervous system [115].

As noted, the existing data indicate that the exposure to ELF-MF may count as a mild stress situation and could be a factor in the development of disturbances of brain stress systems: hypothalamo–pituitary–adrenal (HPA) axis and sympatho–adrenal–medullary (SAM) system [10,16,115,119,120]. Although some findings indicate the deteriorating effects of magnetic fields on hormonal stress response, others failed to exhibit any obvious effects. Continuous long-term (4–6 week) ELF-MF (50 Hz, 0.5 mT) treatment induced some signs of stress: HPA-axis activation (elevated blood glucose level, elevated POMC (the precursor protein for ACTH) mRNA level, and enhanced depression-like behavior in a forced swimming test), although other markers of stress (elevated basal ACTH and corticosterone secretion, adrenal gland hypertrophy, thymus involution, loss of weight gain, and anxiety-like behavior in elevated plus maze) were not observed. This confirms that ELF-MF of the abovementioned intensity creates a weak stress response [10]. In addition, 50 Hz ELF-MF (0.207 μT) significantly raised ACTH, cortisol, and glucose levels in guinea pigs [15]. The concentration of plasma corticosterone level was significantly higher and remained at a similar level in groups of rats exposed to restraint stress (RS) or ELF-MF [17]. Research by Mahdavi et al. [121] showed that exposure to both 1 and 5 Hz ELF-MF of 0.1 mT intensity caused an elevation of ACTH level in rats' plasma, whereas corticosterone level was reduced in both cases. In the animals exposed to 1 Hz ELF-MF, the concentration of adrenaline increased, but in rats exposed to 5 Hz, the level of adrenaline decreased. In rabbits exposed to ELF-MF (10 Hz ELF-MF), the level of blood corticosterone was increased in both the normal and high-cholesterol diet groups [122]. Chronic exposure (1 month) to 50 Hz 100/500 μT ELF-MF significantly raised corticosterone levels in rats' plasma [123]. In mice exposed to 10 μT ELF-MF (1, 4, or 24 h/day for 1 week—short-term exposure), no significant differences in CRH gene expression in hypothalamus were observed, whereas ACTH plasma level was lower regardless of the daily exposure time (1, 4, or 24 h/day for 1 week). Moreover, the expression of pituitary level of POMC was lower in an exposure time-dependent manner, and a statistically significant decrease appeared after 24 h/day exposure [124]. Mostafa et al. [125] showed that 2- and 4-week exposure of rats to ELF-MF (2 G, equivalent to 0.2 mT) significantly increased their plasma corticosterone level. Other studies on mouse model showed that even a relatively low level of EMF (12 nT) can cause corticosterone increase [126]. On the other hand, Kitaoka [16] revealed that levels of ACTH, the hormone that regulates corticosterone secretion, and hypothalamic CRH and pituitary POMC were not changed by ELF-MF (70 Hz, 3 mT). Significant changes were also found in the levels of noradrenaline in various parts of rats' brain: thalamus, hypothalamus, cerebellum and striatum, after 2 and 5 days exposure to 2 mT ELF-MF [115]. In the group of volunteers exposed to ELF-MF (50 Hz, 62 μT, for 2 h/day for 2 days with a 6-day interval) the cortisol level was increased at the beginning of ELF-MF exposure but later it diminished progressively [120]. The workers employed in the live-line procedures (132 kV high-voltage) for more than two years were found to be vulnerable for EM stress with altered adrenaline concentrations [40]. Moreover, exposure of turkey females to ELF-MF (50 Hz, 10 μT) caused NE-activated β-adrenoceptor function decrease, which is known to be involved in the formation of emotional disinterest and depression [126]. The data suggest that the exposure to ELF-MF can establish a new "set-point" for stress-system activity and the direction and dynamics of this process depend on the strength of the field and duration of exposure. The ELF-MF-induced changes in stress hormone levels will initiate cellular adaptation or damage by activation of intrinsic signaling pathways. Consequently, ELF-MF can change the vulnerability of the organism to subsequent stress factors and thus to diseases, mainly related to the nervous system.

Stress is known to strongly affect the immune system. It has been suggested that the potential contribution of ELF-MFs to anxiety or other stress associated disorders is also related to changes in the functioning of the immune system. Moreover, the chronic exposure to ELF-MF appears to also lead to immune system dysfunction, chronic allergic responses, inflammatory responses, and ill health [36]. However, as in other aspects of ELF-MF impact in organism, this factor can also be a double-edged sword and drive the survival-promoting

processes. Importantly, the immune system and the stress systems—HPA axis and SAM—are closely linked to each other. Glucocorticoids and catecholamines are known to modify the secretion of cytokines: proteins that facilitate communication between the immune cells and the cells of the central nervous and endocrine systems [127]. Cytokines have the ability to modulate and activate the HPA axis. Proinflammatory cytokines: IL-1, IL-6, and TNFα, induce corticotropin-releasing hormone (CRH) secretion and they are also involved at every stage of stress reaction [128]. Changes in plasma proinflammatory cytokines were observed after acute continuous exposure (24 h) to ELF-MF with magnetic intensity of 7 mT. The levels of IL-1β, IL-6, and IL-2 were elevated. The number of white and red blood cells and lymphocytes, and the hemoglobin concentration and hematocrit level were increased. However, the repetitive exposure to ELF-MF (1 h/day for 7 days) did not alter either cytokines levels or blood parameters [129]. The change in cytokine production was also noticed in stroke patients treated with ELF-MF (40 Hz, 5 mT ELF-MF) [130]. Following the exposure to ELF-MF, the plasma levels of IL-1β and IL-2 cytokines and the level of IL-1β mRNA expression were increased. In addition, ELF-MF exposure increases the levels of the anti-inflammatory transforming growth factor β (TGF-β) and interleukin-18-binding protein [84]. Thus, the ELF-MF exposure can cause deregulation of the immune system, thereby increasing vulnerability to infectious and autoimmune diseases. Interesting results concerning the effect of ELF-MF on lymphocytes level were obtained by de Kleijn et al. [124]. In mice exposed to 10 μT ELF-MF (1, 4, or 24 h/day for 1 week—short-term exposure, or for 15-week long-term exposure) the increase in CD3+/CD4+ T-lymphocytes was observed only after short-term exposure to ELF-MF. The data suggest that the ELF-MF effect on immune and stress responses may be transient, because no changes in the number of immune cells were observed after long-term exposure. Several authors reported that the ELF-MF-evoked neuroplasticity can be mediated by the effect of magnetic radiation on cytokine level. Cytokines are found to influence the expression of neurotrophins and their receptors. This may indicate the role of inflammatory cytokines in the process of neuroplasticity [130]. The latest reports showed that 1 and 100 μT 50 Hz ELF-MF not only downregulates proinflammatory cytokines (IL-9 and TNF-α), but also activates an inflammation-suppressing cytokine, IL-10. In this case, the most noticeable effect was obtained at the highest value of magnetic induction [131]. The ELF-MF (60 Hz, 10 mT) also mitigated the deficits in ischemic mice, among others in the context of immune function: the levels of inflammatory mediators MMP9 and IL-1β were decreased [102]. The results demonstrate the recovery-stimulating potential of ELF-MF.

5. Association between ELF-MF Exposure and Emotional Behavior and Wellbeing

An association between ELF-MF exposure and emotional behavior has been indicated in many studies. The animal studies have shown that chronic exposure to ELF-MF may induce an anxiogenic and/or a depression-like effect. Dysfunction of stress systems can evoke negative emotional state and can potentiate fear- and anxiety-related behaviors [90,132]. Therefore, it is reasonable to speculate that elevation in ELF-MF-induced anxiety level may be attributed to the effect of ELF-MF on glucocorticoid release following activation of HPA axis and catecholaminergic sympathetic nervous system releasing adrenaline and noradrenaline. These pathways are key biological factors that modulate emotional behavior [90]. Liu et al. [133] reported that ELF-MF exposure (2 mT, 4 h/day for 25 days) had an anxiogenic effect in rats, such as anxiety-like behaviors in open field and elevated plus maze tests. Szemerszky et al. [10] demonstrated that ELF-MF exposure (0.5 mT, 4 weeks) in rats increased their immobility time in a forced swim test. The chronic exposure of mice to ELF-MF (3 mT, total exposure 200 h) induced the depression- and/or anxiety-like behavior (increase in total immobility time in a forced swim test and in the latency to enter the light box in a light–dark transition test). These behavioral disturbances were correlated with high corticosterone secretion [16]. Mice prenatally exposed to ELF-MF (50 Hz, 1 mT) lacked sociability and preference of social novelty, which can be a sign of autism-relevant social abnormalities; however, they did not show anxiety-like behav-

ior [134]. The continuous (21 days) exposure to extremely low-frequency magnetic field (50 Hz, 10 mT) had no significant effect on activity and exploration activity but significantly increased stress and anxiety-related behavior in rats [135]. Quite similar observations in open field and elevated plus maze tests were described by Djordjevic et al. [73] after the exposure to ELF-MF (50 Hz, 10 mT) significantly reduced activity was observed. The noted effects of short-term ELF-MF exposure (50 Hz, 500 μT, 20 min) verified by behavioral tests in rats (elevated plus maze, novel object exploration) appear to suggest that these field parameters may cause some kind of discomfort, influence behavior, and increase passivity and situational anxiety [136]. Increased level of anxiety has also been found in rats exposed to ELF-MF of various flux density (50 Hz; 1, 100, 500, 2000 μT, and 2 mT) [56,137]. In accordance with this, Isogawa et al. [117] observed an anxiogenic effect of rTMS in rats tested in the elevated plus maze. It has also been shown that in rat pups after 6 weeks of exposure to ELF-MF (50 Hz, 3.5 mT, 1 h/day) behavior parameters, such as activity, motion, and response to sound and light, were decreased during exposure, but after exposure they settled back into normal control values [138]. However, the exposure of rats to ELF-MF of lower flux density (100 μT, 50 Hz, for 24 weeks) did not evoke any behavioral changes. The experimental group did not show any anxiety-like behaviors in open field or elevated plus maze. Similarly, depression-like behavior was not detected during tail suspension and forced swim tests [139]. Population studies paid attention to the role of ELF-MF in the development of sleep disorders, anxiety, and depression. It was shown that residential exposure to ELF-MF emitted by a radio–television broadcasting station could increase the anxiety in women [140]. Similarly, power plant workers (chronically exposed to ELF-MF) showed significantly poorer sleep quality than the unexposed group. Moreover, the level of depression symptoms in the exposed group was also significantly higher [14]. Interestingly, magnetic waves in the form of repetitive transcranial magnetic stimulation (rTMS) are used in therapy of depression. Three weeks of daily treatment caused a remission in a significant number of patients resistant to antidepressant treatment [13]. Similarly, 10-day treatment (20 × 2 s trains of 20 Hz stimulation with 58 s intervals) administered to patients with major depressive episodes significantly reduced scores in the Hamilton depression rating scale [141]. Exposure of post-stroke patients to 40 Hz, 7 mT ELF-MF 15 min/day for 4 weeks improved significantly cognitive functions and decreased up to 60% of depression syndromes [86]. As noted earlier, the possible explanation of beneficial effect of rTMS can be the ELF-MF-induced increase of mediators of corticosterone action in the hippocampus, i.e., neurotrophins, since these proteins appear to play a pivotal role in the structure and function of hippocampal neurons.

Behavioral effects of the ELF-MF depend on the length, frequency, and intensity of exposure, and on the initial balance of the brain transmitters [121,142]. Some authors noted reduced activity of animals after ELF-MF exposure, what is known as anxiety-like behavior. Others did not observe any changes following the exposure. Constant exposure to ELF-MF may also cause burdensome symptoms in humans, e.g., sleep disorders or depression. Notwithstanding, ELF-MF with high magnetic induction value (e.g., 7 mT—much higher than average exposure on a daily basis) appears to be effective in depression therapy. ELF-MF also improves cognitive functions in patients with a history of neurological injuries. However, again it should be noted that a considerable variety in the values of magnetic induction and exposure time were used in the research on ELF-MF effect.

6. Conclusions

Currently, people living in urbanized societies are exposed to the influence of various environmental stressors, including ELF-MF. The level of exposure can be different for individual groups and depends on the place of residence and occupation. The studies presented in this article indicate the possibility of changes at various levels of the organism organization as a result of exposure to ELF-MF. Effect of the field is observed in molecular and cellular responses, complex physiological processes such as activation of HPA axis and sympathetic system, as well as in behavioral and mood changes. As a result of exposure to

ELF-MF, the homeostasis is disturbed in a way that is similar to the effect of application of any other stressor. The effects of ELF-MF are associated with the occurrence of various ailments, such as anxiety and sleep or mood disorders, but this type of stimulation is also successfully used in the therapy of depressive disorders as an alternative for drug-resistant or post-stroke patients. There is considerable evidence that ELF-MF-induced processes include interplay between the monoaminergic system, glucocorticoids, and neurotrophins [143]. Therefore, it is conceivable that changes in any of these elements of stress response may ultimately lead to changes in brain function and can be reflected in behavior. The ELF-MF-evoked initial disruption in homeostasis triggers an overcompensation response to re-establish homeostasis, which results in a bidirectional effects at the subsequent stages of response. Recent findings have elucidated the cellular signaling pathways and molecular mechanisms that mediate the character of response, which typically involve free radicals, antioxidants, protein chaperones (e.g., Hsp70) and growth factors (e.g., BDNF), hormones (mainly corticosterone and noradrenaline), cytokines, and neurotransmitters. Despite many studies on bioeffects of ELF-MF exposure, the picture is still not clear and unambiguous. However, we can try to make some general conclusions.

Summarizing the observations cited above, when the organism is subjected to the influence of ELF-MF, typical reactions for stimulation with a stress factor can be observed; however, the changes can evolve in both directions: detrimental or beneficial. It is possible to hypothesize that, as in the case of other stressors, while the exposure to milder ELF-MF (lower intensity and shorter duration) could promote neural plasticity, the chronic stressful conditions (high intensity and long-term duration) could sensitize limbic circuits resulting in greater susceptibility to damage. Some existing studies suggest that ELF-MF of low density creates the weak stress response and improves the brain function [10,15,121], but the effects of high density ELF-MF are definitely stronger as far as stress systems activation and behavioral impairment are concerned [16,119,135]. According to Directive 2013/35/EU and ICNIRP, 2010, the ELF-MF of flux density below 1 mT does not cause any changes in the organism, the field in the range of 1–6 mT may induce some temporary changes in the functioning of the nervous system, but the consequence of higher values of ELF-MF flux density can be permanent. However, other factors such as temporal features of exposure or individual hypersensitivity [144] are also important and can determine the consequences of this kind of stress on the organism. The review of literature concerning the ELF-MF impact on the organism showed that biological effects are often discussed in relation to intensity (T), frequency (Hz) of the field, and duration of exposure. However, the quantification of the electromagnetic phenomena in the organism—dosimetry—is of high importance for proper determining ELF-MF effects. Establishing reliable and reproducible measurement procedures is required. Thus, experimental studies should include the detailed characterization of internal electromagnetic fields in addition to other parameters of ELF-MF exposure. Improvement in the process of validation of physical aspects related to ELF-MF exposure is necessary to achieve the reliable answers to questions concerning the effects of ELF-MF on organism.

Still, there is no answer to the question of where the threshold for ELF-MF exposure lies, above which the adaptive possibilities of the organism are exceeded and when the direction of ELF-MF-induced processes turn into pathology. A better understanding of effects of ELF-MF at the cellular, molecular, physiological, and behavioral levels fills the gap in our knowledge of ELF-MF effects on stress response of systems activity. It is important to recognize the risk concerning the effects of magnetic flux density of ELF-MF on development of stress-related and neurodegenerative disorders. Understanding the fundamental mechanisms of these differential responses in neurons will lead to a new approach in risk assessment of ELF-MF exposure. On the other hand, the property of ELF-MF supporting rehabilitation will be successfully used in the development of novel approaches for the prevention and treatment of many different diseases.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-3425/11/2/174/s1, Table S1: The influence of extremely low frequency magnetic field (ELF-MF) on different aspects of the organism function; http://doi.org/10.5281/zenodo.4457845.

Author Contributions: Visualization (schemes, tables, and graphical abstract) and writing—original draft preparation, A.K.; conceptualization, writing—original draft preparation, and supervision, J.R. Both authors have read and agreed to the published version of the manuscript

Funding: Project realized from NCS grant No. 2017/25/B/NZ7/00638 and POWER.03.05.00-00-Z302/17-00.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks). *Potential Health Effects of Exposure to Electromagnetic Fields (EMF)*; European Commission: Luxembourg, 2015; pp. 1–288.
2. Touitou, Y.; Selmaoui, B. The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system. *Dialogues Clin. Neurosci.* **2012**, *14*, 381–399. [CrossRef] [PubMed]
3. Giorgi, G.; Lecciso, M.; Capri, M.; Lukas Yani, S.; Virelli, A.; Bersani, F.; Del Re, B. An evaluation of genotoxicity in human neuronal-type cells subjected to oxidative stress under an extremely low frequency pulsed magnetic field. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2014**, *775*, 31–37. [CrossRef] [PubMed]
4. De Groot, M.W.; Kock, M.D.; Westerink, R.H. Assessment of the neurotoxic potential of exposure to 50Hz extremely low frequency electromagnetic fields (ELF-EMF) in naive and chemically stressed PC12 cells. *Neurotoxicology* **2014**, *44*, 358–364. [CrossRef] [PubMed]
5. Golbach, L.A.; Philippi, J.G.; Cuppen, J.J.; Savelkoul, H.F.; Verburg-van Kemenade, B.M. Calcium signalling in human neutrophil cell lines is not affected by low-frequency electromagnetic fields. *Bioelectromagnetics* **2015**, *36*, 430–443. [CrossRef] [PubMed]
6. Nakayama, M.; Nakamura, A.; Hondou, T.; Miyata, H. Evaluation of cell viability, DNA single-strand breaks, and nitric oxide production in LPS-stimulated macrophage RAW264 exposed to a 50-Hz magnetic field. *Int. J. Radiat. Biol.* **2016**, *92*, 583–589. [CrossRef]
7. Zhu, K.; Lv, Y.; Cheng, Q.; Hua, J.; Zeng, Q. Extremely Low Frequency Magnetic Fields Do Not Induce DNA Damage in Human Lens Epithelial Cells In Vitro. *Anat. Rec.* **2016**, *299*, 688–697. [CrossRef] [PubMed]
8. Burman, O.; Marsella, G.; Di Clemente, A.; Cervo, L. The effect of exposure to low frequency electromagnetic fields (EMF) as an integral part of the housing system on anxiety-related behaviour, cognition and welfare in two strains of laboratory mouse. *PLoS ONE* **2018**, *13*, e0197054. [CrossRef]
9. Baan, R.; Grosse, Y.; Lauby-Secretan, B.; El Ghissassi, F.; Bouvard, V.; Benbrahim-Tallaa, L.; Guha, N.; Islami, F.; Galichet, L.; Straif, K.; et al. Carcinogenicity of radiofrequency electromagnetic fields. *Lancet Oncol.* **2011**, *12*, 624–626. [CrossRef]
10. Szemerszky, R.; Zelena, D.; Barna, I.; Bárdos, G. Stress-related endocrinological and psychopathological effects of short- and long-term 50Hz electromagnetic field exposure in rats. *Brain Res. Bull.* **2010**, *81*, 92–99. [CrossRef]
11. Blank, M.; Goodman, R. Electromagnetic fields stress living cells. *Pathophysiology* **2009**, *16*, 71–78. [CrossRef]
12. Tasset, I.; Medina, F.J.; Jimena, I.; Agüera, E.; Gascón, F.; Feijóo, M.; Sánchez-López, F.; Luque, E.; Peña, J.; Drucker-Colín, R.; et al. Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: Effects on neurotrophic factors and neuronal density. *Neuroscience* **2012**, *209*, 54–63. [CrossRef] [PubMed]
13. George, M.S.; Lisanby, S.H.; Avery, D.; McDonald, W.M.; Durkalski, V.; Pavlicova, M.; Anderson, B.; Nahas, Z.; Bulow, P.; Zarkowski, P.; et al. Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: A sham-controlled randomized trial. *Arch. Gen. Psychiatry* **2010**, *67*, 507–516. [CrossRef] [PubMed]
14. Hosseinabadi, M.B.; Khanjani, N.; Ebrahimi, M.H.; Haji, B.; Abdolahfard, M. The effect of chronic exposure to extremely low-frequency electromagnetic fields on sleep quality, stress, depression and anxiety. *Electromagn. Biol. Med.* **2019**, *38*, 96–101. [CrossRef] [PubMed]
15. Sedghi, H.; Zare, S.; Hayatgeibi, H.; Alivandi, S.; Ebadi, A.G. Effects of 50 Hz Magnetic Field on Some Factors of Immune System in the Male Guinea Pigs. *Am. J. Immunol.* **2005**, *1*, 37–41. [CrossRef]
16. Kitaoka, K.; Kitamura, M.; Aoi, S.; Shimizu, N.; Yoshizaki, K. Chronic exposure to an extremely low-frequency magnetic field induces depression-like behavior and corticosterone secretion without enhancement of the hypothalamic-pituitary-adrenal axis in mice. *Bioelectromagnetics* **2013**, *34*, 43–51. [CrossRef]
17. Martínez-Sámano, J.; Flores-Poblano, A.; Verdugo-Díaz, L.; Juárez-Oropeza, M.A.; Torres-Durán, P.V. Extremely low frequency electromagnetic field exposure and restraint stress induce changes on the brain lipid profile of Wistar rats. *BMC Neurosci.* **2018**, *19*, 31. [CrossRef]

18. McEwen, B.S. Physiology and neurobiology of stress and adaptation: Central role of the brain. *Physiol. Rev.* **2007**, *87*, 873–904. [CrossRef] [PubMed]
19. Hajós-Korcsok, E.; Robinson, D.D.; Yu, J.H.; Fitch, C.S.; Walker, E.; Merchant, K.M. Rapid habituation of hippocampal serotonin and norepinephrine release and anxiety-related behaviors, but not plasma corticosterone levels, to repeated footshock stress in rats. *Pharmacol. Biochem. Behav.* **2003**, *74*, 609–616. [CrossRef]
20. Selye, H. *The Stress of Life*; McGraw-Hill: New York, NY, USA, 1956.
21. Selye, H. *Stress without Distress*; Springer: Boston, MA, USA, 1974.
22. Lazarus, R.S.; Folkman, S. *Stress, Appraisal, and Coping*; Springer: New York, NY, USA, 1984.
23. Calabrese, E.J.; Mattson, M.P. Hormesis provides a generalized quantitative estimate of biological plasticity. *J. Cell Commun. Signal.* **2011**, *5*, 25–38. [CrossRef]
24. Zimmermann, A.; Bauer, M.A.; Kroemer, G.; Madeo, F.; Carmona-Gutierrez, D. When less is more: Hormesis against stress and disease. *Microb. Cell* **2014**, *1*, 150–153. [CrossRef]
25. Krugers, H.J.; Karst, H.; Joels, M. Interactions between noradrenaline and corticosteroids in the brain: From electrical activity to cognitive performance. *Front. Cell Neurosci.* **2012**, *6*. [CrossRef] [PubMed]
26. Pierzchała-Koziec, K.; Zubel-Łojek, J.; Ocłoń, E.; Latacz, A.; Kępys, B. Emotional stress induces sex-specific sympatho-adrenomedullary responses in lambs. *Acta Biol. Crac. Série Zool.* **2015**, *57*, 39–45.
27. Tafet, G.E.; Bernardini, R. Psychoneuroendocrinological links between chronic stress and depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2003**, *27*, 893–903. [CrossRef]
28. Nesse, R.M.; Bhatnagar, S.; Young, E.A. Evolutionary Origins and Functions of the Stress Response. In *Stress: Concepts, Cognition, Emotion, and Behavior*; Fink, G., Ed.; Academic Press: London, UK, 2016; Volume 1, pp. 95–101.
29. Black, C.N.; Bot, M.; Révész, D.; Scheffer, P.G.; Penninx, B. The association between three major physiological stress systems and oxidative DNA and lipid damage. *Psychoneuroendocrinology* **2017**, *80*, 56–66. [CrossRef] [PubMed]
30. De Kloet, E.R.; Vreugdenhil, E.; Oitzl, M.S.; Joëls, M. Brain corticosteroid receptor balance in health and disease. *Endocr. Rev.* **1998**, *19*, 269–301. [CrossRef] [PubMed]
31. Rogalska, J. Mineralocorticoid and glucocorticoid receptors in hippocampus: Their impact on neurons survival and behavioral impairment after neonatal brain injury. *Vitam. Horm.* **2010**, *82*, 391–419. [CrossRef] [PubMed]
32. Arnett, M.G.; Muglia, L.M.; Laryea, G.; Muglia, L.J. Genetic Approaches to Hypothalamic-Pituitary-Adrenal Axis Regulation. *Neuropsychopharmacology* **2016**, *41*, 245–260. [CrossRef]
33. Mushak, P. Temporal stability of chemical hormesis (CH): Is CH just a temporary stop on the road to thresholds and toxic responses? *Sci. Total Environ.* **2016**, *569–570*, 1446–1456. [CrossRef]
34. Cuccurazzu, B.; Leone, L.; Podda, M.V.; Piacentini, R.; Riccardi, E.; Ripoli, C.; Azzena, G.B.; Grassi, C. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. *Exp. Neurol.* **2010**, *226*, 173–182. [CrossRef]
35. Komaki, A.; Khalili, A.; Salehi, I.; Shahidi, S.; Sarihi, A. Effects of exposure to an extremely low frequency electromagnetic field on hippocampal long-term potentiation in rat. *Brain Res.* **2014**, *1564*, 1–8. [CrossRef]
36. Johansson, O. Disturbance of the immune system by electromagnetic fields-A potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment. *Pathophysiology* **2009**, *16*, 157–177. [CrossRef] [PubMed]
37. Corallo, C.; Battisti, E.; Albanese, A.; Vannoni, D.; Leoncini, R.; Landi, G.; Gagliardi, A.; Landi, C.; Carta, S.; Nuti, R.; et al. Proteomics of human primary osteoarthritic chondrocytes exposed to extremely low-frequency electromagnetic fields (ELF EMFs) and to therapeutic application of musically modulated electromagnetic fields (TAMMEF). *Electromagn. Biol. Med.* **2014**, *33*, 3–10. [CrossRef] [PubMed]
38. Li, S.S.; Zhang, Z.Y.; Yang, C.J.; Lian, H.Y.; Cai, P. Gene expression and reproductive abilities of male Drosophila melanogaster subjected to ELF-EMF exposure. *Mutat. Res.* **2013**, *758*, 95–103. [CrossRef] [PubMed]
39. Merla, C.; Liberti, M.; Consales, C.; Denzi, A.; Apollonio, F.; Marino, C.; Benassi, B. Evidences of plasma membrane-mediated ROS generation upon ELF exposure in neuroblastoma cells supported by a computational multiscale approach. *Biochim. Biophys. Acta Biomembr.* **2019**, *1861*, 1446–1457. [CrossRef] [PubMed]
40. Tiwari, R.; Lakshmi, N.K.; Bhargava, S.C.; Ahuja, Y.R. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations. *Electromagn. Biol. Med.* **2015**, *34*, 56–62. [CrossRef]
41. Duan, W.; Liu, C.; Zhang, L.; He, M.; Xu, S.; Chen, C.; Pi, H.; Gao, P.; Zhang, Y.; Zhong, M.; et al. Comparison of the genotoxic effects induced by 50 Hz extremely low-frequency electromagnetic fields and 1800 MHz radiofrequency electromagnetic fields in GC-2 cells. *Radiat. Res.* **2015**, *183*, 305–314. [CrossRef]
42. Yin, C.; Luo, X.; Duan, Y.; Duan, W.; Zhang, H.; He, Y.; Sun, G.; Sun, X. Neuroprotective effects of lotus seedpod procyanidins on extremely low frequency electromagnetic field-induced neurotoxicity in primary cultured hippocampal neurons. *Biomed. Pharmacother.* **2016**, *82*, 628–639. [CrossRef]
43. Ferroni, L.; Tocco, I.; De Pieri, A.; Menarin, M.; Fermi, E.; Piattelli, A.; Gardin, C.; Zavan, B. Pulsed magnetic therapy increases osteogenic differentiation of mesenchymal stem cells only if they are pre-committed. *Life Sci.* **2016**, *152*, 44–51. [CrossRef]
44. Haghighat, N.; Abdolmaleki, P.; Parnian, J.; Behmanesh, M. The expression of pluripotency and neuronal differentiation markers under the influence of electromagnetic field and nitric oxide. *Mol. Cell Neurosci.* **2017**, *85*, 19–28. [CrossRef]

45. Emre, M.; Cetiner, S.; Zencir, S.; Unlukurt, I.; Kahraman, I.; Topcu, Z. Oxidative stress and apoptosis in relation to exposure to magnetic field. *Cell Biochem. Biophys.* **2011**, *59*, 71–77. [CrossRef]
46. Juszczak, K.; Kaszuba-Zwoinska, J.; Thor, P.J. Pulsating electromagnetic field stimulation of urothelial cells induces apoptosis and diminishes necrosis: New insight to magnetic therapy in urology. *J. Physiol. Pharmacol.* **2012**, *63*, 397–401. [PubMed]
47. Rezaei-Tavirani, M.; Hasanzadeh, H.; Seyyedi, S.; Ghoujeghi, F.; Semnani, V.; Zali, H. Proteomic Analysis of Extremely Low-Frequency ElectroMagnetic Field (ELF-EMF) with Different Intensities in Rats Hippocampus. *Arch. Neurosci.* **2018**, *5*, e62954. [CrossRef]
48. Yang, M.L.; Ye, Z.M. Extremely low frequency electromagnetic field induces apoptosis of osteosarcoma cells via oxidative stress. *J. Zhejiang Univ.* **2015**, *44*, 323–328. (In Chinese)
49. Liu, X.; Zuo, H.; Wang, D.; Peng, R.; Song, T.; Wang, S.; Xu, X.; Gao, Y.; Li, Y.; Wang, S.; et al. Improvement of spatial memory disorder and hippocampal damage by exposure to electromagnetic fields in an Alzheimer's disease rat model. *PLoS ONE* **2015**, *10*, e0126963. [CrossRef] [PubMed]
50. Wei, J.; Tong, J.; Yu, L.; Zhang, J. EMF protects cardiomyocytes against hypoxia-induced injury via heat shock protein 70 activation. *Chem. Biol. Interact.* **2016**, *248*, 8–17. [CrossRef] [PubMed]
51. Huang, Y.; Liu, Z.; Liu, W.; Yin, C.; Ci, L.; Zhao, R.; Yang, X. Short communication: Salivary haptoglobin and chromogranin A as non-invasive markers during restraint stress in pigs. *Res. Vet. Sci.* **2017**, *114*, 27–30. [CrossRef]
52. D'amico, M.A.; Ghinassi, B.; Izzicupo, P.; Manzoli, L.; Di Baldassarre, A. Biological function and clinical relevance of chromogranin A and derived peptides. *Endocr. Connect.* **2014**, *3*, R45–R54. [CrossRef]
53. Touitou, Y.; Lambrozo, J.; Mauvieux, B.; Riedel, M. Evaluation in humans of ELF-EMF exposure on chromogranin A, a marker of neuroendocrine tumors and stress. *Chronobiol. Int.* **2020**, *37*, 60–67. [CrossRef]
54. Corraliza-Gomez, M.; Sanchez, D.; Ganfornina, M.D. Lipid-Binding Proteins in Brain Health and Disease. *Front. Neurol.* **2019**, *10*, 1152. [CrossRef]
55. Martínez-Sámano, J.; Torres-Durán, P.V.; Juárez-Oropeza, M.A.; Verdugo-Díaz, L. Effect of acute extremely low frequency electromagnetic field exposure on the antioxidant status and lipid levels in rat brain. *Arch. Med. Res.* **2012**, *43*, 183–189. [CrossRef]
56. Karimi, S.A.; Salehi, I.; Shykhi, T.; Zare, S.; Komaki, A. Effects of exposure to extremely low-frequency electromagnetic fields on spatial and passive avoidance learning and memory, anxiety-like behavior and oxidative stress in male rats. *Behav. Brain Res.* **2019**, *359*, 630–638. [CrossRef] [PubMed]
57. Sun, Y.; Huang, X.; Wang, Y.; Shi, Z.; Liao, Y.; Cai, P. Lipidomic alteration and stress-defense mechanism of soil nematode Caenorhabditis elegans in response to extremely low-frequency electromagnetic field exposure. *Ecotoxicol. Environ. Saf.* **2019**, *170*, 611–619. [CrossRef] [PubMed]
58. Che, Y.; Zhou, Z.; Shu, Y.; Zhai, C.; Zhu, Y.; Gong, S.; Cui, Y.; Wang, J.F. Chronic unpredictable stress impairs endogenous antioxidant defense in rat brain. *Neurosci. Lett.* **2015**, *584*, 208–213. [CrossRef] [PubMed]
59. Pejic, S.; Stojikjkovic, V.; Todorovic, A.; Gavrilovic, L.; Pavlovic, I.; Popovic, N.; Pajovic, S.B. Antioxidant Enzymes in Brain Cortex of Rats Exposed to Acute, Chronic and Combined Stress. *Folia Biol.* **2016**, *64*, 189–195. [CrossRef]
60. Pizzino, G.; Irrera, N.; Cucinotta, M.; Pallio, G.; Mannino, F.; Arcoraci, V.; Squadrito, F.; Altavilla, D.; Bitto, A. Oxidative Stress: Harms and Benefits for Human Health. *Oxid. Med. Cell Longev.* **2017**, *2017*, 8416763. [CrossRef]
61. Srivastava, K.K.; Kumar, R. Stress, oxidative injury and disease. *Indian J. Clin. Biochem.* **2015**, *30*, 3–10. [CrossRef]
62. Frahm, J.; Mattsson, M.O.; Simkó, M. Exposure to ELF magnetic fields modulate redox related protein expression in mouse macrophages. *Toxicol. Lett.* **2010**, *192*, 330–336. [CrossRef]
63. Garip, A.I.; Akan, Z. Effect of ELF-EMF on number of apoptotic cells; correlation with reactive oxygen species and HSP. *Acta Biol. Hung.* **2010**, *61*, 158–167. [CrossRef]
64. Mannerling, A.C.; Simkó, M.; Mild, K.H.; Mattsson, M.O. Effects of 50-Hz magnetic field exposure on superoxide radical anion formation and HSP70 induction in human K562 cells. *Radiat. Environ. Biophys.* **2010**, *49*, 731–741. [CrossRef]
65. Vannoni, D.; Albanese, A.; Battisti, E.; Aceto, E.; Giglioni, S.; Corallo, C.; Carta, S.; Ferrata, P.; Fioravanti, A.; Giordano, N. In vitro exposure of human osteoarthritic chondrocytes to ELF fields and new therapeutic application of musically modulated electromagnetic fields: Biological evidence. *J. Biol. Regul. Homeost. Agents* **2012**, *26*, 39–49.
66. Calcabrini, C.; Mancini, U.; De Bellis, R.; Diaz, A.R.; Martinelli, M.; Cucchiarini, L.; Sestili, P.; Stocchi, V.; Potenza, L. Effect of extremely low-frequency electromagnetic fields on antioxidant activity in the human keratinocyte cell line NCTC 2544. *Biotechnol. Appl. Biochem.* **2017**, *64*, 415–422. [CrossRef] [PubMed]
67. Sun, Y.; Shi, Z.; Wang, Y.; Tang, C.; Liao, Y.; Yang, C.; Cai, P. Coupling of oxidative stress responses to tricarboxylic acid cycle and prostaglandin E2 alterations in Caenorhabditis elegans under extremely low-frequency electromagnetic field. *Int. J. Radiat. Biol.* **2018**, *94*, 1159–1166. [CrossRef] [PubMed]
68. Akdag, M.Z.; Dasdag, S.; Ulukaya, E.; Uzunlar, A.K.; Kurt, M.A.; Taşkin, A. Effects of extremely low-frequency magnetic field on caspase activities and oxidative stress values in rat brain. *Biol. Trace Elem. Res.* **2010**, *138*, 238–249. [CrossRef] [PubMed]
69. Goraca, A.; Ciejka, E.; Piechota, A. Effects of extremely low frequency magnetic field on the parameters of oxidative stress in heart. *J. Physiol. Pharmacol.* **2010**, *61*, 333–338. [PubMed]
70. Budziosz, J.; Stanek, A.; Sieroń, A.; Witkoś, J.; Cholewka, A.; Sieroń, K. Effects of Low-Frequency Electromagnetic Field on Oxidative Stress in Selected Structures of the Central Nervous System. *Oxid. Med. Cell Longev.* **2018**, *2018*, 1427412. [CrossRef] [PubMed]

71. Duan, Y.; Wang, Z.; Zhang, H.; He, Y.; Lu, R.; Zhang, R.; Sun, G.; Sun, X. The preventive effect of lotus seedpod procyanidins on cognitive impairment and oxidative damage induced by extremely low frequency electromagnetic field exposure. *Food Funct.* **2013**, *4*, 1252–1262. [CrossRef]
72. Luo, X.; Chen, M.; Duan, Y.; Duan, W.; Zhang, H.; He, Y.; Yin, C.; Sun, G.; Sun, X. Chemoprotective action of lotus seedpod procyanidins on oxidative stress in mice induced by extremely low-frequency electromagnetic field exposure. *Biomed. Pharmacother.* **2016**, *82*, 640–648. [CrossRef]
73. Djordjevic, N.Z.; Paunović, M.G.; Peulić, A.S. Anxiety-like behavioural effects of extremely low-frequency electromagnetic field in rats. *Environ. Sci. Pollut. Res. Int.* **2017**, *24*, 21693–21699. [CrossRef]
74. Martínez-Sámano, J.; Torres-Durán, P.V.; Juárez-Oropeza, M.A.; Elías-Viñas, D.; Verdugo-Díaz, L. Effects of acute electromagnetic field exposure and movement restraint on antioxidant system in liver, heart, kidney and plasma of Wistar rats: A preliminary report. *Int. J. Radiat. Biol.* **2010**, *86*, 1088–1094. [CrossRef]
75. Kuzay, D.; Ozer, C.; Sirav, B.; Canseven, A.G.; Seyhan, N. Oxidative effects of extremely low frequency magnetic field and radio frequency radiation on testes tissues of diabetic and healthy rats. *Bratisl. Lek. Listy* **2017**, *118*, 278–282. [CrossRef]
76. El-Helaly, M.; Abu-Hashem, E. Oxidative stress, melatonin level, and sleep insufficiency among electronic equipment repairers. *Indian J. Occup. Environ. Med.* **2010**, *14*, 66–70. [CrossRef] [PubMed]
77. Zhang, Y.; Zhang, D.; Zhu, B.; Zhang, H.; Sun, Y.; Sun, C. Effects of dietary green tea polyphenol supplementation on the health of workers exposed to high-voltage power lines. *Environ. Toxicol. Pharmacol.* **2016**, *46*, 183–187. [CrossRef] [PubMed]
78. Hosseinabadi, M.B.; Khanjani, N. The Effect of Extremely Low-Frequency Electromagnetic Fields on the Prevalence of Musculoskeletal Disorders and the Role of Oxidative Stress. *Bioelectromagnetics* **2019**, *40*, 354–360. [CrossRef] [PubMed]
79. Morabito, C.; Rovetta, F.; Bizzarri, M.; Mazzoleni, G.; Fanò, G.; Mariggiò, M.A. Modulation of redox status and calcium handling by extremely low frequency electromagnetic fields in C2C12 muscle cells: A real-time, single-cell approach. *Free Radic. Biol. Med.* **2010**, *48*, 579–589. [CrossRef] [PubMed]
80. Ehnert, S.; Fentz, A.K.; Schreiner, A.; Birk, J.; Wilbrand, B.; Ziegler, P.; Reumann, M.K.; Wang, H.; Falldorf, K.; Nussler, A.K. Extremely low frequency pulsed electromagnetic fields cause antioxidative defense mechanisms in human osteoblasts via induction of $\bullet O^{2-}$ and H_2O_2. *Sci. Rep.* **2017**, *7*, 14544. [CrossRef] [PubMed]
81. Patruno, A.; Tabrez, S.; Pesce, M.; Shakil, S.; Kamal, M.A.; Reale, M. Effects of extremely low frequency electromagnetic field (ELF-EMF) on catalase, cytochrome P450 and nitric oxide synthase in erythro-leukemic cells. *Life Sci.* **2015**, *121*, 117–123. [CrossRef]
82. Lewicka, M.; Henrykowska, G.A.; Pacholski, K.; Szczęsny, A.; Dziedziczak-Buczyńska, M.; Buczyński, A. The impact of electromagnetic radiation of different parameters on platelet oxygen metabolism—In vitro studies. *Adv. Clin. Exp. Med.* **2015**, *24*, 31–35. [CrossRef]
83. Song, K.; Im, S.H.; Yoon, Y.J.; Kim, H.M.; Lee, H.J.; Park, G.S. A 60 Hz uniform electromagnetic field promotes human cell proliferation by decreasing intracellular reactive oxygen species levels. *PLoS ONE* **2018**, *13*, e0199753. [CrossRef]
84. Reale, M.; Kamal, M.A.; Patruno, A.; Costantini, E.; D'Angelo, C.; Pesce, M.; Greig, N.H. Neuronal cellular responses to extremely low frequency electromagnetic field exposure: Implications regarding oxidative stress and neurodegeneration. *PLoS ONE* **2014**, *9*, e104973. [CrossRef]
85. Falone, S.; Marchesi, N.; Osera, C.; Fassina, L.; Comincini, S.; Amadio, M.; Pascale, A. Pulsed electromagnetic field (PEMF) prevents pro-oxidant effects of H2O2 in SK-N-BE(2) human neuroblastoma cells. *Int. J. Radiat. Biol.* **2016**, *92*, 281–286. [CrossRef]
86. Cichoń, N.; Bijak, M.; Miller, E.; Saluk, J. Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients. *Bioelectromagnetics* **2017**, *38*, 386–396. [CrossRef] [PubMed]
87. Cichoń, N.; Rzeźnicka, P.; Bijak, M.; Miller, E.; Miller, S.; Saluk, J. Extremely low frequency electromagnetic field reduces oxidative stress during the rehabilitation of post-acute stroke patients. *Adv. Clin. Exp. Med.* **2018**, *27*, 1285–1293. [CrossRef] [PubMed]
88. Fulda, S.; Gorman, A.M.; Hori, O.; Samali, A. Cellular Stress Responses: Cell Survival and Cell Death. *Int. J. Cell Biol.* **2010**. [CrossRef] [PubMed]
89. Soleimani, A.F.; Zulkifli, I.; Omar, A.R.; Raha, A.R. The relationship between adrenocortical function and Hsp70 expression in socially isolated Japanese quail. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2012**, *161*, 140–144. [CrossRef] [PubMed]
90. Cirulli, F.; Alleva, E. The NGF saga: From animal models of psychosocial stress to stress-related psychopathology. *Front. Neuroendocrinol.* **2009**, *30*, 379–395. [CrossRef] [PubMed]
91. Hagena, H.; Hansen, N.; Manahan-Vaughan, D. β-Adrenergic Control of Hippocampal Function: Subserving the Choreography of Synaptic Information Storage and Memory. *Cereb. Cortex* **2016**, *26*, 1349–1364. [CrossRef]
92. Amaroli, A.; Chessa, M.G.; Bavestrello, G.; Bianco, B. Effects of an extremely low-frequency electromagnetic field on stress factors: A study in Dictyostelium discoideum cells. *Eur. J. Protistol.* **2013**, *49*, 400–405. [CrossRef]
93. Wyszkowska, J.; Shepherd, S.; Sharkh, S.; Jackson, C.W.; Newland, P.L. Exposure to extremely low frequency electromagnetic fields alters the behaviour, physiology and stress protein levels of desert locusts. *Sci. Rep.* **2016**, *6*, 36413. [CrossRef]
94. Zeni, O.; Simkó, M.; Scarfi, M.R.; Mattsson, M.O. Cellular Response to ELF-MF and Heat: Evidence for a Common Involvement of Heat Shock Proteins? *Front. Public Health* **2017**, *5*, 280. [CrossRef]
95. Sharma, D.; Masison, D.C. Hsp70 structure, function, regulation and influence on yeast prions. *Protein Pept. Lett.* **2009**, *16*, 571–581. [CrossRef]

96. Perez, F.P.; Zhou, X.; Morisaki, J.; Jurivich, D. Electromagnetic field therapy delays cellular senescence and death by enhancement of the heat shock response. *Exp. Gerontol.* **2008**, *43*, 307–316. [CrossRef] [PubMed]
97. Gutzeit, H.O. Biological Effects of ELF-EMF Enhanced Stress Response: New Insights and New Questions. *Electro Magn.* **2001**, *20*, 15–26. [CrossRef]
98. Goodman, R.; Lin-Ye, A.; Geddis, M.S.; Wickramaratne, P.J.; Hodge, S.E.; Pantazatos, S.P.; Blank, M.; Ambron, R.T. Extremely low frequency electromagnetic fields activate the ERK cascade, increase hsp70 protein levels and promote regeneration in Planaria. *Int. J. Radiat. Biol.* **2009**, *85*, 851–859. [CrossRef] [PubMed]
99. Miranda, M.; Morici, J.F.; Zanoni, M.B.; Bekinschtein, P. Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. *Front. Cell Neurosci.* **2019**, *13*, 363. [CrossRef]
100. Bathina, S.; Das, U.N. Brain-derived neurotrophic factor and its clinical implications. *Arch. Med. Sci.* **2015**, *11*, 1164–1178. [CrossRef] [PubMed]
101. Li, Y.; Yan, X.; Liu, J.; Li, L.; Hu, X.; Sun, H.; Tian, J. Pulsed electromagnetic field enhances brain-derived neurotrophic factor expression through L-type voltage-gated calcium channel- and Erk-dependent signaling pathways in neonatal rat dorsal root ganglion neurons. *Neurochem. Int.* **2014**, *75*, 96–104. [CrossRef]
102. Cichoń, N.; Bijak, M.; Czarny, P.; Miller, E.; Synowiec, E.; Sliwinski, T.; Saluk-Bijak, J. Increase in Blood Levels of Growth Factors Involved in the Neuroplasticity Process by Using an Extremely Low Frequency Electromagnetic Field in Post-stroke Patients. *Front. Aging Neurosci.* **2018**, *10*, 294. [CrossRef]
103. Urnukhsaikhan, E.; Mishig-Ochir, T.; Kim, S.C.; Park, J.K.; Seo, Y.K. Neuroprotective Effect of Low Frequency-Pulsed Electromagnetic Fields in Ischemic Stroke. *Appl. Biochem. Biotechnol.* **2017**, *181*, 1360–1371. [CrossRef]
104. Catterall, W.A.; Few, A.P. Calcium channel regulation and presynaptic plasticity. *Neuron* **2008**, *59*, 882–901. [CrossRef]
105. Ardeshirylajimi, A.; Soleimani, M. Enhanced growth and osteogenic differentiation of Induced Pluripotent Stem cells by Extremely Low-Frequency Electromagnetic Field. *Cell Mol. Biol.* **2015**, *61*, 36–41.
106. Grassi, C.; D'Ascenzo, M.; Torsello, A.; Martinotti, G.; Wolf, F.; Cittadini, A.; Azzena, G.B. Effects of 50 Hz electromagnetic fields on voltage-gated Ca2+ channels and their role in modulation of neuroendocrine cell proliferation and death. *Cell Calcium* **2004**, *35*, 307–315. [CrossRef] [PubMed]
107. Luo, F.L.; Yang, N.; He, C.; Li, H.L.; Li, C.; Chen, F.; Xiong, J.X.; Hu, Z.A.; Zhang, J. Exposure to extremely low frequency electromagnetic fields alters the calcium dynamics of cultured entorhinal cortex neurons. *Environ. Res.* **2014**, *135*, 236–246. [CrossRef] [PubMed]
108. Sun, Z.C.; Ge, J.L.; Guo, B.; Guo, J.; Hao, M.; Wu, Y.C.; Lin, Y.A.; La, T.; Yao, P.T.; Mei, Y.A.; et al. Extremely Low Frequency Electromagnetic Fields Facilitate Vesicle Endocytosis by Increasing Presynaptic Calcium Channel Expression at a Central Synapse. *Sci. Rep.* **2016**, *6*, 21774. [CrossRef] [PubMed]
109. Zhao, Q.R.; Lu, J.M.; Yao, J.J.; Zhang, Z.Y.; Ling, C.; Mei, Y.A. Neuritin reverses deficits in murine novel object associative recognition memory caused by exposure to extremely low-frequency (50 Hz) electromagnetic fields. *Sci. Rep.* **2015**, *5*, 11768. [CrossRef]
110. Yang, Y.; Li, L.; Wang, Y.G.; Fei, Z.; Zhong, J.; Wei, L.Z.; Long, Q.F.; Liu, W.P. Acute neuroprotective effects of extremely low-frequency electromagnetic fields after traumatic brain injury in rats. *Neurosci. Lett.* **2012**, *516*, 15–20. [CrossRef]
111. Sakhaie, M.H.; Soleimani, M.; Pourheydar, B.; Majd, Z.; Atefimanesh, P.; Asl, S.S.; Mehdizadeh, M. Effects of Extremely Low-Frequency Electromagnetic Fields on Neurogenesis and Cognitive Behavior in an Experimental Model of Hippocampal Injury. *Behav. Neurol.* **2017**, *2017*, 9194261. [CrossRef]
112. Collard, J.F.; Lazar, C.; Nowé, A.; Hinsenkamp, M. Statistical validation of the acceleration of the differentiation at the expense of the proliferation in human epidermal cells exposed to extremely low frequency electric fields. *Prog. Biophys. Mol. Biol.* **2013**, *111*, 37–45. [CrossRef]
113. Di Loreto, S.; Falone, S.; Caracciolo, V.; Sebastiani, P.; D'Alessandro, A.; Mirabilio, A.; Zimmitti, V.; Amicarelli, F. Fifty hertz extremely low-frequency magnetic field exposure elicits redox and trophic response in rat-cortical neurons. *J. Cell Physiol.* **2009**, *219*, 334–343. [CrossRef]
114. Fathi, E.; Farahzadi, R. Zinc Sulphate Mediates the Stimulation of Cell Proliferation of Rat Adipose Tissue-Derived Mesenchymal Stem Cells Under High Intensity of EMF Exposure. *Biol. Trace Elem. Res.* **2018**, *184*, 529–535. [CrossRef]
115. Chung, Y.H.; Lee, Y.J.; Lee, H.S.; Chung, S.J.; Lim, C.H.; Oh, K.W.; Sohn, U.D.; Park, E.S.; Jeong, J.H. Extremely low frequency magnetic field modulates the level of neurotransmitters. *Korean J. Physiol. Pharmacol.* **2015**, *19*, 15–20. [CrossRef]
116. Sieroń, A.; Labus, Ł.; Nowak, P.; Cieślar, G.; Brus, H.; Durczok, A.; Zagził, T.; Kostrzewa, R.M.; Brus, R. Alternating extremely low frequency magnetic field increases turnover of dopamine and serotonin in rat frontal cortex. *Bioelectromagnetics* **2004**, *25*, 426–430. [CrossRef] [PubMed]
117. Isogawa, K.; Fujiki, M.; Akiyoshi, J.; Tsutsumi, T.; Horinouchi, Y.; Kodama, K.; Nagayama, H. Anxiety induced by repetitive transcranial magnetic stimulation is suppressed by chronic treatment of paroxetine in rats. *Pharmacopsychiatry* **2003**, *36*, 7–11. [CrossRef] [PubMed]
118. Janać, B.; Tovilović, G.; Tomić, M.; Prolić, Z.; Radenović, L. Effect of continuous exposure to alternating magnetic field (50 Hz, 0.5 mT) on serotonin and dopamine receptors activity in rat brain. *Gen. Physiol. Biophys.* **2009**, *28*, 41–46. [PubMed]
119. Jadidi, M.; Firoozabadi, S.M.; Rashidy-Pour, A.; Sajadi, A.A.; Sadeghi, H.; Taherian, A.A. Acute exposure to a 50 Hz magnetic field impairs consolidation of spatial memory in rats. *Neurobiol. Learn. Mem.* **2007**, *88*, 387–392. [CrossRef]

120. Kirschenlohr, H.; Ellis, P.; Hesketh, R.; Metcalfe, J. Gene expression profiles in white blood cells of volunteers exposed to a 50 Hz electromagnetic field. *Radiat. Res.* **2012**, *178*, 138–149. [CrossRef]
121. Mahdavi, S.M.; Sahraei, H.; Yaghmaei, P.; Tavakoli, H. Effects of electromagnetic radiation exposure on stress-related behaviors and stress hormones in male wistar rats. *Biomol. Ther.* **2014**, *22*, 570–576. [CrossRef]
122. Hosseini, E.; Nafisi, S.; Zare, S. The effects of electromagnetic fields on plasma levels of corticosterone, free-T3, free-T4 malonyl-dialdehyde in white male rabbit with normal diet and hyperchlostrol diet. *Vet. Res. Forum* **2011**, *2*, 222–225.
123. Afhami, M.; Bahaoddini, A.; Saadat, M. P20: Investigation the Effect of EMF on Plasma Levels of Corticosterone, Testosterone and Testicular Gene Expression of Gstt1 of Male Rats. *Shefaye Khatam* **2016**, *4*, 43.
124. de Kleijn, S.; Ferwerda, G.; Wiese, M.; Trentelman, J.; Cuppen, J.; Kozicz, T.; de Jager, L.; Hermans, P.W.; Verburg-van Kemenade, B.M. A short-term extremely low frequency electromagnetic field exposure increases circulating leukocyte numbers and affects HPA-axis signaling in mice. *Bioelectromagnetics* **2016**, *37*, 433–443. [CrossRef]
125. Mostafa, R.M.; Mostafa, Y.M.; Ennaceur, A. Effects of exposure to extremely low-frequency magnetic field of 2 G intensity on memory and corticosterone level in rats. *Physiol. Behav.* **2002**, *76*, 589–595. [CrossRef]
126. Laszlo, A.M.; Ladanyi, M.; Boda, K.; Csicsman, J.; Bari, F.; Serester, A.; Molnar, Z.; Sepp, K.; Galfi, M.; Radacs, M. Effects of extremely low frequency electromagnetic fields on turkeys. *Poult. Sci.* **2018**, *97*, 634–642. [CrossRef] [PubMed]
127. Tian, R.; Hou, G.; Li, D.; Yuan, T.F. A possible change process of inflammatory cytokines in the prolonged chronic stress and its ultimate implications for health. *Sci. World J.* **2014**, *2014*, 780616. [CrossRef] [PubMed]
128. Silverman, M.N.; Pearce, B.D.; Biron, C.A.; Miller, A.H. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. *Viral. Immunol.* **2005**, *18*, 41–78. [CrossRef] [PubMed]
129. Wyszkowska, J.; Jędrzejewski, T.; Piotrowski, J.; Wojciechowska, A.; Stankiewicz, M.; Kozak, W. Evaluation of the influence of in vivo exposure to extremely low-frequency magnetic fields on the plasma levels of pro-inflammatory cytokines in rats. *Int. J. Radiat. Biol.* **2018**, *94*, 909–917. [CrossRef] [PubMed]
130. Cichoń, N.; Saluk-Bijak, J.; Miller, E.; Sliwinski, T.; Synowiec, E.; Wigner, P.; Bijak, M. Evaluation of the effects of extremely low frequency electromagnetic field on the levels of some inflammatory cytokines in post-stroke patients. *J. Rehabil. Med.* **2019**, *51*, 854–860. [CrossRef] [PubMed]
131. Mahaki, H.; Jabarivasal, N.; Sardanian, K.; Zamani, A. Effects of Various Densities of 50 Hz Electromagnetic Field on Serum IL-9, IL-10, and TNF-α Levels. *Int. J. Occup. Environ. Med.* **2020**, *11*, 24–32. [CrossRef]
132. Sterner, E.Y.; Kalynchuk, L.E. Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: Relevance to depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2010**, *34*, 777–790. [CrossRef]
133. Liu, T.; Wang, S.; He, L.; Ye, K. Anxiogenic effect of chronic exposure to extremely low frequency magnetic field in adult rats. *Neurosci. Lett.* **2008**, *434*, 12–17. [CrossRef]
134. Alsaeed, I.; Al-Somali, F.; Sakhnini, L.; Aljarallah, O.S.; Hamdan, R.M.; Bubishate, S.A.; Sarfaraz, Z.K.; Kamal, A. Autism-relevant social abnormalities in mice exposed perinatally to extremely low frequency electromagnetic fields. *Int. J. Dev. Neurosci.* **2014**, *37*, 58–64. [CrossRef]
135. Korpinar, M.A.; Kalkan, M.T.; Tuncel, H. The 50 Hz (10 mT) sinusoidal magnetic field: Effects on stress-related behavior of rats. *Bratisl. Lek. Listy* **2012**, *113*, 521–524. [CrossRef]
136. Balassa, T.; Szemerszky, R.; Bárdos, G. Effect of short-term 50 Hz electromagnetic field exposure on the behavior of rats. *Acta Physiol. Hung.* **2009**, *96*, 437–448. [CrossRef] [PubMed]
137. He, L.H.; Shi, H.M.; Liu, T.T.; Xu, Y.C.; Ye, K.P.; Wang, S. Effects of extremely low frequency magnetic field on anxiety level and spatial memory of adult rats. *Chin. Med. J.* **2011**, *124*, 3362–3366. [PubMed]
138. Mattar, F.A.; Bareedy, M.H.; El-Dosouky, M.E.M.; Zaghloul, M.S.; Mahmod, M.S.M. Effects of 6 Weeks Exposure of 3.5 mT (ELF EMF) on Some Animal behaviors in White Albino Rat (Sprague Dawley) Pups. *Zagazig Vet. J.* **2014**, *42*, 74–80. [CrossRef]
139. Lai, J.; Zhang, Y.; Liu, X.; Zhang, J.; Ruan, G.; Chaugai, S.; Chen, C.; Wang, D.W. Effects of extremely low frequency electromagnetic fields (100μT) on behaviors in rats. *Neurotoxicology* **2016**, *52*, 104–113. [CrossRef] [PubMed]
140. Boscolo, P.; Di Giampaolo, L.; Di Donato, A.; Antonucci, A.; Paiardini, G.; Morelli, S.; Vasile, R.; Spagnoli, G.; Reale, M.; Dadorante, V.; et al. The immune response of women with prolonged exposure to electromagnetic fields produced by radiotelevision broadcasting stations. *Int. J. Immunopathol. Pharmacol.* **2006**, *19* (Suppl. 4), 43–48.
141. Berman, R.M.; Narasimhan, M.; Sanacora, G.; Miano, A.P.; Hoffman, R.E.; Hu, X.S.; Charney, D.S.; Boutros, N.N. A randomized clinical trial of repetitive transcranial magnetic stimulation in the treatment of major depression. *Biol. Psychiatry* **2000**, *47*, 332–337. [CrossRef]
142. Janać, B.; Pesić, V.; Jelenković, A.; Vorobyov, V.; Prolić, Z. Different effects of chronic exposure to ELF magnetic field on spontaneous and amphetamine-induced locomotor and stereotypic activities in rats. *Brain Res. Bull.* **2005**, *67*, 498–503. [CrossRef]
143. Roszkowski, M.; Manuella, F.; von Ziegler, L.; Durán-Pacheco, G.; Moreau, J.L.; Mansuy, I.M.; Bohacek, J. Rapid stress-induced transcriptomic changes in the brain depend on beta-adrenergic signaling. *Neuropharmacology* **2016**, *107*, 329–338. [CrossRef]
144. Genuis, S.J.; Lipp, C.T. Electromagnetic hypersensitivity: Fact or fiction? *Sci. Total Environ.* **2012**, *414*, 103–112. [CrossRef]

Table S1. The influence of extremely low frequency magnetic field (ELF-MF) on different aspects of the organism function (papers are listed according to year of publication).

In order to prepare the table the bibliography research in PubMed was performed using the following keywords in varied combinations:: “electromagnetic field”, “stress”, “corticosterone“, “noradrenaline”, “HPA axis”, “oxidative stress”, “cellular damage”, “BDNF”, “HSP70”, “neurotransmitters”, “cytokines”, “plasticity”, “viability”, “recovery”; “behavior”. We also used Boolean operator “and” to receive the most relevant search results. All articles written in English were manually screened, and the appropriate were identified. The articles published from 2010 to 2020 were taken into consideration. The papers concerning the effects of extremely low frequency magnetic field on a wide spectrum of molecular, neuronal, hormonal and behavioural stress responses were considered and only information related to ELF-MF effects are included in the Table.

	References/ Area of interest	**Model**	**Parameters of exposure to ELF-MF**	**Procedure**	**Effects**
1.	Akdag et al., 2010 **Oxidative stress** **Cell death**	rat (males, 4 months old, n= 10)	50 Hz, 100 µT/500 µT	1.Rats were exposed to ELF-MF 2 h/day for 10 months. 2.Parameters assessed: after the last exposure -active caspase-3 - apoptotic index -MDA, TOS, OSI, MPO -CAT, TAC	•active caspase-3 and MPO activity not changed •CAT activity ↑ •OSI, TOS, MDA only after exposure to 500 µT ↑ •TAC was lower only after exposure to 500 µT ↓
2.	Cuccurazzu et al., 2010 **Calcium,** **Proliferation** **Proteins** **Genes** **Neuroplasticity**	C57BL/6 mouse (males, adult, exposure n= 38)	50 Hz, 1 mT	1.Mice were exposed (1) 7 h/day for 4 days, (2) 1 h/day, 3 h/day, 7 h/day for 7 days, (3) 7 h/day for 7 days. 2.Parameters assessed: 7h/day for 4 days: -expression of pro-neuronal genes (*Mash1, NeuroD2, Hes1*); Expression of gene encoding Ca_v1- calcium channel subunits, Expression of proteins (NeuroD1, NeuroD2, Ca_v1 channels) 1 h/day, 3h /day, 7h /day for 7 days: -Cell proliferation, Immune reactivity 7 h/day for 7 days: -LTP	•gene expression of *Mash1, NeuroD2, Hes1* and gene encoding Ca_v1 calcium channels↑ •protein expression of NeuroD1, NeuroD2 and Ca_v1 ↑ •neurogenesis ↑ •newly generated immature neurons had survived and became mature dentate gyrus granule cells •LTP ↑
3.	El-Helaly and Abu-Hashem, 2010 **Oxidative stress** **Behaviour**	human (mean age 36.88 years old, n= 50)	50 Hz/60 Hz 0.06 µT-0.86 µT,	1.Workers chronically exposed to ELF-MF (mean employment duration 9-12 years) 2.Parameters assessed: -sleep sufficiency -plasma melatonin level -MDA level	•melatonin ↓ •MDA ↑ •sleep insufficiency ↑

4.	Frahm et al., 2010 **Oxidative stress** **Proteins**	mouse macrophages from bone marrow	50 Hz, 1 mT	1.Cells were exposed to ELF-MF from 5 min to 24 h. 2.Parameters assessed: -reactive oxygen species after 5 , 15, 30 and 45 min of ELF-MF exposure. -regulatory proteins and proteins involved in the response to oxidative stress levels (gp91phox, clathrin and adaptin) after 15, 30, 45 min and 1, 2, 4 or 24 h of exposure -the levels of PI3K, PKB and PP2A after 5, 10, 15, 30, 45 min and 1 or 24 h of exposure.	•ROS production ↑ •clathrin, adaptin, PI3K, PKB, PP2A ↓ •not changed or slightly increased level of gp91phox after short term exposures (< 2h)
5.	Garip and Akan, 2010 **Oxidative stress** **Cell death** **Viability** **Proteins**	K562 human leukemia cell line	50 Hz, 1 mT	1.Experimental groups: -cells treated with H_2O_2 to induce apoptosis -cells exposed to ELF-MF for 3 h -cells H_2O_2 treated and exposed to ELF-MF for 3 h 2. Parameters assessed: - number of apoptotic cells, - ROS and Hsp70	•ELF-MF exposure alone ↓ in number of apoptotic cells. •in H_2O_2 treated cells ELF-MF significantly enhanced apoptosis level •viability of ELF-MF exposed cells not changed •H2O2-induced decreased cells viability not altered by ELF-MF. •ROS level ↑ in all ELF-MF exposed groups •level of Hsp70 ↑ in all ELF-MF exposed groups
6.	George et al., 2010 **Behaviour**	depressive patients (mean age 47,7 years old, n= 92)	10 Hz, 120% of motor threshold 3000 stimuli per session	1.Patients subjected to 3-week treatment with rTMS (40 min/day in a 5-day sequence- 15 sessions). 2.Parameters assessed: -Hamilton Scale for Depression score	•Hamilton Scale for Depression score ↑ •the odds of attaining remission ↑
7.	Goraca et al., 2010 **Oxidative stress**	rat (males, 2-3 months old, n= 7)	40 Hz, 7 mT	1. Rats were exposed to ELF-MF 30 min/day or 60 min/day for 14 days. 2. Parameters assessed (after the last exposure) - TAC level in plasma - TBARS, H_2O_2, total free sulphydryl groups and GSH concentrations in heart tissue.	•ELF-MF exposure 30 min/day – no effect •ELF-MF exposure 60 min/day: -TBARS and H_2O_2 concentrations ↑ -GSH, total free -SH groups, TAC level ↓
8.	Mannerling et al., 2010 **Oxidative stress** **Proliferation** **Viability** **Proteins**	K562 human leukaemia cell line	50 Hz, 0.025 mT/0.05 mT/ 0.1 mT	1.Cells were exposed to ELF-MF of 0.1 mT for 1 h or to heat shock (42ºC) (positive control), then incubated 24 h. Parameters assessed: proliferation, viability and cell cycle distribution 2.Cells were exposed to ELF-MF of several flux densities	•0.1 mT ELF-MF no effect on proliferation or cell cycle •Hsp70 expression after ELF-MF at several flux densities ↑ •free radical release ↑ at each flux density

				Parameter assessed: -Hsp70 level and superoxide anion radical immediately, 6, 12 and 24 h after exposure.	
9.	Martínez-Sámano et al., 2010 **Oxidative stress**	rat (males, adult, n= 6)	60 Hz, 2.4 mT	1.Rats (restrained and unrestrained) were exposed to ELF-MF for 2 h. 2.Parameters assessed: -GSH, CAT, SOD and TBARS in liver, heart, kidney and plasma immediately after exposure	•CAT activity and TBARS levels not changed in all groups •SOD activity ↓ in plasma unrestrained ELF-MF exposed group and unchanged in the restrained group exposed to ELF-MF •GSH concentration ↓in unrestrained exposed group in heart, kidney and plasma and in restrained group exposed to ELF-MF in heart and liver
10.	Morabito et al, 2010 **Oxidative stress** **Calcium**	C2C12 cells (myoblasts)	50 Hz, 0,1 mT/1 mT	1.Cells were exposed to ELF-MF for 30 min. 2.Parameters assessed: -reactive oxygen species (intracellular superoxide anion, hydrogen peroxide) -TAS, activity of antioxidant enzymes -cell damage markers (protein carbonyl content, MDA); -mitochondrial membrane potential -Ca^{2+} signaling	•0.1 mT ELF-MF no effect on ROS production •1 mT: - superoxide anion level not changed; hydrogen peroxide and TAS ↑ -MDA and protein carbonyl content not changed -activity of GPx ↑ -activity of CAT ↑ •basal intracellular Ca^{2+} after 0.1 and 1 mT exposure ↑
11.	Szemerszky et al., 2010 **Behaviour** **Stress hormones**	rat (males, adult, n= 8)	50 Hz, 0.5 mT	1.Rats were subjected into short term (8 h/day for 5 days) exposure to ELF-MF. Parameters assessed: -elevated plus maze (48 h after exposure) -ACTH, POMC and CORT (48 h after exposure and 2 days later) 2.Rats were subjected into long term (24 h/day for 6 weeks) exposure to ELF-MF. Parameters assessed: -forced swim test (in the 4th week) -week elevated plus maze (in the 6th test) -ACTH, POMC and CORT (in the 6th test)	•elevated plus maze behaviour – not changed •helpless behaviour ↑ •POMC ↑ (long term exposure) •ACTH and CORT levels – not changed
12.	Emre et al., 2011 **Oxidative stress** **Cell death**	rat (males, adult, n= 10)	1 Hz/10 Hz/ 20 Hz/40 Hz, 1.5 mT pulsed ELF-MF	1.Rats were exposed to ELF-MF of frequencies 1 Hz, 10 Hz, 20 Hz and 40 Hz in sequence with 4-min and 1-min intervals between each frequency 1 h/days for 30 days. 2.Parameters assessed (after the last exposure):	•ALT, AST and ALP activities as well as albumin, bilirubin and total protein levels ↑ •MDA concentration and SOD activity were increased ↑ •necrotic cell ↓

	Proteins			-ALT, AST,ALP activities, albumin, bilirubin and total protein levels in serum. -MDA concentration and SOD activity in liver -apoptotic and necrotic cells	•apoptotic cells ↑
13.	He et al., 2011 **Behaviour**	rat (males, adult, n= 10)	50 Hz, 2 mT	1.Rats were exposed to ELF-MF for 1 h or 4 h per day for 4 weeks. 2. Parameters assessed : - behaviour in open field test, elevated plus maze and Morris water maze after last exposure	4-weeks exposure to ELF-MF for 4 h/day: •anxiety- like behaviours ↑ •latency to find hidden platform in Morris water maze ↓ •long-term memory of former location of platform ↑ •short-term memory and locomotor activity – not changed
14.	Hosseini et al., 2011 **Oxidative stress Stress hormones**	rabbit (males, adult, n= 8)	10 Hz pulsed ELF-MF	1.Rabbits with normal and high-cholesterol diet were exposed to ELF-MF 2 h/day for 5 days. 2.Parameters assessed (12 h after the last exposure) -plasma levels of CORT, free-T3, free-T4 and MDA	•CORT, free-T3 and free-T4 in exposed rabbits with normal and high-cholesterol diet ↑ •MDA in exposed rabbits with high-cholesterol diet ↓
15.	Juszczak et al., 2012 **Cell death**	rat urothelial cultured cells	50 Hz, 45 mT pulsed ELF-MF	1.Urothelial cells were exposed to pulsating ELF-MF three times for 4 h with 24-h intervals. 2. Parameters assessed: -apoptotic and necrotic cells after exposure	•apoptosis ↑ •necrosis ↓
16.	Kirschenlohr et al., 2012 **Cell death Proliferation Stress hormones Genes**	human (males, 20-30 years old, n= 17)	50 Hz, 62 μT	1.Group of volunteers was exposed to ELF-MF for 2 h/ day for 2 days with 6 days interval. 2.Parameters assessed: -expression of genes related to stress response, cell proliferation, apoptotic genes and CORT concentration in plasma at the time points: 0 min, 5 min, 10 min, 20 min, 40 min, 80 min and 120 min of experiment	•no gene response •CORT level ↑ the beginning of ELF-MF exposure but diminished progressively
17.	Kitaoka et al., 2012 **Behaviour Stress hormones Genes**	mouse (males, 4 weeks old, n= 5-10)	60 Hz, 3 mT	1.Mice were exposed to ELF-MF 8 h/day for 25 days. 2.Parameters assessed: -behaviour in open field test, elevated plus maze, light–dark transition test and forced swim test (after exposure) -noradrenaline, ACTH and CORT, glucose, the expression of genes related to stress response (week after behavioural tests)	•anxiety- like behaviours ↑ •helpless behaviour ↑ •ACTH – unchanged •CORT ↑ •glucose - unchanged •CYP17A1 expression ↑ •noradrenaline release and adrenal tyrosine hydroxylase expression - unchanged

18.	Korpinar et al., 2012 **Behaviour**	rat (males, adult, n= 38)	50 Hz, 10 mT	1.Rats were exposed to ELF-MF 24h/day for 21 days. 2.Parameters assessed (after exposure) : -behaviour in elevated plus-maze and hole-board tests	•stress and anxiety- like behaviours ↑ •activity and exploration – not affected
19.	Martínez-Sámano et al., 2012 **Oxidative stress Stress hormones Lipids**	rat (males, 45 days old, n= 8)	60 Hz, 2.4 mT	1.Rats (restrained (ELF-MF+RS) and unrestrained (ELF-MF)) were exposed to ELF-MF for 2 h. 2.Parameters assessed (after exposure) -SOD and CAT activities, reduced GSH, NO, -total cholesterol, and triacylglycerol levels, TBARS content in total lipids in brains -plasma CORT concentrations.	•SOD and CAT activities ↓ in ELF-MF and ELF-MF+MR exposed rats •GSH and NO levels ↓ in ELF-MF+RS treated group •total cholesterol and triacylglycerol levels not affected •TBARS in total lipids ↑ in ELF-MF+RS group •CORT level not changed in ELF-MF group
20.	Tasset et al., 2012 **Oxidative stress Neuroplasticity Behaviour Neurotransmitters**	rat (males, 3 months old, n= 8)	60 Hz, 0.7 mT	1.Rats (3-Nitropropionic acid administrated -3NP to induce Huntington disease) were exposed to ELF-MF 2 h in the morning and 2 h in the afternoon for 21 days. 2.Parameters assessed (after exposure) -behavioural changes in open field and forced swim tests -BDNF, GDNF and dopamine levels -production od lipid peroxidation products 8-hydroxy-2'-deoxyguanosine (8-OHdG) and GSH as well as capsase-3 and LDH activities	ELF-MF effects in 3NP treated rats: •neutralization of behavioural disturbances •recovery of dopamine levels •BDNF, GDNF ↑ •cell damage and oxidative stress markers ↓ •reversed neurodegeneration
21.	Vannoni et al., 2012 **Oxidative stress Proliferation Viability**	human osteoarthritic chondrocytes	100 Hz ELF-MF or Musically Modulated Electromagnetic Fields	1.Cultured cells were exposed 30 min/day for 15 days to ELF-MF or a system of Therapeutic Application of Musically Modulated Electromagnetic Fields (TAMMEF) of variable frequency and intensity. Parameters assessed: -cell survival and proliferation, expression of ERK1/2 proteins 3, 7 and 15 days of exposure -ROS and reduced GSH production during 15 days of exposure. -Mitochondrial transmembrane potential 2.Cells were stimulated with IGF-1 or IL-1β (model of osteoarthritis) and treated for 12 h ELF-MF or TAMMEF. Parameters assessed:	•cell proliferation ↑ both types of field •expression of ERK1/2 proteins ↑ in ELF-MF exposed cells •apoptosis level and mitochondrial transmembrane potential unchanged by both types of field •at the beginning of the ELF-MF treatment, the levels of ROS and reduced GSH ↑, but returned to lower levels during consecutive days of exposure •ROS production not altered by ELF-MF in IGF-1 and IL-1β stimulated cells

				-ROS and reduced GSH production, -mitochondrial transmembrane potential	•restored mitochondrial transmembrane potential and GSH ↑ after both types of exposure in IGF-1 and IL-1β induced cells
22.	Yang et al., 2012 **Cell death** **Proteins** **Behaviour**	rat (males, n= 32)	15 Hz, 0.1 mT/0.3 mT/0.5 mT	1. Rats (traumatic brain injury model) were exposed ELF-MF for 30 min, 1 h, 6 h, 12 h, 18 h, 24 h or 30 h. From 30 min to 30 h after ELF-MF exposure animals were injected with kainic acid to induce apoptosis. 2. Parameters assessed: -Morris water maze - 24 h after kainic acid injection -apoptosis level, brain water content and blood-brain barrier damages -HIF-1 protein expression	•memory ↑ •apoptosis ↓ •brain water content ↓ •blood-brain barrier damages ↓ •HIF-1 protein expression ↓
23.	Amaroli et al., 2013 **Oxidative stress** **Viability** **Proteins**	protozoan *Dictyostelium discoideum*	50 Hz 300 μT	*1.D. discoideum* cells were exposed to ELF-MF for 24 h. *2.*Parameters assessed: -cell growth, pseudocholinesterase activity and Hsp70-related molecules and -CAT and GPx activities immediately after exposure and 24 h later	•cell growth ↓ •activity of pseudocholinesterase ↑ •Hsp70-related molecules ↑ •CAT and GPx activities unchanged •effect transient - all altered parameters returned to their control values 24-h after ELF-MF exposure
24.	Collard et al., 2013 **Cell death** **Proliferation** **Genes**	human epidermis cultures	40 Hz pulsed ELF-MF	1.Epidermis cultures were exposed to pulsed ELF-MF 40 min/day for 11 days. 2.Parameters assessed: expression of genes involved in proliferation, differentiation, apoptosis, cell migration and stress response 4 and 7 days of exposure and at the day after the end of the exposure	•expression of genes involved in proliferation, differentiation, apoptosis, cell migration and stress response (*DKK1, SPRR3, NDRG4, CHEK1*) ↑
25.	Corallo et al., 2013 **Oxidative stress** **Proteins** **Immune response** **Viability**	human osteoarthritic chondrocytes	100 Hz ELF-MF or Musically Modulated Electromagnetic Fields	1.Cultured cells were exposed 30 min/day for 14 days to ELF-MF (100 Hz) or a system of Therapeutic Application of Musically Modulated Electromagnetic Fields (TAMMEF). 2.Parameters assessed: -cell viability at days 2, 7 and 14 -proteomic analysis	•cell viability ↓ in ELF-MF exposed cells •protein involved in inflammatory response: S100-A10 ↑ after TAMMEF exposure and S100-A11 ↑ after ELF-MF exposure •cystatin-B proteinase inhibitor ↑ both types of field •MnSOD ↓ after ELF-MF exposure and ↑ after TAMMEF •pattern of proteins associated with cell metabolism - changed by both types of field
26.	Duan et al., 2013	mouse	50 Hz, 8 mT	1.Mice were exposed to ELF-MF (4 h/day for 28 days).	•learning and memory abilities ↓ •MDA, ROS, NO and NOS ↑

	Oxidative stress Behaviour	(males, 3 weeks old, n= 10)		2.Parameters assessed: -learning and memory (Morris water maze) after exposure then -ROS, MDA and NO levels as well as NOS, SOD, CAT and GPx activities	•activities of SOD, CAT and GPx ↓
27.	Li et al., 2013 **Genes**	*Drosophila melanogaster* (males, after eclosion or from egg stage, n= 20- pre-screening, 60- further analysis)	50 Hz, 3 mT	1.Insects were treated with short- term (8 h/16 h/24 h/48 h/ 72 h) and long- term (lifetime) ELF-MF exposure. 2.Parameters assessed: in three-day old flies -transcriptomic analysis of genes	•short-term exposure - affected genes involved in metabolic processes, cytoskeletal organization, mitotic spindle organization, cell death, protein modification and proteolysis. •long-term exposure - changed expression of genes involved in metabolic processes, response to stress, mitotic spindle organization, aging, cell death and cellular respiration.
28.	Alsaeed et al., 2014 **Behaviour**	mouse (males, n= 8)	50 Hz, 1 mT	1.Pregnant females were exposed to ELF-MF in the last week of gestation, their offspring was then exposed to the same field conditions 7 days after birth. 2.Parameters assessed (8-11 weeks of age): - sociability (Crawley's test, preference for social novelty test - behaviour in open field test, elevated plus maze, hole-board - olfactory abilities and motor coordination	•sociability and preference of social novelty – lacked •locomotion – unchanged •anxiety-like behaviour – not showed •exploratory behaviour ↓ •olfactory abilities and motor coordination - unchanged
29.	Giorgi et al., 2014 **DNA damage**	human neuroblastoma BE(2)C cells	50 Hz, 1 mT pulsed ELF-MF	1.Cell were pre-treated with H_2O_2 (300 μM, 1 h) and then incubated under 1 mT pulsed ELF-MF 1, 24, 48 and 72 h after H_2O_2 treatment. 2.Parameters assessed: -DNA damage -cytotoxicity.	•H_2O_{2-} - induced DNA damages – not influenced •cytotoxic effect of H_2O_2.- not affected
30.	de Groot et al., 2014 **Oxidative stress Calcium**	pheochromocytoma (PC12) cells	50 Hz, 1 μT-1000 μT block-pulsed ELF-MF	1.Chemically stressed or untreated PC12 cells were exposed to ELF-MF for 30 min or 48 h. 2.Parameters assessed: -changes in $[Ca^{2+}]_i$ -ROS and membrane integrity (in cells exposed for 48 h)	•basal or depolarization-evoked $[Ca2+]_i$ - unchanged •ROS level as well as membrane integrity - unchanged

31.	Komaki et al., 2014 **Neuroplasticity**	rat (males, adult, n= 10)	50 Hz, 100 μT	1.Rats were exposed 2 h/day for 3 months. 2.Parameters assessed (the day after the last exposure) -induction of LTP (excitatory postsynaptic potential, compound action potential) -paired-pulse ratio	•excitatory postsynaptic potential slope ↑ •compound action potential ↑ •paired-pulse ratio – not changed
32.	Li et al., 2014 **Neuroplasticity** **Calcium** **Viability**	dorsal root ganglion neurons	50 Hz, 0.1 mT/1 mT/ 10 mT/100 mT pulsed ELF-MF	1.Cells were exposed to ELF-MF 2 h/day for 1 or 3 days. Cells were treated with calcium channel blockers, calcium stores inhibitors, ERK inhibitor, phospholipase C inhibitors, calcium chelators, calcium store releaser, IP3 blocker, D-AP5 and MK801. 2.Parameters assessed: -protein expression of BDNF -gene expression of *Bdnf* -intracellular calcium concentration -cell viability	•gene expression of *Bdnf* ↑ for 1-100 μT after 1 day and for 0,1-100 μT after 3 days •intracellular calcium concentration ↑ for 1 mT after 1 day •cell viability – not changed •gene expression of *Bdnf* and intracellular calcium concentration ↓ after treatment with calcium chelators and calcium channels blocker (1 mT, after 3 days) •gene expression of *Bdnf* ↓ after treatment with ERK inhibitor (1 mT, after 3 days)
33.	Luo et al., 2014 **Calcium**	entorhinal cortex neurons	50 Hz, 1 mT/3 mT	1.Cells were exposed to ELF-MF for 24 h (5 min on and 10 min off). 2.Parameters assessed: -whole cell currents including high-voltage and low-voltage activated calcium channels -intracellular concentration of calcium	•whole cell currents and high and low-voltage activated calcium channels – not changed •basal levels of the calcium concentration – not changed •calcium concentration ↓ in response to potassium stimulus
34.	Mahdavi et al., 2014 **Behaviour** **Stress hormones**	rat (males, n= 8)	1 Hz/ 5 Hz, 0.1 mT	1.Rats were exposed to 1 or 5 Hz 0,1 mT ELF-MF for 1, 3, 7, 14 or 21 days. 2.Parameters assessed (after the end of each exposure) -behaviour in open field -plasma levels of glucose, ACTH, CORT and adrenaline	•locomotor activity, rearing and sniffing in open field for 1 Hz – not changed, for 5 Hz ↑ •CORT ↓ •ACTH ↑ •adrenaline ↑ for 1 Hz •glucose ↓ for 1 Hz, and ↑ for 5 Hz
35.	Mattar et al., 2014 **Behaviour**	rat (pups aged 2, 4 or 6 weeks, n= 20)	50 Hz, 3.5 mT	1.Pups were exposed to ELF-MF for 6 weeks (1h/day for 6 days). 2.Parameters assessed (during and after exposure): -behaviour (activity, inactivity, motion, response to sound, response to light, eating -extended of hair -redness of limbs -protrusion of testis and penis	•during exposure: -activity, motion, response to sound and light ↓ -inactivity, redness of limbs, protrusion of testis and penis ↑ •after exposure: -activity, motion, response to sound and light, extended of hair (after some time return to normal), redness of limbs (after some time

					return to normal), protrusion of testis and penis (after some time return to normal) ↑ -inactivity ↓
36.	Reale et al., 2014 **Oxidative stress** **Viability** **Immune response**	human SH-SY5Y cells	50 Hz, 1 mT	1.Cell were exposed to ELF-MF for 1, 3, 6 or 24 h. Some cells were co-treated with H_2O_2. 2.Parameters assessed: -cell viability, -NOS and CAT activities, $O_2^{\bullet-}$ production -enzymatic kinetic parameters related to CAT and CYP-450 -expression of cyto/chemokines	•cell morphology and viability – not affected •NOS and CAT activities ↑ •kinetic parameters characterizing CAT activity-total velocity and rate of decrease in enzyme reaction ↑ •CYP-450 activity and $O_2^{\bullet-}$ production ↑ •TGF-β and IL-18BP expression ↑ •CAT activity ↓ and ↑ $O_2^{\bullet-}$ production in cells co-treated with H_2O_2
37.	Ardeshirylajimi and Soleimani, 2015 **Calcium** **Proliferation** **Genes** **Viability**	human pluripotent stem cells (iPSCs)	50 Hz, 1.5 mT pulsed ELF-MF	1.Cells were exposed to ELF-MF 8 h/day for 1, 3, 5, 7 or 14 days. 2.Parameters assessed: -cell viability, cell division, cell proliferation and mineralization of extracellular matrix -alkaline phosphatase activity intracellular calcium content (after 7 and 14 days of exposure). -Expressions of osteogenesis-related genes: collagen type 1, runt-related transcription factor 2 (Runx2), osteocalcin, osteonectin, alkaline phosphatase, osteoprotegerin, matrix metalloproteinases 1 and 3 (after 7 and 14 days of exposure)	•proliferation ↑ •mineralization of the extracellular matrix ↑ •calcium content, alkaline phosphatase activity ↑ •the gene expression of most osteogenesis-related genes ↑
38.	Chung et al., 2015 **Neurotransmitters**	rat (males, n= 10)	60 Hz, 2 mT	1.Rats were exposed to ELF-MF constantly for 2 or 5 days. 2.Parameters assessed in cerebellum, cortex, hippocampus, thalamus and striatum: -noradrenaline, vanillylmandelic acid, serotonin, 5-hydroxyindoleacetic acid, dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid -aspartic acid, glutamate, glutamine, glycine, taurine, tyrosine, gamma aminobutyric acid -NO	•noradrenaline and vanillylmandelic acid ↑ in all brain structures except of cortex •serotonin ↑ after 5 days in the striatum and thalamus •5-hydroxyindoleacetic acid ↑ in the striatum and hippocampus after 5 days and in thalamus after 2 and 5 days •dopamine ↑ in the thalamus after 5 days •aspartic acid ↓ in the cortex after 5 days •glutamate ↑ in the thalamus after 5 days •glutamine ↓ in the cortex and cerebellum, and ↑ in the striatum and thalamus after 5 days •glycine ↓ in the cortex and hippocampus, and ↑ in the striatum and thalamus after 5 days

					•taurine ↓ in the cortex and hippocampus •tyrosine ↑ in the thalamus after 5 days •gamma aminobutyric acid ↓ in the cortex, cerebellum and hippocampus, and ↑ in the striatum and thalamus •NO ↑ in the stratum after 2 and 5 days, and in the thalamus and hippocampus after 5 days
39.	Duan et al., 2015 **DNA damage** **Viability**	mouse spermatocyte - derived GC-2 cell line	50 Hz, 1 mT/2 mT/ 3 mT	1.Cells were exposed to 50 Hz ELF-MF of magnetic flux densities: 1, 2 and 3 mT for 24 h with intermittency cycles of 5 min field on and 10 min field off. 2.Parameters assessed: -cell viability and DNA damages	•cell viability - not affected •DNA strand breaks ↑ (only 3 mT)
40.	Golbach et al., 2015 **Calcium**	human neutrophil-like cell lines HL-60 and PLB-985	50 Hz, 5 µT/300 µT/500 µT or 50 Hz, 2.5 mT	1.First variant: Cells were exposed to 5, 300 or 500 µT for 30 min (short-term exposure) or subjected to real-time exposure during flow cytometry (50 Hz 2.5 mT). Second variant cells were exposed to 300 µT or 500 µT ELF-MF for 4-5 days. 2.Parameters assessed: -intracellular calcium mobilization -genes expression (calcium influx pathway genes). -morphology of HL-60 cells after four-day exposure.	regardless of magnetic flux density value •calcium mobilization – not altered •calcium signaling - unchanged •gene-expression patterns of calcium-signaling related genes- not altered •phenotypic changes – not found
41.	Lewicka et al., 2015 **Oxidative stress**	human blood platelets	1 kHz, 0.5 mT 50 Hz, 10 mT 1 kHz, 220 V/m	1.Human blood platelets were exposed to different sources of electromagnetic radiation: car electronics (1 kHz 0.5 mT), physiotherapy equipment (50 Hz 10 mT) or LCD monitors (1 kHz 220 V/m) for 30 min. 2.Parameters assessed (before and after exposure) CAT activity and MDA concentration	•CAT activity and MDA concentration ↑ (the most significant changes after exposure to car electronics)
42.	Li et al., 2015 **Oxidative stress**	human (males mean age 30.9, females mean age 29.8, n= 310)	Occupational exposure 0.62 – 30.19 µT (500 kV) 0.51 – 60.11 µT (220 kV)	1.Workers occupationally exposed to ELF-MF were included to research. 2.Parameters assessed: -plasma levels of TAS and MDA as well as GPx and SOD activities -genotoxicity.	•oxidative stress parameters – not changed •genotoxicity – not induced
43.	Liu et al., 2015 **Behaviour**	rat	50 Hz, 400 µT	1.Rats - Alzheimer's Disease Model exposed to ELF-MF 24 h/day for 60 days.	•pathological damages of hippocampus ↓ •spatial learning and memory disorder ↓

	Proteins	(males, 8 weeks old, n= 16)		1. Parameters assessed: -behaviour in Morris water maze- five consecutive days after exposure -brain damage (15 min/5 days after exposure) -proteomic analysis (15 min after exposure)	•changes in the expression of proteins involved in synaptic transmission (SNCG, SNAP-25b), protein degradation (UCH-L1,UBE2N), oxidative stress (CFL1, PRDX5, PRDX6), energy metabolism (DUSP3, DDT, PDHE1-B, ECH), inflammation (FABP), Tau aggregation (Tpi1, EFHD2), and brain injury (MBP).
44.	Patruno et al., 2015 **Oxidative stress**	myelogenous leukemia cell line K562	50 Hz, 1mT	1.Cells were exposed to ELF-MF for 1, 3, 6, 9, 12, 18 or 24 h. 2.Parameters assessed: -activities and kinetic parameters of CAT, iNOS and CYP-450	•activities of CAT and CYP-450 ↑ •iNOS ↓
45.	Tiwari et al., 2015 **Oxidative stress** **Stress hormones** **DNA damage**	human (males, 20-58 years old, n= 142)	occupational exposure to 132 kV high-voltage substations	1.Workers were exposed to ELF-MF for more than 2 years of occupational exposure. 2.Parameters assessed: -levels of plasma adrenaline, oxidative stress markers (MDA, NO) and -DNA damages	•adrenaline ↑ •DNA damages ↑ •MDA and NO ↑
46.	Yang and Ye, 2015 **Oxidative stress** **Cell death** **Proliferation** **Viability**	human osteosarcoma MG-63 cells	50 Hz, 1mT	2.Cells were exposed to 1 mT ELF-MF for 1, 2 or 3 h. 3.Parameters assessed: -proliferation and apoptosis rate -ROS level -expression of p38MAPK	•cells viability ↓ •cell growth ↓ •apoptosis ↑ •ROS ↑ •activation of p38MAPK (kinase implicated in pathological processes)
47.	Zhao et al., 2015 **Behaviour** **Neuroplasticity**	ICR mouse (females, 3 to 4 weeks old n = at least 10)	50 Hz, 1 mT	1.Mice were exposed to ELF-MF of 1 mT for 12 h/day for up to 21 days. 2.Parameters assessed: -recognition memory ability -locomotor activity -dendritic spine densities of hippocampal CA1 pyramidal cells	•exposure to 1 mT ELF-MF for 7 days: -object recognition ↓ -locomotor activity not changed •exposure to 1 mT ELF-MF for 7 or 10 days: -dendritic spine density of neurons in the hippocampus ↓
48.	Falone et al., 2016 **Oxidative stress** **Viability**	human neuroblastoma SH-SY5Y cell line	75 Hz, 2 mT pulsed ELF-MF	1.Cells were exposed to ELF-MF 3 times during 5 days (day 1, 3, 5). 24h after the last exposure cells were treated with H_2O_2 for 10 or 30 min. 2.Parameters assessed: -cell viability,	•ELF-MF alone (without H_2O_2) did not affect the oxidative status and viability of the cells •in H2O2 treated cells ELF-MF: -cell viability ↑ -ROS level ↓

				-ROS level and MnSOD activity	-MnSOD activity ↑
49.	Ferroni et al., 2016 **Calcium Genes**	human mesenchymal stem cell (MSCs)	100 Hz, less than 40 μT pulsed ELF-MF	1.Cells were cultured in several types of medium: adipogenic, osteogenic, neural or glial differentiative medium and basal medium and then exposed to pulsed ELF-MF 24 min/day for 21 days. 2.Parameters assessed: -gene expression of adipogenic, neuronal, glial, and osteogenic markers -expression of angiogenic and mechano-transduction markers (HIF1A, VEGFA, VEGFB, VEGFC, CDC42, RHO). -intracellular lipid content and ALP activity -presence of calcium depots and osteoids	•cells in basal medium: -mRNA level of genes related to angiogenesis ↑ -the levels of HIF1A, VEGFA, VEGFB, VEGFC, CDC42, RHO expression ↑ -expression of adipogenic, neuronal, glial and osteogenic - unchanged. •cells in adipogenic medium: -intracellular lipid content and adipogenic differentiation markers – not changed •cells in neural/glial differentiative medium -levels of genes involved in neuronal and glial commitment - not changed •cells in osteogenic medium -osteogenic markers ↑ -ALP activity ↑ -accumulation of calcium depots ↑
50.	de Kleijn et al., 2016 **Stress hormones**	BalB/c mice (males, 6 weeks old, n= 6-20)	signal contained multiple frequencies 20 Hz-5000 Hz, 10 μT	1.Mice were subjected into short term (1 week) and long term (15 weeks) exposures for 1, 4 or 24 h/day. 2.Parameters assessed (after exposure) -leukocyte numbers -hypothalamic CRH, plasma ACTH, pituitary POMC, expression of CYP11A1 in adrenal glands (short term exposure)	•effect only short-term exposure (1 week): -number of leukocytes ↑ (24h/day) -CRH – not affected -ACTH ↓ (4 h/day) -POMC ↓ (24 h/day) -CYP11A1 expression – not altered
51.	Lai et al., 2016 **Behaviour**	rat (males, adult, n= 10)	50 Hz, 100 μT	1.Rats were exposed to ELF-MF 20 h/day for 24 weeks. 2. Parameters assessed: after exposure -behaviour in open field, Morris water maze, tail suspension test, elevated plus maze, forced swim test, fear conditioning test - brain morphology and histology.	•behaviour - not affected •brain morphology and histology - not affected
52.	Luo et al., 2016 **Oxidative stress**	ICR mouse (males, 3 weeks old, n= 12)	50 Hz, 8 mT	1.Mice were exposed to ELF-MF 4 h/day for 28 days. 2.Parameters assessed (after the last exposure) -SOD, CAT, GPx, GR and GST activities and MDA level in blood and cerebral cortex	•antioxidant enzymes ↓ •MDA level ↑
53.	Nakayama et al., 2016	macrophage RAW264 cells	50 Hz, 0.5 mT	1.Cells were exposed to ELF-MF for 24 h. 2.Parameters assessed: -DNA damages	•cell viability - not affected •NO production – not affected •DNA damage – not found

	Oxidative stress **DNA damage** **Viability**			-cell viability -NO level	
54.	Sun et al., 2016 **Calcium** **Neuroplasticity**	C57 mouse (males, females, pups, n= 4-30)	50 Hz, 1 mT	1.Young mice were exposed to ELF-MF from birth to 8-10 postnatal days. 2.Parameters assessed (after exposure) -protein and gene expression of calcium ion channels (total, P/Q, N and R subtypes) -exocytosis -frequency and amplitude of excitatory postsynaptic currents -size of the readily releasable pool -slow and rapid endocytosis of synaptic vesicles -endocytosis overshoot -bulk endocytosis -post-tetanic potentiation	•calcium influx ↑ •endocytosis ↑ •no effect on the size of the readily releasable pool or exocytosis •post-tetanic potentiation ↑ •protein and gene expression of P/Q and N subtypes of calcium channels ↑
55.	Wei et al., 2016 **Cell death** **Calcium** **Proteins**	cardiomyocytes isolated from neonatal rats	15 Hz, 2 mT	1.Cells were exposed to ELF-MF for 30 min and incubated in hypoxic conditions for 12 h (ELF-MF+hypoxia), part of cells was pre-treated with Hsp70 inhibitor and exposed to the same conditions. 2.Parameters assessed: -Morphology -protein content -cytotoxicity -concentration of free intracellular calcium -expression of *Hsp70* -expression of pro-apoptotic proteins (Bax, capsase-3) -expression of anti-apoptotic protein Bcl-2	•effects of ELF-MF on cells under hypoxic conditions: -cytotoxicity ↓ -protein content ↓ -expression of Bax and caspase-3 ↓ -expression of Bcl-2 ↑ -hypertrophy ↓ -$[Ca^{2+}]_i$ oscillation baseline ↓ -amplitude of $[Ca^{2+}]_i$ oscillation ↑ -expression of *Hsp70* ↑ •Hsp70 inhibitor suppresses ELF-MF induced-cardioprotection
56.	Wyszkowska et al., 2016 **Behaviour** **Proteins**	*Schistocerca gregaria* desert locust (males/females, adult 4-9 days post-moult, n= 36)	50 Hz, 1 mT/4 mT/7 mT	1.Insects were exposed to ELF-MF for 24 h. 2. Parameters assessed: -walking behaviour and muscle force dynamics -action potential and excitatory postsynaptic potential (EPSP) properties of the fast extensor tibiae motor neuron (FETi) -Hsp70 level	•motor activity ↓ (4 and 7 mT) •muscle force ↓ •latency and duration of the FETi action potential ↑ (7 mT) •Hsp70 ↑ (7 mT)

57.	Yin et al., 2016 **Oxidative stress** **Cell death** **DNA damage** **Viability** **Calcium**	rat hippocampal neurons	50 Hz, 8 mT	1.Cells were exposed to ELF-MF for 90 min. 2.Parameters assessed: -cell viability and morphology -MDA level and SOD activity -intracellular Ca^{2+} level -mitochondrial membrane potential -intracellular ROS level -DNA damages -cell cycle and apoptosis	•cell viability ↓ •morphologically - changed, •SOD activity ↓ •MDA and ROS levels ↑ •Ca^{2+} level ↑ •depolarization of mitochondrial membrane potential ↑ •apoptosis and DNA damages ↑ •number of cells in G0/G1 phase ↓
58.	Zhang et al., 2016 A **Oxidative stress** **DNA damage**	human (n= 190)	ELF-MF of 110-420 kV	1.Workers exposed to high-voltage power lines. 2.Parameters assessed: oxidative stress (8-isoprostane) oxidative damage to DNA (8-hydroxy-2-deoxy-guanosine, 8-OHdG) in urine	•8-isoprostane and 8-OhdG levels ↑
59.	Zhu et al., 2016 **DNA damage**	human lens epithelial cells	50 Hz, 0.4 mT	1.Cells were exposed to ELF-MF for 2, 6, 12, 24 or 48 h. 2.Parameters assessed: -DNA damages	•DNA fragmentation – not found
60.	Calcabrini et al., 2017 **Oxidative stress**	human keratinocyte cell line NCTC 2544	50 Hz, 25 µT- 200 µT	1.Cells were exposed to ELF-MF for 1, 2 or 4 h. 2.Parameters assessed: -ROS production -GSH content, lipid peroxidation and antioxidant defense activity (after 1, 2 and 4 h of exposure to 50 and 100 µT)	•ROS production (1 h, 50 and 100 µT) ↑ •GSH content ↓ (1 and 2 h of) ↑ (4 h) •antioxidant enzymes activities (SOD, GPx, GR) ↓ •TBARS level ↑
61.	Cichoń et al., 2017 **Oxidative stress** **Behaviour**	post stroke patients (mean age 68 years old, n= 23	40 Hz, 7 mT various pulse shape quantities	1.Post stroke patients subjected to 15 min therapy 5 days/week for 4 weeks. 2.Parameters assessed: -CAT, SOD activities -plasma TAS -functional and mental status	•CAT, SOD activities ↑ •TAS level - not changed •functional and mental status ↑
62.	Djordjevic et al., 2017 **Oxidative stress** **Behaviour**	rat (males, 3 months old, n= 5)	50 Hz, 10 mT	1.Rats were exposed to ELF-MF 24 h/day for 7 days. 2. Parameters assessed (24 h after the last exposure) -behaviour in open field and elevated plus maze -concentrations of $O_2^{\bullet-}$, NO_2^- and $ONOO^-$ in hypothalamic tissue	•activity in elevated plus maze and open field ↓ •$O_2^{\bullet-}$ and NO_2^- ↑ •$ONOO^-$ - not changed

63.	Ehnert et al., 2017 **Oxidative stress**	human osteoblasts	16 Hz, 6-282 μT pulsed ELF-MF	1. Osteoblasts were exposed to pulsed ELF-MF for 7 min (single or repetitive exposure 7 min/day for 5 days (>3). 2. Parameters assessed: -formation of $O_2^{\bullet -}$, H_2O_2, HO•, $ONOO^-$, GSH content -expression of *SOD1*, *SOD2*, *CAT*, *GPX1, GPX3, GPX4* and *GSR* -SOD2, CAT, GPXs and GSR proteins	•single exposure: -$O_2^{\bullet -}$ and H_2O_2 ↑, -HO•, $ONOO^-$ and GSH content – not affected •repetitive exposure to ELF-MF: -ROS production ↓ -*SOD2*, *CAT*, *GPX3,* and *GSR* ↑
64.	Haghighat et al., 2017 **Proliferation Proteins Genes**	rat bone marrow mesenchymal stem cells (BMSC)	50 Hz, 20 mT	1.Cells were exposed to ELF-MF for 1 week. 2.Parameters assessed: -expression of genes responsible for pluripotency and neuronal differentiation as well as their proteins level -cell morphology	•cell proliferation ↓ •cell length and multi-polarization of cells ↑ (neurons' morphology) •expression of genes responsible for pluripotency and neuronal differentiation ↓
65.	Kuzay et al., 2017 **Oxidative stress**	rat (males, adult, n= 6)	50 Hz, 8.2 mT	1.Rats – diabetic model were exposed 20 min/day for 1 month for 5 days a week. 2.Parameters assessed (after the last exposure) -testicular tissue levels of MDA, total NO and GSH	•MDA and NO level ↑ •GSH level ↓
66.	Sakhaie et al., 2017 **Neuroplasticity Behaviour**	mouse (males, 6-7 weeks old, n=14)	50 Hz, 1 mT	1.Neurotoxin-injected mice were exposed to ELF-MF 6h/day for 6 days. 2.Parameters assessed: -neuronal maturation (24 h after the last exposure) -neurogenesis (24 h after the last exposure) -memory (Morris water maze test) (32 days after exposure)	ELF-MF reversed: •learning and memory abilities impairment •neuronal maturation impairment •neurogenesis decrease
67.	Urnukhsaikhan et al., 2017 **Neuroplasticity Cell death Immune response Behaviour**	C57B6 mice (males, 8 weeks old, n= 18)	60 Hz, 10 mT pulsed ELF-MF	1.Ischemic mice were exposed to ELF-MF 6 h/day for 14 days. 2.Parameters assessed: -motor abilities (rotarod tests, 3, 7, 11, and 14 days after surgery) After 1, 3 or 14 days -expression of anti-apoptotic *Bcl-xL* and pro-apoptotic *Bax* and *Bad* -expression of Bad, phosphorylated Bad, Bax, caspase 3, Bcl-xL, -BDNF, TrkB, PKB, phosphorylated PKB -inflammation related MMP9, IL-1β and IL-6β infarct volume	•motor abilities ↑ •expression of BDNF, TrkB and phosphorylated PKB ↑ •expression of pro-apoptotic Bad, Bax and caspase 3 ↓ •expression of anti-apoptotic Bcl-xL ↑ •inflammatory mediators MMP9 and IL-1β ↓
68.	Budziosz et al., 2018	rat	50 Hz, 10 kV/m, 4.3 pT	1.Rats were exposed to ELF-MF 22 h/day for 28 days	•MDA level and TOS in central nervous system - not changed

	Oxidative stress	(males, 10 weeks old, n= 10)		2.Parameters assessed (24 h after the last exposure) -TOS, MDA, SOD and its isoenzymes (CuZnSOD, MnSOD), CAT, GPx, GR, GST and TAC	•activities of antioxidant enzymes ↓(except for frontal cortex CAT, GPx and hippocampal GR) •non-enzymatic antioxidants – not affected (except the frontal cortex).
69.	Burman et al, 2018 **Behaviour**	BALB/cAnNCrl mouse C57BL/6NCrl mouse (females, juvenile 6-8 weeks old, n= 5)	5-100 Hz	1.Mice were exposed to ELF-MF in 6 week period. 2.Parameters assessed: -food and water uptake (every week) -indirect behavioural and physical measures: injury/wound scores [present/absent]; barbering score [present/absent]; whisker trimming score [present/absent], body weight (g) (every week) -position of mice within the cage (every week) -behaviour in open field test and novel-object recognition test (after the end of the exposure)	•no effect found
70.	Cichoń et al., 2018 A **Oxidative stress**	human (males, females, mean age 68 years old, n= 23)	40 Hz, 5 mT various pulse shape quantities	1.Post-stroke patients were subjected to magnetotherapy (15 min x 20 treatments). Parameters assessed: -protein carbonyl groups, thiol groups, MDA level	•carbonyl groups and MDA levels ↓ •thiol groups level ↑
71.	Cichoń et al., 2018 B **Neuroplasticity** **Immune response** **Behaviour**	post stroke patients (mean age 48 years old, n= 25)	40 Hz, 5 mT various pulse shape quantities	1.Post stroke patients subjected to 15 min therapy, 10 sessions with an interval of 14 days. 2.Parameters assessed in plasma: -BDNF, Expression of *BDNF,* VEGF level -cytokines: HGF, SCF, SDF-1α, β-NGF and LIF -neurologic deficits -functional and cognitive status -level of depression	•level of BDNF ↑ •expression of *BDNF* ↑ •level of VEGF ↑ •HGF and SCF levels ↑ •SDF-1α level – not changed •neurologic deficits ↓ •functional and cognitive status ↑ •depressive syndrome ↓
72.	Fathi and Farahzadi, 2018 **Proliferation**	rat adipose tissue-derived mesenchymal stem cells (rADSCs)	50 Hz, 20 mT	1.rADSCs were first cultured in adipogenic, osteogenic, chondrogenic and neurogenic mediums and exposed to ELF-MF 30 min/day for 21 days. 2.Parameters assessed: -cell proliferation -population double time	•cell proliferation ↓ •population double time - prolonged
73.	Laszlo et al., 2018	turkey (females, adult, n= 40)	50 Hz, 10 μT pulsed ELF-MF	1.Turkeys were exposed 20 min every 8 h for 3 weeks. 2.Parameters assessed:	•activity ↓ •hemoglobin, SGOT, SGPT, AP, γGT and LDH – not changed

	Behaviour **Stress hormones**			-the behaviour (relaxation, play, competition, aggression). -hemoglobin, SGOT, SGPT, AP, γGT, LDH (blood , every week) -level of cAMP to detect noradrenaline-activated β-adrenoreceptor function (blood , every week)	•noradrenaline-activated β-adrenoceptor function ↓
74.	Martínez-Sámano et al., 2018 **Oxidative stress** **Stress hormones** **Lipids**	rat (males, 8 weeks old, n= 6)	60 Hz, 2.4 mT	1.Rats (restrained (ELF-MF+RS) and unrestrained (ELF-MF)) were exposed to ELF-MF for 2 h/day for 21 days. 2.Parameters assessed (after the last exposure) -total cholesterol, and triacylglycerol levels, TBARS -plasma CORT concentrations, -total free fatty acids and fatty acid methyl esters (FAMEs)	•ELF-MF group: -total cholesterol and triacylglycerol levels not affected -CORT level ↑ -total lipids in cerebellum and total cholesterol in cortex ↑ -polar lipids in cortex ↓ -polyunsaturated fatty acids in cerebellum ↓ and ↑ in subcortical structures. •TBARS in total lipids ↑ in both groups •concentrations of non-esterified fatty acids in subcortical structures (RS+ELF-MF) ↑
75.	Rezaie-Tavirani et al, 2018 **Proteins**	rat (males, adult, n= not provided)	50 Hz, 0.5 mT/ 1 mT	1.Rats were exposed to 0.5 mT or 1 mT 3 h/day for 2 or 4 weeks. 2.Parameters assessed: -proteome profile -protein-protein interaction -molecular function	•0.5 mT: -64 spots up-regulated -40 spots down-regulated •1 mT: -86 spots up-regulated -65 spots down-regulated •expression of Sptan1 and Dpysl2 ↓ by 0.5 mT •expression of Dpysl2 ↑ by 1 mT after 2 weeks •expression of Tpi1 and Lap3 ↓ through time •expression Tppp ↓ by 1 mT through time •general protein expression ↓ with increasing ELF-MF intensity and time •identified proteins are related to apoptosis, stress response and number of metabolic processes.
76.	Song et al., 2018 **Oxidative stress** **Proliferation**	HeLa cells IMR-90 fibroblasts	60 Hz, 3 mT/ 6 mT	Variants of experiments: 1.Cells were exposed to 6 mT ELF-MF for 30 or 60 min.	•single exposure to 6 mT and repetitive exposure to 3 or 6 mT: -DNA damages and change of cell viability - not found. •exposure to 6 mT for total 168 h:

	DNA damage Viability			2.Cells were exposed to 3 and 6 mT ELF-MF 30 min every 24 h for 72 h or 30 min 8 times a day for 3 days. 3.Cells exposed to 6 mT for 72 h were additionally exposed for subsequent 96 h. Parameters assessed: DNA damage, cell viability cell cycle and ROS level (during continuous exposure) 4.Cells were co-incubated with GOx, (to induce H_2O_2 and reduce proliferation) and exposed to ELF-MF for 1, 6, 12 and 24 h Parameters assessed: phosphorylation of PKB and Erk1/2	-cells viability and cell cycle progression ↑ -ROS level ↓ •in GOx – treated cells, ELF-MF: -mitigated anti-proliferative effect -phosphorylation of PKB and ERK1/2 ↑
77.	Sun et al., 2018 **Oxidative stress**	*Caenorhabditis elegans* (n= 90300)	50 Hz, 3 mT	1.Worms were exposed to ELF-MF from egg stage reaching the fourth larva (L4) stage (about 48 h). 2.Parameters assessed: -expression of the genes involved in TCA cycle (associated with tumor growth). PGE_2, ROS, TAC level -SOD and CAT activities	•TCA cycle enzyme ↓ •arachidonic acid and PGE_2 ↑ •expression of PGE_2 synthase ↑ •ROS level ↑ •TAC level ↓ •SOD and CAT activities – not changed
78.	Wyszkowska et al., 2018 **Immune response**	rat (males, adults, n= 6)	50 Hz, 7 mT	1.Rats were subjected to acute (24 h) or repetitive exposure (1 h/day for 7 days). 2.Parameters assessed (plasma): -cytokines: IL-1β, IL-2, IL-6, IL-10 -number of: total white blood cells, lymphocytes, monocytes, granulocytes red blood cells, platelets -hemoglobin -Hematocrit	•24 h exposure: -IL-1β, IL-2, IL-6 ↑ -white blood cells, lymphocytes, red blood cells, hemoglobin and hematocrit ↑ •repetitive exposure 1 h/day for 7 days – no effect
79.	Cichoń et al., 2019 **Immune response**	post stroke patients (mean age 44,8 years old, n= 25)	40 Hz, 5 mT pulsed ELF-MF	1.Post stroke patients subjected to 15 min therapy, 10 sessions with an interval of 14 days. 2.Parameters assessed: -Plasma levels of cytokines: IL-1β, IL-2, IFN-γ, TGF-β -Expression of *IL-1β*	•level of IL-1β ↑ •expression level of *IL-1β* ↑ •level of IL-2 ↑ •IFN-γ and TGF-β levels – not changed
80.	Hosseinabadi and Khanjani, 2019	human (20-50 years old, n= 152)	Mean: 4,09 V/m, 16.27 μT	1.Power plant workers (occupational exposure) 2.Parameters assessed: -serum TAC, MDA, SOD, CAT and GPx	•oxidative stress ↑

	Oxidative stress				
81.	Hosseinabadi et al., 2019 **Behaviour**	human (30-40 years old, n= 132)	occupational exposure	1.Participants were exposed to ELF-MF (the 8-h time-weighted average). 2.Parameters assessed: -sleep quality -depression, anxiety and stress levels	•sleep quality ↓ •stress, depression and anxiety ↑ (linear relation (trend) with increased exposure)
82.	Karimi et al., 2019 **Oxidative stress** **Behaviour**	rat (males, adult, n= 12)	50 Hz, 1 μT/100 μT/500 μT/ 2000 μT	1.Rats were exposed to ELF-MF 2 h/day for 60 days. 2.Parameters assessed: -elevated plus maze (the day after the last exposure) and Morris water maze test (the day after elevated plus maze) -the passive avoidance test (one week after Morris water maze) -MDA, TAC, TOS and total thiol molecules (after behavioural tests)	•lipid peroxidation ↑ (100 μT and 500 μT) •TAC ↑ (1 μT and 500 μT) •total thiol molecules ↑ (all induction values) •TOS ↑ (500 μT). •memory retention ↑ (100 μT and 2000 μT, water maze) •memory retention ↑ (100 μT, 500 μT and 2000 μT, passive avoidance test) •anxiety ↑ (all induction values)
83.	Merla et al., 2019 **Oxidative stress**	SH-SY5Y neuroblastoma cells	50 Hz, 1 mT	1.Cells were treated with DPI an inhibitor of the plasma membrane enzyme NADPH oxidase (Nox) and then exposed for ELF-MF for 24 h. 1.Parameters assessed: -source of ROS production	•ROS generation ↑ •plasma membrane Nox is involved in redox imbalance elicited by ELF-MF
84.	Sun et al., 2019 **Proteins** **Genes** **Lipids**	*Caenorhabditis elegans* (n= 40000)	50 Hz, 3 mT	1.Worms were placed under 3 mT ELF-MF till reaching the L4 stage (48 h). 2.Parameters assessed: -total triacylglycerols (TGs) level (proteomic and transcriptomic profiling)	•in glycerolipids (GLs) category: -total triacylglycerols (TGs) ↑ -diacylglycerols (DGs) ↓ •other identified lipid categories showed no regular pattern of changes -stress response related genes expressions ↑ -the most enriched protein functions were: defense response, reproduction and lipid transport
85.	Mahaki et al., 2020 **Immune response**	rat (males, adults, n= 16)	50 Hz, 1 μT/100 μT/500 μT/2000 μT	1.Rats were exposed to ELF-MF 2h/day for 60 days at two phases: at pre- and post-stimulation of the immune system. 2.Parameters assessed: -serum levels of cytokines: IL-9, IL-10, TNF-α	•before immunization: -IL-9 ↓ for 100 μT -TNF-α ↓ for 100 μT-2000 μT -IL-10 ↑ for 1 μT and 100 μT •after immunization: -IL-9, TNF-α ↓ for 1 μT and 100 μT -no significant differences in IL-10 level

					•post-immunization levels compared to pre-immunization: -IL-9, TNF-α ↑ for 100 μT-2000 μT -IL-10 ↑ for 2000 μT
86.	Touitou et al., 2020 **Proteins**	human (males, mean 38 years old, n= 15)	50 Hz, mean 0.9 μT	1.Men chronically exposed to ELF-MF (1-20 years). 2.Parameters assessed: -serum level of CgA	•CgA levels ↓ in participants exposed to highest level of exposure.

Abbreviations: 8-OHdG- 8-hydroxy-2'-deoxyguanosine; **$[Ca]^{2+}_i$** – intracellular ionized calcium; **ACTH** - adrenocorticotropic hormone; **ALP**- alkaline phosphatase; **ALT**- alanine transaminase; **AP**- alkaline phosphatase; **AST**- aspartate transaminase; **Bad**- Bcl-2-associated death promoter; **Bax**- Bcl-2-associated X protein; **Bcl-2**- B cell leukemia/lymphoma-2; **Bcl-xL**- B-cell lymphoma-extra-large; **BDNF**- brain derived neurotrophic factor; **cAMP**- 3′5′-cyclic-adenosine-monophosphate; **CAT**- catalase; **CDC42**- cell division control protein 42 homolog; **CFL1**- cofilin 1; **CgA**- chromogranin A; ***CHEK1***- checkpoint kinase 1 encoding gene; **CORT**- corticosterone/cortisol; **CRH**- corticotropin-releasing hormone; **CuZnSOD (SOD3)**- copper-zinc-superoxide dismutase; **CYP11A1**- cytochrome P450 11A1; **CYP17A1**- cytochrome P450 17A1/steroid 17α-monooxygenase; **CYP-450**- cytochromes P450; **D-AP5**- 5-phosphono-D-norvaline; **DDT**- D-dopachrome decarboxylase; ***DKK1***- dickkopf-related protein 1 encoding gene; **DPI**- diphenyleneiodonium; **Dpysl2**- dihydropyrimidinase-related protein 2; **DUSP3**- dual specificity phosphatase 3; **ECH**- 2-Enoyl-Coa Hydratase; **EFHD2**- EF-hand domain-containing protein D2; **ERK**- extracellular signaling-regulated kinase; **FABP**- fatty acid-binding protein; **free-T3**- free triiodothyronine; **free-T4**- free thyroxine; **GDNF**- glial cell-derived neurotrophic factor; **GOx**- glucose oxidase; **GPx**- glutathione peroxidase; **GR**- glutathione reductase; **GSH**- glutathione; **GSR**- glutathione-disulfide reductase; **GST**-glutathione S-transferase; **HGF**- hepatocyte growth factor; **HIF-1**- hypoxia-inducible factor 1; **HIF-1A**- hypoxia-inducible factor 1-alpha; **HO•** - hydroxyl radicals; **Hsp70**- heat shock protein 70; **IFN-γ**- interferon-γ; **IGF-1**- insulin-like growth factor 1; **IL-10**- interleukin 10; **IL-18BP**- interleukin-18-binding protein; **IL-1β**- interleukin 1β; **IL-2**- interleukin 2; **IL-6**- interleukin 6; **IL-6β**- interleukin 6β; **iNOS**- inducible nitric oxide synthase; **IP3**- inositol trisphosphate; **Lap3**-cytosol aminopeptidase; **LDH**- lactate dehydrogenase; **LIF**- leukemia inhibitory factor; **LTP**- long-term potentiation; **MBP**- myelin basic protein; **MDA**-malondialdehyde; **MK801**- dizocilpine; **MMP9**- matrix metallopeptidase 9; **MnSOD (SOD2)**- manganese-dependent superoxide dismutase; **MPO**-myeloperoxidase; ***NDRG4***- N-myc downregulated gene 4; **NO**- nitric oxide; **NO_2^-** - nitrite; **NOS**- nitric oxide synthase; **$O_2^{\bullet-}$** -superoxide anion; **$ONOO^-$** - peroxynitrite; **OSI**- oxidative stress index; **p38MAPK**- p38 mitogen-activated protein kinase; **PDHE1-B**- pyruvate dehydrogenase E1; **PGE_2** – prostaglandin E_2; **PI3K**- phosphoinositide 3-kinase; **PKB**- protein kinase B; **POMC**- pro-opiomelanocortin; **PP2A**- protein phosphatase 2; **PRDX5**- peroxiredoxin-5; **PRDX6**-peroxiredoxin-6; **RHO**- ras homolog family; **ROS**- reactive oxygen species; **rTMS**- repetitive transcranial magnetic stimulation; **S100-A10**- S100 calcium-binding protein A10; **S100-A11**- S100 calcium-binding protein A11; **SCF**- stem cell factor; **SDF-1α**- stromal derived factor-1α; **SGOT**- serum glutamic-oxaloacetic transaminase; **SGPT**- serum glutamic-pyruvic transaminase; **SNAP-25b**- synaptosomal-associated protein 25b; **SNCG**- synuclein gamma; **SOD**-superoxide dismutase; ***SPRR3***- small proline-rich protein 3 encoding gene; **Sptan1**- spectrin alpha chain; **TAC**- total antioxidant capacity; **TAS**- total antioxidant status; **TBARS**- thiobarbituric acid reactive substances; **TCA cycle**- tricarboxylic acid cycle/ the Krebs cycle; **TGF-β**- transforming growth factor β; **TOS**- total oxidant status; **Tpi1**- triose phosphate isomerase; **Tppp**- tubulin polymerization-promoting protein; **TrkB**- tyrosine receptor kinase B; **UBE2N**-ubiquitin-conjugating enzyme E2 N; **UCH-L1**- ubiquitin carboxy-terminal hydrolase L1; **VEGF**- vascular endothelial growth factor; **VEP**- visual evoked potential; **β-NGF**- β nerve growth factor; **γGT**- gamma-glutamyl transpeptidase

References

1. Akdag, M.Z.; Dasdag, S.; Ulukaya, E.; Uzunlar, A.K.; Kurt, M.A.; Taşkin, A. Effects of extremely low-frequency magnetic field on caspase activities and oxidative stress values in rat brain. Biol Trace Elem Res 2010, 138(1-3), 238-249; doi: 10.1007/s12011-010-8615-3
2. Cuccurazzu, B.; Leone, L.; Podda, M.V.; Piacentini, R.; Riccardi, E.; Ripoli, C.; Azzena, G.B.; Grassi, C. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol 2010, 226(1), 173-82; doi: 10.1016/j.expneurol.2010.08.022
3. El-Helaly, M.; Abu-Hashem, E. Oxidative stress, melatonin level, and sleep insufficiency among electronic equipment repairers. Indian J Occup Environ Med 2010, 14(3), 66-70; doi:10.4103/0019-5278.75692
4. Frahm, J.; Mattsson, M.O.; Simkó, M. Exposure to ELF magnetic fields modulate redox related protein expression in mouse macrophages. Toxicol Lett 2010, 192(3), 330-336; doi: 10.1016/j.toxlet.2009.11.010
5. Garip, A.I.; Akan, Z. Effect of ELF-EMF on number of apoptotic cells; correlation with reactive oxygen species and HSP. Acta Biol Hung 2010, 61(2), 158-167; doi: 10.1556/ABiol.61.2010.2.4
6. George, M.S.; Lisanby, S.H.; Avery, D.; McDonald, W.M.; Durkalski, V.; Pavlicova, M.; Anderson, B.; Nahas, Z.; Bulow, P.; Zarkowski, P.; Holtzheimer, P.E. 3rd; Schwartz, T.; Sackeim, H.A. Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: a sham-controlled randomized trial. Arch Gen Psychiatry 2010, 67(5), 507-516; doi: 10.1001/archgenpsychiatry.2010.46
7. Goraca, A.; Ciejka, E.; Piechota, A. Effects of extremely low frequency magnetic field on the parameters of oxidative stress in heart. J Physiol Pharmacol 2010, 61(3), 333-338
8. Mannerling, A.C.; Simkó, M.; Mild, K.H.; Mattsson, M.O. Effects of 50-Hz magnetic field exposure on superoxide radical anion formation and HSP70 induction in human K562 cells. Radiat Environ Biophys 2010, 49(4), 731-741; doi: 10.1007/s00411-010-0306-0
9. Martínez-Sámano, J.; Torres-Durán, P.V.; Juárez-Oropeza, M.A.; Elías-Viñas, D.; Verdugo-Díaz, L. Effects of acute electromagnetic field exposure and movement restraint on antioxidant system in liver, heart, kidney and plasma of Wistar rats: a preliminary report. Int J Radiat Biol 2010, 86(12), 1088-1094; doi: 10.3109/09553002.2010.501841
10. Morabito, C.; Rovetta, F.; Bizzarri, M.; Mazzoleni, G.; Fanò, G.; Mariggiò, M.A. Modulation of redox status and calcium handling by extremely low frequency electromagnetic fields in C2C12 muscle cells: A real-time, single-cell approach. Free Radic Biol Med 2010, 48(4), 579-589; doi: 10.1016/j.freeradbiomed.2009.12.005
11. Szemerszky, R.; Zelena, D.; Barna, I.; Bárdos, G. Stress-related endocrinological and psychopathological effects of short- and long-term 50Hz electromagnetic field exposure in rats. Brain Res Bull 2010, 81(1), 92-99; doi: 10.1016/j.brainresbull.2009.10.015
12. Emre, M., Cetiner, S.; Zencir, S.; Unlukurt, I.; Kahraman, I.; Topcu, Z. Oxidative stress and apoptosis in relation to exposure to magnetic field. Cell Biochem Biophys 2011, 59(2), 71-77; doi: 10.1007/s12013-010-9113-0
13. He, L.H.; Shi, H.M.; Liu, T.T.; Xu, Y.C.; Ye, K.P.; Wang, S. Effects of extremely low frequency magnetic field on anxiety level and spatial memory of adult rats. Chin Med J (Engl) 2011, 124(20), 3362-3366
14. Hosseini, E.; Nafisi, S.; Zare, S. The effects of electromagnetic fields on plasma levels of corticosterone, free-T3, free-T4 malonyldialdehyde in white male rabbit with normal diet and hyperchlostrol diet, Veterinary Research Forum 2011, 2(4), 222- 225
15. Juszczak, K.; Kaszuba-Zwoinska, J.; Thor, P.J. Pulsating electromagnetic field stimulation of urothelial cells induces apoptosis and diminishes necrosis: new insight to magnetic therapy in urology. J Physiol Pharmacol 2012, 63(4), 397-401

16. Kirschenlohr, H.; Ellis, P.; Hesketh, R.; Metcalfe, J. Gene expression profiles in white blood cells of volunteers exposed to a 50 Hz electromagnetic field. Radiat Res 2012, 178(3), 138-149; doi: 10.1667/rr2859.1
17. Kitaoka, K.; Kitamura, M.; Aoi, S.; Shimizu, N.; Yoshizaki, K. Chronic exposure to an extremely low-frequency magnetic field induces depression-like behavior and corticosterone secretion without enhancement of the hypothalamic-pituitary-adrenal axis in mice. Bioelectromagnetics 2013, 34(1), 43-51; doi: 10.1002/bem.21743
18. Korpinar, M.A.; Kalkan, M.T.; Tuncel, H. The 50 Hz (10 mT) sinusoidal magnetic field: effects on stress-related behavior of rats. Bratisl Lek Listy 2012, 113(9), 521-524; doi: 10.4149/bll_2012_117
19. Martínez-Sámano, J.; Torres-Durán, P.V.; Juárez-Oropeza, M.A.; Verdugo-Díaz, L. Effect of acute extremely low frequency electromagnetic field exposure on the antioxidant status and lipid levels in rat brain. Arch Med Res 2012 , 43(3), 183-9; doi: 10.1016/j.arcmed.2012.04.003
20. Tasset, I.; Medina, F.J.; Jimena, I.; Agüera, E.; Gascón, F.; Feijóo, M.; Sánchez-López, F.; Luque, E.; Peña, J.; Drucker-Colín, R.; Túnez ,I. Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: effects on neurotrophic factors and neuronal density. Neuroscience 2012, 209, 54-63; doi: 10.1016/j.neuroscience.2012.02.034
21. Vannoni, D.; Albanese, A.; Battisti, E.; Aceto, E.; Giglioni, S.; Corallo, C.; Carta, S.; Ferrata, P.; Fioravanti, A.; Giordano, N. In vitro exposure of human osteoarthritic chondrocytes to ELF fields and new therapeutic application of musically modulated electromagnetic fields: biological evidence. J Biol Regul Homeost Agents 2012, 26(1), 39-49
22. Yang, Y.; Li, L.; Wang, Y.G.; Fei, Z.; Zhong, J.; Wei, L.Z.; Long, Q.F.; Liu, W.P. Acute neuroprotective effects of extremely low-frequency electromagnetic fields after traumatic brain injury in rats. Neurosci Lett 2012, 516(1), 15-20; doi: 10.1016/j.neulet.2012.03.022
23. Amaroli, A.; Chessa, M.G.; Bavestrello, G.; Bianco, B. Effects of an extremely low-frequency electromagnetic field on stress factors: a study in Dictyostelium discoideum cells. Eur J Protistol 2013, 49(3), 400-405; doi:10.1016/j.ejop.2012.12.002
24. Collard, J.F.; Lazar, C.; Nowé, A.; Hinsenkamp, M. Statistical validation of the acceleration of the differentiation at the expense of the proliferation in human epidermal cells exposed to extremely low frequency electric fields. Prog Biophys Mol Biol 2013, 111(1), 37-45; doi: 10.1016/j.pbiomolbio.2012.12.004
25. Corallo, C.; Battisti, E.; Albanese, A.; Vannoni, D.; Leoncini, R.; Landi, G.; Gagliardi, A.; Landi, C.; Carta, S.; Nuti, R.; Giordano, N. Proteomics of human primary osteoarthritic chondrocytes exposed to extremely low-frequency electromagnetic fields (ELF EMFs) and to therapeutic application of musically modulated electromagnetic fields (TAMMEF). Electromagn Biol Med 2014, 33(1), 3-10; doi: 10.3109/15368378.2013.782316
26. Duan, Y.; Wang, Z.; Zhang, H.; He, Y.; Lu, R.; Zhang, R.; Sun, G.; Sun, X. The preventive effect of lotus seedpod procyanidins on cognitive impairment and oxidative damage induced by extremely low frequency electromagnetic field exposure. Food Funct 2013, 4(8), 1252-1262; doi: 10.1039/c3fo60116a
27. Li, S.S.; Zhang, Z.Y.; Yang, C.J.; Lian, H.Y.; Cai, P. Gene expression and reproductive abilities of male Drosophila melanogaster subjected to ELF-EMF exposure. Mutat Res 2013, 758(1-2), 95-103; doi: 10.1016/j.mrgentox.2013.10.004
28. Alsaeed, I.; Al-Somali, F.; Sakhnini, L.; Aljarallah, O.S.; Hamdan, R.M.; Bubishate, S.A.; Sarfaraz, Z.K.; Kamal, A. Autism-relevant social abnormalities in mice exposed perinatally to extremely low frequency electromagnetic fields. Int J Dev Neurosci 2014, 37, 58-64; doi: 10.1016/j.ijdevneu.2014.06.010
29. Giorgi, G.; Lecciso, M.; Capri, M.; Lukas Yani, S.; Virelli, A.; Bersani, F.; Del Re, B. An evaluation of genotoxicity in human neuronal-type cells subjected to oxidative stress under an extremely low frequency pulsed magnetic field. Mutat Res Genet Toxicol Environ Mutagen 2014, 775-776, 31-7; doi: 10.1016/j.mrgentox.2014.10.003

30. de Groot, M.W.; Kock, M.D.; Westerink, R.H. Assessment of the neurotoxic potential of exposure to 50Hz extremely low frequency electromagnetic fields (ELF-EMF) in naive and chemically stressed PC12 cells. Neurotoxicology 2014, 44, 358-364; doi: 10.1016/j.neuro.2014.07.00
31. Komaki, A.; Khalili, A.; Salehi, I.; Shahidi, S.; Sarihi, A. Effects of exposure to an extremely low frequency electromagnetic field on hippocampal long-term potentiation in rat. Brain Res 2014, 1564, 1-8; doi: 10.1016/j.brainres.2014.03.041
32. Li, Y.; Yan, X.; Liu, J.; Li, L.; Hu, X.; Sun, H.; Tian, J. Pulsed electromagnetic field enhances brain-derived neurotrophic factor expression through L-type voltage-gated calcium channel- and Erk-dependent signaling pathways in neonatal rat dorsal root ganglion neurons. Neurochem Int 2014, 75, 96-104; doi: 10.1016/j.neuint.2014.06.004
33. Luo, F.L.; Yang, N.; He, C.; Li, H.L.; Li, C.; Chen, F.; Xiong, J.X.; Hu, Z.A.; Zhang, J. Exposure to extremely low frequency electromagnetic fields alters the calcium dynamics of cultured entorhinal cortex neurons. Environ Res 2014, 135, 236-246; doi: 10.1016/j.envres.2014.09.023
34. Mahdavi, S.M.; Sahraei, H.; Yaghmaei, P.; Tavakoli, H. Effects of electromagnetic radiation exposure on stress-related behaviors and stress hormones in male wistar rats. Biomol Ther (Seoul) 2014, 22(6), 570-576; doi:10.4062/biomolther.2014.054
35. Mattar, F.A.; Bareedy, M.H.; El-Dosouky, M.E.M.; Zaghloul, M.S.; Mahmod, M.S.M. Effects of 6 Weeks Exposure of 3.5 mT (ELF EMF) on Some Animal behaviors in White Albino Rat (Sprague Dawley) Pups, Zagazig Veterinary Journal 2014, 42(1), 74-80
36. Reale, M.; Kamal, M.A.; Patruno, A.; Costantini, E.; D'Angelo, C.; Pesce, M.; Greig, N.H. Neuronal cellular responses to extremely low frequency electromagnetic field exposure: implications regarding oxidative stress and neurodegeneration. PLoS One 2014, 9(8), e104973; doi: 10.1371/journal.pone.0104973
37. Ardeshirylajimi, A.; Soleimani, M. Enhanced growth and osteogenic differentiation of Induced Pluripotent Stem cells by Extremely Low-Frequency Electromagnetic Field. Cell Mol Biol (Noisy-le-grand) 2015, 61(1), 36-41
38. Chung, Y.H.; Lee, Y.J.; Lee, H.S.; Chung, S.J.; Lim, C.H.; Oh, K.W.; Sohn, U.D.; Park, E.S.; Jeong, J.H. Extremely low frequency magnetic field modulates the level of neurotransmitters. Korean J Physiol Pharmacol 2015, 19(1), 15-20; doi: 10.4196/kjpp.2015.19.1.15
39. Duan, W.; Liu, C.; Zhang, L.; He, M.; Xu, S.; Chen, C.; Pi, H.; Gao, P.; Zhang, Y.; Zhong, M.; Yu, Z.; Zhou, Z. Comparison of the genotoxic effects induced by 50 Hz extremely low-frequency electromagnetic fields and 1800 MHz radiofrequency electromagnetic fields in GC-2 cells. Radiat Res 2015, 183(3), 305-314; doi: 10.1667/RR13851.1
40. Golbach, L.A.; Philippi, J.G.; Cuppen, J.J.; Savelkoul, H.F.; Verburg-van Kemenade, B.M. Calcium signalling in human neutrophil cell lines is not affected by low-frequency electromagnetic fields. Bioelectromagnetics 2015, 36(6), 430-43; doi: 10.1002/bem.21924
41. Lewicka, M.; Henrykowska, G.A.; Pacholski, K.; Szczęsny, A.; Dziedziczak-Buczyńska, M.; Buczyński, A. The impact of electromagnetic radiation of different parameters on platelet oxygen metabolism - in vitro studies. Adv Clin Exp Med 2015, 24(1), 31-35; doi: 10.17219/acem/38169
42. Li, L.; Xiong, D.F.; Liu, J.W.; Li, Z.X.; Zeng, G.C.; Li, H.L. A cross-sectional study on oxidative stress in workers exposed to extremely low frequency electromagnetic fields. Int J Radiat Biol 2015, 91(5), 420-425; doi: 10.3109/09553002.2015.1012304. PMID: 25651040
43. Liu, X.; Zuo, H.; Wang, D.; Peng, R.; Song, T.; Wang, S.; Xu, X.; Gao, Y.; Li, Y.; Wang, S.; Wang, L.; Zhao, L. Improvement of spatial memory disorder and hippocampal damage by exposure to electromagnetic fields in an Alzheimer's disease rat model. PLoS One 2015, 10(5):e0126963; doi: 10.1371/journal.pone.0126963
44. Patruno, A.; Tabrez, S.; Pesce, M.; Shakil, S.; Kamal, M.A.; Reale, M. Effects of extremely low frequency electromagnetic field (ELF-EMF) on catalase, cytochrome P450 and nitric oxide synthase in erythro-leukemic cells. Life Sci 2015, 121, 117-123; doi: 10.1016/j.lfs.2014.12.003
45. Tiwari, R.; Lakshmi, N.K.; Bhargava, S.C.; Ahuja, Y.R. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations. Electromagn Biol Med 2015, 34(1), 56-62; doi: 10.3109/15368378.2013.869755

46. Yang, M.L.; Ye, Z.M. [Extremely low frequency electromagnetic field induces apoptosis of osteosarcoma cells via oxidative stress]. Zhejiang Da Xue Xue Bao Yi Xue Ban 2015, 44(3), 323-328. Chinese
47. Zhao, Q.R.; Lu, J.M.; Yao, J.J.; Zhang, Z.Y.; Ling, C.; Mei, Y.A. Neuritin reverses deficits in murine novel object associative recognition memory caused by exposure to extremely low-frequency (50 Hz) electromagnetic fields. Sci Rep 2015, 5, 11768; doi: 10.1038/srep11768
48. Falone, S.; Marchesi, N.; Osera, C.; Fassina, L.; Comincini, S.; Amadio, M.; Pascale, A. Pulsed electromagnetic field (PEMF) prevents pro-oxidant effects of H2O2 in SK-N-BE(2) human neuroblastoma cells. Int J Radiat Biol 2016, 92(5), 281-286; doi: 10.3109/09553002.2016.1150619
49. Ferroni, L.; Tocco, I.; De Pieri, A.; Menarin, M.; Fermi, E.; Piattelli, A.; Gardin, C.; Zavan, B. Pulsed magnetic therapy increases osteogenic differentiation of mesenchymal stem cells only if they are pre-committed. Life Sci 2016, 152, 44-51; doi: 10.1016/j.lfs.2016.03.020
50. de Kleijn, S.; Ferwerda, G.; Wiese, M.; Trentelman, J.; Cuppen, J.; Kozicz, T.; de Jager, L.; Hermans, P.W.; Verburg-van Kemenade, B.M. A short-term extremely low frequency electromagnetic field exposure increases circulating leukocyte numbers and affects HPA-axis signaling in mice. Bioelectromagnetics 2016, 37(7), 433-443; doi: 10.1002/bem.21998
51. Lai, J.; Zhang, Y.; Liu, X.; Zhang, J.; Ruan, G.; Chaugai, S.; Chen, C.; Wang, D.W. Effects of extremely low frequency electromagnetic fields (100μT) on behaviors in rats. Neurotoxicology 2016, 52, 104-113; doi: 10.1016/j.neuro.2015.11.010
52. Luo, X.; Chen, M.; Duan, Y.; Duan, W.; Zhang, H.; He, Y.; Yin, C.; Sun, G.; Sun, X. Chemoprotective action of lotus seedpod procyanidins on oxidative stress in mice induced by extremely low-frequency electromagnetic field exposure. Biomed Pharmacother 2016, 82, 640-648; doi: 10.1016/j.biopha.2016.06.005
53. Nakayama, M.; Nakamura, A.; Hondou, T.; Miyata, H. Evaluation of cell viability, DNA single-strand breaks, and nitric oxide production in LPS-stimulated macrophage RAW264 exposed to a 50-Hz magnetic field. Int J Radiat Biol 2016, 92(10), 583-589; doi: 10.1080/09553002.2016.1206224
54. Sun, Z.C.; Ge, J.L.; Guo, B.; Guo, J.; Hao, M.; Wu, Y.C.; Lin, Y.A.; La, T.; Yao, P.T.; Mei, Y.A.; Feng, Y.; Xue, L. Extremely Low Frequency Electromagnetic Fields Facilitate Vesicle Endocytosis by Increasing Presynaptic Calcium Channel Expression at a Central Synapse. Sci Rep 2016, 6, 21774; doi: 10.1038/srep21774
55. Wei, J.; Tong, J.; Yu, L.; Zhang, J. EMF protects cardiomyocytes against hypoxia-induced injury via heat shock protein 70 activation. Chem Biol Interact 2016, 248, 8-17; doi: 10.1016/j.cbi.2016.02.003
56. Wyszkowska, J.; Shepherd, S.; Sharkh, S.; Jackson, C.W.; Newland, P.L. Exposure to extremely low frequency electromagnetic fields alters the behaviour, physiology and stress protein levels of desert locusts. Sci Rep 2016, 6, 36413; doi: 10.1038/srep36413
57. Yin, C.; Luo, X.; Duan, Y.; Duan, W.; Zhang, H.; He, Y.; Sun, G.; Sun, X. Neuroprotective effects of lotus seedpod procyanidins on extremely low frequency electromagnetic field-induced neurotoxicity in primary cultured hippocampal neurons. Biomed Pharmacother 2016 , 82, 628-639; doi: 10.1016/j.biopha.2016.05.032
58. Zhang, Y.; Zhang, D.; Zhu, B.; Zhang, H.; Sun, Y.; Sun, C. Effects of dietary green tea polyphenol supplementation on the health of workers exposed to high-voltage power lines. Environ Toxicol Pharmacol 2016, 46, 183-187; doi: 10.1016/j.etap.2016.07.016
59. Zhu, K.; Lv, Y.; Cheng, Q.; Hua, J.; Zeng, Q. Extremely Low Frequency Magnetic Fields Do Not Induce DNA Damage in Human Lens Epithelial Cells In Vitro. Anat Rec (Hoboken) 2016, 299(5), 688-697; doi: 10.1002/ar.23312
60. Calcabrini, C.; Mancini, U.; De Bellis, R.; Diaz, A.R.; Martinelli, M.; Cucchiarini, L.; Sestili, P.; Stocchi, V.; Potenza, L. Effect of extremely low-frequency electromagnetic fields on antioxidant activity in the human keratinocyte cell line NCTC 2544. Biotechnol Appl Biochem 2017, 64(3), 415-422; doi: 10.1002/bab.1495

61. Cichoń, N.; Bijak, M.; Miller, E.; Saluk, J. Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients. Bioelectromagnetics 2017, 38(5), 386-396; doi: 10.1002/bem.22055
62. Djordjevic, N.Z.; Paunović, M.G.; Peulić, A.S. Anxiety-like behavioural effects of extremely low-frequency electromagnetic field in rats. Environ Sci Pollut Res Int 2017, 24(27), 21693-21699; doi: 10.1007/s11356-017-9710-1
63. Ehnert, S.; Fentz, A. K.; Schreiner, A.; Birk, J.; Wilbrand, B.; Ziegler, P.; Reumann, M. K.; Wang, H.; Falldorf, K.; Nussler, A. K. Extremely low frequency pulsed electromagnetic fields cause antioxidative defense mechanisms in human osteoblasts via induction of •O2- and H2O2. Scientific reports 2017, 7(1), 14544; doi.org/10.1038/s41598-017-14983-9
64. Haghighat, N.; Abdolmaleki, P.; Parnian, J.; Behmanesh, M. The expression of pluripotency and neuronal differentiation markers under the influence of electromagnetic field and nitric oxide. Mol Cell Neurosci 2017, 85, 19-28; doi: 10.1016/j.mcn.2017.08.005
65. Kuzay, D.; Ozer, C.; Sirav, B.; Canseven, A.G.; Seyhan, N. Oxidative effects of extremely low frequency magnetic field and radio frequency radiation on testes tissues of diabetic and healthy rats. Bratisl Lek Listy 2017, 118(5), 278-282; doi: 10.4149/BLL_2017_055
66. Sakhaie, M.H.; Soleimani, M.; Pourheydar, B.; Majd, Z.; Atefimanesh, P.; Asl, S.S.; Mehdizadeh, M. Effects of Extremely Low-Frequency Electromagnetic Fields on Neurogenesis and Cognitive Behavior in an Experimental Model of Hippocampal Injury. Behav Neurol 2017, 2017, 9194261; doi: 10.1155/2017/9194261
67. Urnukhsaikhan, E.; Mishig-Ochir, T.; Kim, S.C.; Park, J.K.; Seo, Y.K. Neuroprotective Effect of Low Frequency-Pulsed Electromagnetic Fields in Ischemic Stroke. Appl Biochem Biotechnol 2017, 181(4), 1360-1371; doi: 10.1007/s12010-016-2289-z
68. Budziosz, J.; Stanek, A.; Sieroń, A.; Witkoś, J.; Cholewka, A.; Sieroń, K. Effects of Low-Frequency Electromagnetic Field on Oxidative Stress in Selected Structures of the Central Nervous System. Oxid Med Cell Longev 2018, 2018:1427412; doi: 10.1155/2018/1427412
69. Burman, O.; Marsella, G.; Di Clemente, A.; Cervo, L. The effect of exposure to low frequency electromagnetic fields (EMF) as an integral part of the housing system on anxiety-related behaviour, cognition and welfare in two strains of laboratory mouse. PLoS One 2018, 13(5):e0197054; doi: 10.1371/journal.pone.0197054
70. Cichoń, N.; Rzeźnicka, P.; Bijak, M.; Miller, E.; Miller, S.; Saluk, J. Extremely low frequency electromagnetic field reduces oxidative stress during the rehabilitation of post-acute stroke patients. Adv Clin Exp Med 2018A, 27(9), 1285-1293; doi: 10.17219/acem/73699
71. Cichoń, N.; Bijak, M.; Czarny, P.; Miller, E.; Synowiec, E.; Sliwinski, T.; Saluk-Bijak, J. Increase in Blood Levels of Growth Factors Involved in the Neuroplasticity Process by Using an Extremely Low Frequency Electromagnetic Field in Post-stroke Patients. Front Aging Neurosci 2018B, 10, 294; doi: 10.3389/fnagi.2018.00294
72. Fathi, E.; Farahzadi, R. Zinc Sulphate Mediates the Stimulation of Cell Proliferation of Rat Adipose Tissue-Derived Mesenchymal Stem Cells Under High Intensity of EMF Exposure. Biol Trace Elem Res 2018, 184(2), 529-535; doi: 10.1007/s12011-017-1199-4
73. Laszlo, A.M.; Ladanyi, M.; Boda, K.; Csicsman, J.; Bari, F.; Serester, A.; Molnar, Z.; Sepp, K.; Galfi, M.; Radacs, M. Effects of extremely low frequency electromagnetic fields on turkeys. Poult Sci 2018, 97(2), 634-642; doi: 10.3382/ps/pex304
74. Martínez-Sámano, J.; Flores-Poblano, A.; Verdugo-Díaz, L.; Juárez-Oropeza, M.A.; Torres-Durán, P.V. Extremely low frequency electromagnetic field exposure and restraint stress induce changes on the brain lipid profile of Wistar rats. BMC Neurosci 2018, 19(1), 31; doi: 10.1186/s12868-018-0432-1
75. Rezaei-Tavirani, M.; Hasanzadeh, H.; Seyyedi, S.; Ghoujeghi, F.; Semnani, V.; Zali, H. Proteomic Analysis of Extremely Low-Frequency ElectroMagnetic Field (ELF-EMF) With Different Intensities in Rats Hippocampus, Arch Neurosci 2018, 5(1):e62954; doi: 10.5812/archneurosci.62954

76. Song, K.; Im, S.H.; Yoon, Y.J.; Kim, H.M.; Lee, H.J.; Park, G.S. A 60 Hz uniform electromagnetic field promotes human cell proliferation by decreasing intracellular reactive oxygen species levels. PLoS One 2018, 13(7), e0199753; doi:10.1371/journal.pone.0199753
77. Sun, Y.; Shi, Z.; Wang, Y.; Tang, C.; Liao, Y.; Yang, C.; Cai, P. Coupling of oxidative stress responses to tricarboxylic acid cycle and prostaglandin E2 alterations in Caenorhabditis elegans under extremely low-frequency electromagnetic field. Int J Radiat Biol 2018, 94(12), 1159-1166; doi: 10.1080/09553002.2019.1524943
78. Wyszkowska, J.; Jędrzejewski, T.; Piotrowski, J.; Wojciechowska, A.; Stankiewicz, M.; Kozak W. Evaluation of the influence of in vivo exposure to extremely low-frequency magnetic fields on the plasma levels of pro-inflammatory cytokines in rats. Int J Radiat Biol 2018, 94(10), 909-917; doi: 10.1080/09553002.2018.1503428
79. Cichoń, N.; Saluk-Bijak, J.; Miller, E.; Sliwinski, T.; Synowiec, E.; Wigner, P.; Bijak, M. Evaluation of the effects of extremely low frequency electromagnetic field on the levels of some inflammatory cytokines in post-stroke patients. J Rehabil Med 2019, 51(11), 854-860; doi: 10.2340/16501977-262
80. Hosseinabadi, M.B.; Khanjani, N. The Effect of Extremely Low-Frequency Electromagnetic Fields on the Prevalence of Musculoskeletal Disorders and the Role of Oxidative Stress. Bioelectromagnetics 2019A, 40(5), 354-360; doi: 10.1002/bem.22198
81. Hosseinabadi, M.B.; Khanjani, N.; Ebrahimi, M.H.; Haji, B.; Abdolahfard, M. The effect of chronic exposure to extremely low-frequency electromagnetic fields on sleep quality, stress, depression and anxiety. Electromagn Biol Med 2019B, 38(1), 96-101; doi: 10.1080/15368378.2018.1545665
82. Karimi, S.A.; Salehi, I.; Shykhi, T.; Zare, S.; Komaki, A. Effects of exposure to extremely low-frequency electromagnetic fields on spatial and passive avoidance learning and memory, anxiety-like behavior and oxidative stress in male rats. Behav Brain Res 2019, 359, 630-638; doi: 10.1016/j.bbr.2018.10.002
83. Merla, C.; Liberti, M.; Consales, C.; Denzi, A.; Apollonio, F.; Marino, C.; Benassi, B. Evidences of plasma membrane-mediated ROS generation upon ELF exposure in neuroblastoma cells supported by a computational multiscale approach. Biochim Biophys Acta Biomembr 2019, 1861(8), 1446-1457; doi: 10.1016/j.bbamem.2019.06.005
84. Sun, Y.; Huang, X.; Wang, Y.; Shi, Z.; Liao, Y.; Cai, P. Lipidomic alteration and stress-defense mechanism of soil nematode Caenorhabditis elegans in response to extremely low-frequency electromagnetic field exposure. Ecotoxicol Environ Saf 2019, 170, 611-619; doi: 10.1016/j.ecoenv.2018.11.137
85. Mahaki, H.; Jabarivasal, N.; Sardanian, K.; Zamani, A. Effects of Various Densities of 50 Hz Electromagnetic Field on Serum IL-9, IL-10, and TNF-α Levels. Int J Occup Environ Med 2020, 11(1), 24-32; doi: 10.15171/ijoem.2020.1572
86. Touitou, Y.; Lambrozo, J.; Mauvieux, B.; Riedel, M. Evaluation in humans of ELF-EMF exposure on chromogranin A, a marker of neuroendocrine tumors and stress. Chronobiol Int 2020, 37(1), 60-67; doi: 10.1080/07420528.2019.1683857

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2022, Article ID 1031211, 14 pages
https://doi.org/10.1155/2022/1031211

Research Article

Bidirectional Effect of Repeated Exposure to Extremely Low-Frequency Electromagnetic Field (50 Hz) of 1 and 7 mT on Oxidative/Antioxidative Status in Rat's Brain: The Prediction for the Vulnerability to Diseases

Angelika Klimek,[1] **Anna Nowakowska**,[1] **Hanna Kletkiewicz**,[1] **Joanna Wyszkowska**,[1] **Justyna Maliszewska**,[1] **Milena Jankowska**,[1] **Lukasz Peplowski**,[2] **and Justyna Rogalska**[1]

[1]*Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, 87-100, Poland*
[2]*Department of Biophysics, Institute of Physics, Faculty of Physics, Astronomy and Informatics Nicolaus Copernicus University in Torun, 87-100, Poland*

Correspondence should be addressed to Angelika Klimek; klimek@doktorant.umk.pl

Received 20 December 2021; Accepted 11 May 2022; Published 14 June 2022

Academic Editor: Ana Lloret

Studies reported evidence for opposite effects of extremely low-frequency electromagnetic field (EMF): harmful, including the oxidative stress induction, and beneficial, such as the activation of antioxidant defense. People's exposure to EMF is often repeated or prolonged, and it is important to consider the cumulative effect of such kind of exposure on the organism. If changes evoked by repeated exposure to EMF are permanent, responsiveness to other stress factors can be modified. The aims of our study were (1) to evaluate changes in the levels of oxidative stress and antioxidant defense markers in the prefrontal cortex of adult rats after repeated exposure to 1 and 7 mT EMF and (2) to assess whether repeated EMF exposure can modify oxidative/antioxidative status in response to other stress factors. Rats were exposed to EMF 1 h/day for 7 days, one, twice, or three times. After each exposure, 8-isoprostanes, protein carbonyl groups, and the total antioxidant capacity were assessed. Part of the animals, after EMF treatment, was exposed to another stress factor—open field. Results showed that repeated exposure changed the oxidative/antioxidative status depending on the intensity of the EMF and the number of exposures. 1 mT EMF created weak changes in the oxidative status in the brain; however, 7 mT EMF moved the balance to a clearly higher level. The changes in the oxidative status after 1 mT EMF were enough to reduce, and after 7 mT EMF to intensify oxidative processes in response to the next stress. We concluded that the organism might adapt to "weak" EMF, while "strong" EMF exceeds the adaptive capacity of the organism and sensitizes it to subsequent stress, and thus may modulate vulnerability to diseases. Our results also provide new insights into the possible therapeutic properties of the magnetic field, as 1 mT EMF appears to have a potentially protective impact on the brain.

1. Introduction

The influence of the extremely low-frequency electromagnetic field (EMF) on living organisms is still being widely investigated. EMF includes frequencies in the range of 3-300 Hz, which also correspond to physiological brain oscillations [1]. The most common frequencies values in our environment are 50 Hz and 60 Hz [2, 3]. Nowadays, people are increasingly exposed to this type of electromagnetic field due to the increasing number of electrical devices in our urbanized environment. EMF exposure has been proved to be an important risk factor in the development of diseases

affecting the nervous system, such as anxiety or depression [4]. The brain is an organ extremely sensitive to oxidative stress because of the high amount of unsaturated lipids, energetics dependent on mitochondria, neurotransmitters' metabolism generating hydrogen peroxide (H_2O_2), the ability of neurotransmitters to autooxidation, and engagement of reactive oxygen species in Ca^{2+} signaling [5]. Oxidative stress underlies many neurodegenerative and neuropsychiatric disorders [6].

The results of studies concerning the effect of EMF (50/60 Hz) on oxidative stress and antioxidant defense are often contradictory or insufficient. It means that the answer to the question whether EMF has a beneficial or detrimental impact on the organism is not obvious. Some authors reported evidence for the harmful effects of EMF, including oxidative stress induction [7–9]. The increased level of one of the most common markers of lipid peroxidation, malondialdehyde (MDA), was noticed in a several studies on humans and animal models exposed to EMF [7, 8]. In addition, oxidative damages are often accompanied by a decrease in antioxidant defense [8–10]. On the other hand, the increase of reports on the benefits associated with exposure to EMF such as activation of antioxidant systems is observed [11–13]. These protective effects of EMF are also used in medicine, e.g., for the treatment of brain damages [13]. It is also important that EMF exposure experienced by people is often repeated or prolonged, but most research evaluates the effect of the single period of EMF exposure.

EMF is recognized as a mild stress factor [2, 14], which as other stress factors can result in different activations of stress response systems; it means that exposure to EMF can establish a new "set-point" for stress systems activity. However, the course of this phenomenon (the direction and dynamics) depends on the intensity of the electromagnetic field. As a consequence, the EMF-induced changes in stress hormones can boost "cellular hormesis" by activation of signaling pathways including oxidative/antioxidative processes in different ways [15].

Here, we propose the hypothesis that the impact of an extremely low-frequency electromagnetic field on oxidant/antioxidant balance is not definitely negative, and the direction and dynamics of EMF-induced changes depend on the value of magnetic flux density as well as on the number of exposures. During repeated exposure to some stress factor, the organism responds to each individual stressor; however, consequently, the cumulative effect of all exposures is observed. As a result, temporary or permanent regulatory changes can be evoked [16]. The first exposure to stress generates the stress-induced initial disruption in homeostasis. As the response, the processes to reestablish the balance are triggered and they require gene expression and protein synthesis that progresses over time; thus, the temporal feature of the response to repetitive stress is essential [17]. We assumed that the first exposure to EMF changes the oxidative/antioxidative status; thus, each next EMF exposure would overlap its level established under the previous EMF exposure.

Therefore, the first aim of the study was to evaluate the changes in the levels of oxidative stress as well as the antioxidant defense markers in the prefrontal cortex of adult rats after repeated exposure to EMF of two different values of magnetic flux density (1 and 7 mT). The prefrontal cortex seems to be more sensitive to oxidative stress than other brain structures and concerning its role in the development of nervous system-related diseases; it appears to be relevant for research of the impact of EMF on oxidative/antioxidative status [18]. We have chosen to evaluate the effects of EMF of 1 and 7 mT. According to European Union Directive 2013/35/EU, the threshold value for magnetic flux density of the minor transient changes in the brain is set to a value of 1 mT EMF for 50 Hz. On the other hand, the exposure of workers to 50 Hz magnetic fields must be limited to the value 6 mT [19]. It has been shown that exposure to EMF of intensity higher than 6 mT causes measurable biological effects, e.g., increased lipid peroxidation [20, 21], DNA damage [22], and neuronal networks synchrony firing generation [23].

Then, we assumed that if the changes evoked by repeated exposure to EMF are permanent, the responsiveness to the other kinds of stress can be modified. It means that EMF can change the vulnerability of the organism to subsequent stress factors and thus to diseases, mainly related to the nervous system, in a two-way manner: compensatory or detrimental ones. Intriguingly, many nervous system-related disorders may be accounted for by a 'two-hit model' in which environmental stressors change the central nervous system function in the permanent way leading to changes in vulnerability to a "second hit," in turn leading to the onset of disease [24]. Thus, the second aim of the research was to assess whether the consequence of repeated EMF exposure can be the modification of the oxidative/antioxidative status in response to subsequent, other stress factors.

2. Materials and Methods

2.1. Animals. A total of 175 male adults (3-month-old) Wistar rats weighing 300-350 g were used throughout the experimentation. The number of animals in the research was planned in accordance with 3R principles (replacement, reduction, and refinement; EU Directive 2010/63/EU) [25]. Rats were housed in plastic cages in a temperature/humidity/light-controlled laboratory conditions ($22 \pm 2^{\circ}$C, humidity $55 \pm 10\%$, 12 : 12 h light : dark cycle with lights on at 7:00 a.m.). Rodent laboratory feed and drinking water were provided ad libitum. All experimental procedures were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decision number: 3/2018).

2.2. In Vivo Electromagnetic Field Exposure System. Animals were exposed to 50 Hz sinusoidal electromagnetic field of 1 mT and 7 mT (root mean square (RMS)) flux density, generated in a coil of 0.1 m in radius, designed by EiE (Elektronika i Elektromedycyna Sp. J., Otwock, Poland). This exposure system has been described in detail previously [3, 26]. The EMF was regulated during exposure by input current to the coil, and the magnetic flux density was measured before each experiment with a Gauss meter (Model GM2, AlphaLab, Inc., USA). The nonhomogeneity of the EMF within the area containing the animal's cage was < 15% (Figure 1). The temperature during all

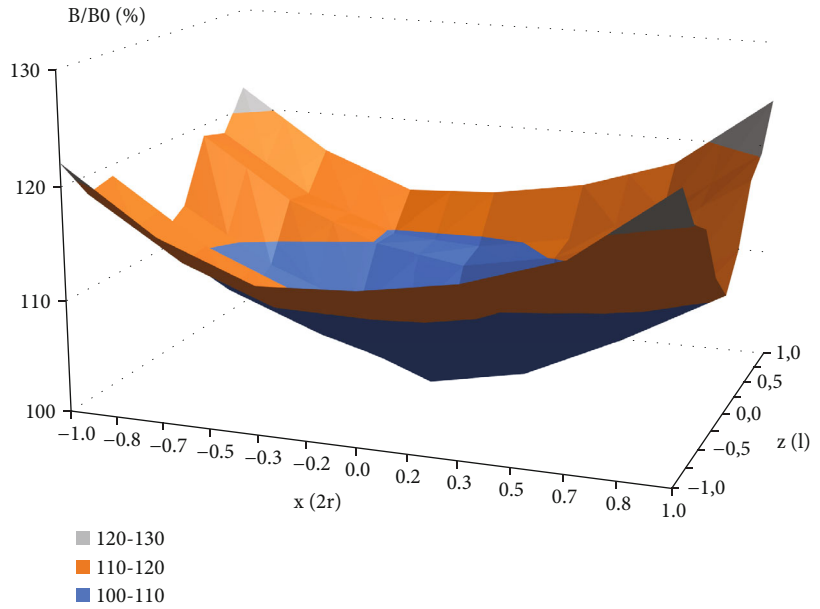


Figure 1: The plot of the mean value of magnetic flux density within the area of the animal's cage in the coil. Abbreviations: *B*: magnetic flux density vector, *B*/*B*0: normalized magnetic flux density relative to the value in the geometrical centre of the coil; *z*/*l*: normalized distance from the coil centre along *z*-axis; *x*/2*r*: normalized distance from the solenoid centre along *x*-axis; *l*: coil length; *r*: coil radius

experiments was monitored using thermocouples mounted in the animal cages, and it was set to 26 ± 1°C.

2.3. Experimental Design. One week after habituation to the laboratory environment, the rats in individual plastic cages (12 cm × 20 cm × 14 cm, with a perforated plexiglass cover and wood shavings bedding) were put into the centre of the EMF coil. Exposure to "low" (1 mT) and "high" (7 mT) EMF was performed for three periods described as *E*1, *E*2, and *E*3. Each period included 7-day exposure, 1 h/1day. Rats subjected to the same experimental procedure except electromagnetic field exposure were used as the control.

The experimental design included two sets of experiments (Figure 2). During the first set of experiments, a part of rats ($n = 88$) after each period of EMF or control exposure (*E*1, *E*2, and *E*3) was decapitated to estimate the effect of EMF on oxidant/antioxidant status (8-isoprostanes (8-epi PGF2α), protein carbonyl (CP) groups, and level of total antioxidant capacity (TAC)—described in this research as "basal" (B) level) (Figure 2(a)). The remaining rats from each group ($n = 87$) were used during the second set of experiments (Figure 2(b)). The experiments as previously included one to three periods of EMF or control exposure (*E*1, *E*2, or *E*3), and after each of them the part of animals was exposed to another kind of stress factor—open field (OF) (size of the box: 100 cm × 100 cm, duration of the test: 5 min). It is a stress-induced procedure (exposure to a novel, open, light environment), which was proved to evoke the activation of brain stress systems. Then, the animals were decapitated for assessment of stress-induced changes in oxidant/antioxidant parameters as a consequence of previous exposure to EMF. To avoid the influence of the circadian rhythm on the results, decapitation was performed between 10:00 and 12:00 am.

The rats were divided into six groups: (1) EMF/B/1mT: animals exposed to EMF (50 Hz, 1 mT), in which the basal (B) level of markers was assessed; (2) EMF/OF/1mT: animals exposed to EMF (50 Hz, 1 mT) and exposed to open field test (OF); (3) EMF/B/7mT: animals exposed to EMF (50 Hz, 7 mT) in which the basal (B) level of markers was assessed; (4) EMF/OF/7mT: animals exposed to EMF (50 Hz, 7 mT) and exposed to open field test (OF); (5) C/B: control animals subjected to the same experimental procedure as the experimental groups 1 and 3, except EMF exposure; (6) C/OF: control animals subjected to the same experimental procedure as the experimental groups 2 and 4, except EMF exposure.

2.4. Sample Collection. After decapitation, the part of the brain (prefrontal cortex) was quickly dissected. Each fragment of tissue was weighed and immediately frozen in liquid nitrogen and stored at −80°C until further biochemical analysis. For determination of oxidant/antioxidant status, brain samples were homogenized on ice in phosphate buffer pH 7.4. After centrifugation for 10 minutes at 12000 x g, the supernatants were collected in Eppendorf tubes and stored until they were used for the assessment of the level of protein carbonyl (CP) groups and 8-isoprostanes (8-epi PGF2α) as a result of proteins and phospholipid peroxidation, respectively, as well as the total nonenzymatic antioxidant capacity (TAC).

2.5. Determination of the Markers of Oxidant/Antioxidant Status Level. Protein carbonyl (CP) groups and 8-isoprostanes (8-epi PGF2α) as well as total nonenzymatic antioxidant capacity (TAC) concentrations were determined with commercial

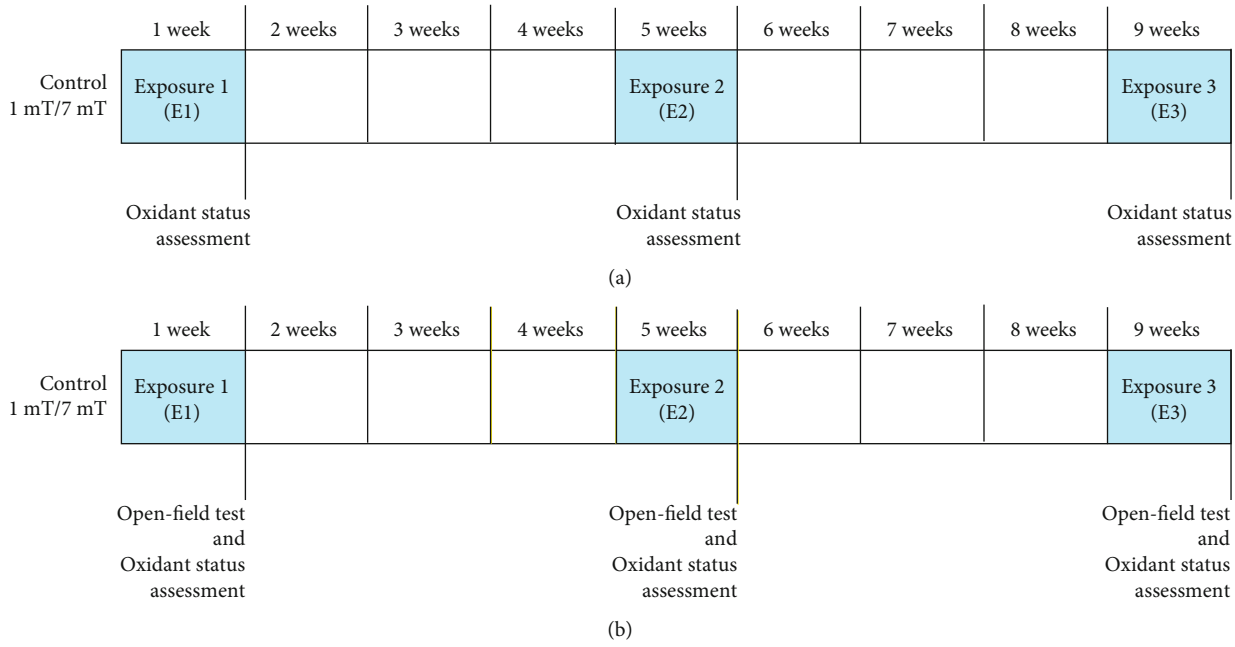


Figure 2: Experimental design. (a) First set of experiments: assessment of basal level of oxidative stress markers and antioxidants. (b) Second set of experiments: assessment of open field-induced level of oxidative stress markers and antioxidants.

kits according to the manufacturers' instructions. Each sample was assayed in triplicate. Colorimetric changes in the assay were detected using a multimode microplate reader Epoch 2 (BioTek Instruments, Inc., Winooski, UT, USA).

2.5.1. Determination of Protein Peroxidation. The level of end product of protein peroxidation-protein carbonyl (CP) groups was assayed using a Protein Carbonyl Content Assay Kit (Sigma-Aldrich, No MAK094, USA) based on their reaction with 2,4-dinitrophenylohydrazine (DNPH). Finally, dinitrophenyl (DNP) hydrazone adducts were formed and then were detected spectrophotometrically at 375 nm. The level of DNP was proportional to the concentrations of CP. The amount of carbonyl in the sample well was calculated per 1 mg of protein. Results were expressed as nanomoles per mg of protein.

2.5.2. Determination of Phospholipid Peroxidation. The level of end product of phospholipid peroxidation–8-isoprostanes (8-epi PGF2α) was assayed using 8-isoprostane ELISA Kit (Cayman Chemical, No 516351, USA). 8-Isoprostane has been proposed as a marker of oxidative stress and antioxidant deficiency. Isoprostanes are prostaglandin (PG) isomers that are generated from polyunsaturated fatty acids, mainly from arachidonic acid by a nonenzymatic process that involves peroxidation of membrane phospholipids by free radicals and reactive oxygen species. The absorbance of samples was measured at 406 nm. The concentration of 8-epi PGF2α was expressed as picograms per milliliter of sample.

2.5.3. Determination of Total Antioxidant Capacity. TAC was determined using a Total Antioxidant Capacity Assay Kit (Sigma-Aldrich, No MAK187, USA) in which concentrations of both small molecules and protein antioxidants were determined. Determination of TAC is based on reduction Cu^{2+} to Cu^{+} by both small molecules and proteins but using the Protein Mask prevents Cu^{2+} reduction by proteins. Finally, the amount of reduced Cu^{+} ion enabling the analysis of small molecule antioxidants. The reduced Cu^{+} ions chelate with a colorimetric probe. The absorbance (peak at 570 nm) is proportional to the TAC level in Trolox equivalents (a water-soluble vitamin E analogue used as an antioxidant standard). Results were calculated as nanomoles per milliliter of sample.

2.6. Data Analysis. To analyze the effect of repeated exposure to EMF on the markers of oxidative stress and total antioxidant capacity, we applied a general linear model (GLM) allowing to determine the effect of a combination of two fixed categorical factors: EMF intensity and number of exposures (1 mT, 7 mT vs. *E*1, *E*2, and *E*3). The levels of the open field-induced oxidative stress and antioxidant markers were compared to their basal levels for each group, respectively: C/B vs. C/OF, EMF/B/1mT vs. EMF/OF/1mT, and EMF/B/7mT vs. EMF/OF/7mT using GLM with open field/basal level and number of exposures as fixed categorical factors. If necessary the data were log-transformed after checking for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). Differences between compared data were considered significant when $P < 0.05$. The analyses were carried out using SPSS 25.0 package (IBM Inc.).

3. Results

3.1. The Basal Level of Oxidative Stress Markers

3.1.1. Protein Carbonyl (CP) Groups. The intensity of EMF, as well as the number of exposures, had an influence on

Table 1: Results of statistical analysis of the oxidative markers concentrations in the prefrontal cortex.

	Dependent variable	Effect	*df*	*F*	*P*
a	CP basal level	Intensity of the electromagnetic field (mT)	2	22.133	*<0.001*
		Number of exposures (*E*1-*E*3)	2	16.681	*<0.001*
		(mT) × (*E*1-*E*3)	4	9.851	*<0.001*
		Error	68		
b	8-epi PGF2*α* basal level	Intensity of the electromagnetic field (mT)	2	15.231	*<0.001*
		Number of exposures (*E*1-*E*3)	2	0.191	0.827
		(mT) × (*E*1-*E*3)	4	2.343	0.063
		Error	73		
c	Open field-induced CP level	Intensity of the electromagnetic field (mT)	2	190.737	*<0.001*
		Number of exposures (*E*1-*E*3)	2	19.793	*<0.001*
		(mT) × (*E*1-*E*3)	4	12.051	*<0.001*
		Error	71		
d	Open field-induced 8-epi PGF2*α* level	Intensity of the electromagnetic field (mT)	2	13.921	*<0.001*
		Number of exposures (*E*1-*E*3)	2	0.657	0.522
		(mT) × (*E*1-*E*3)	4	7.753	*<0.001*
		Error	66		

F: GLM; statistically significant *P* value: indicated in italic; CP: protein carbonyl groups.

the basal level of CP groups in the prefrontal cortex. The CP groups' level increased with increasing EMF intensity, and the subsequent exposures enhanced the effect. There was also a significant interaction between these two factors (Table 1(a), Figure 3(a)). After first (*E*1) and second exposures (*E*2) to EMF of 1 mT the level of CP groups in the rat's brain did not differ from the control level; however, CP level after *E*2 was significantly higher than that after *E*1 ($P < 0.01$). After the third exposure (*E*3), CP level increased significantly compared to the control group ($P < 0.001$) and was also significantly higher than that noticed after *E*1 ($P < 0.001$) and *E*2 ($P < 0.01$). In animals exposed to EMF of 7 mT after *E*1, we observed a slight elevation in the level of the CP groups in comparison to control values, after *E*2 as well as after *E*3; however, the increase in CP level was significant ($P < 0.001$). Moreover, the statistically significant increase in the CP level after *E*2 was observed in comparison to that observed after *E* 1 ($P < 0.01$), whereas after *E*3, the CP level was not significantly different from the value determined after *E*2. In animals exposed to EMF of 7 mT, the level of CP was enhanced in comparison to the value in the EMF/B/1mT group after *E*1 and *E*2 ($P < 0.001$) and the difference disappeared after *E*3.

3.1.2. 8-Isoprostanes (8-epi PGF2α). The level of 8-epi PGF2*α* after exposure to EMF was dependent only on the strength of the electromagnetic field (Table 1(b), Figure 3(b)). The exposure to EMF of 1 mT did not significantly affect the level of 8-epi PGF2*α* regardless of the number of exposures. Also, the analysis showed no clear differences between values of this parameter observed after *E*1 to *E*3. However, the tendency to decrease in the level of 8-epi PGF2*α* with each subsequent exposure was observed (Figure 3(b)). Otherwise, in the EMF/B/7mT group, the tendency to increase in the level of 8-epi PGF2*α* with each next exposure was noticed (Figure 3(b)). After *E*3, the level of 8-epi PGF2*α* was clearly higher in the group exposed to EMF of 7 mT compared to the control group as well as to that in the EMF/B/1mT group ($P < 0.001$).

3.2. The Open Field-Induced Level of Oxidative Stress Markers

3.2.1. Protein Carbonyl (CP) Groups. The direction of changes in CP level in animals exposed to EMF of 1 mT and 7 mT after exposure to subsequent stress factor—open field test (EMF/OF/1mT and EMF/OF/7mT groups) coincided with their basal level observed only after exposure to electromagnetic field (EMF/B/1mT and EMF/B/7mT groups). Both intensity of the EMF and the number of exposures had an effect on the open field-induced level of CP groups. The interaction of both factors was also significant (Table 1(c), Figure 4(a)). There were no significant differences between the EMF/OF/1mT and C/OF groups after *E*1 and *E*2, but after *E*3 the level of CP in the group exposed to EMF of 1 mT was even lower in comparison to the value in the respective control group (C/OF) ($P < 0.001$). After a single exposure to EMF of 1 mT, the CP level was higher than that noticed after *E*2 and *E*3 ($P < 0.001$), while no significant difference was noticed between values after *E*2 and *E*3. The lowest value of CP groups in the EMF/OF/1mT group was observed after *E*3. The level of CP in the EMF/OF/7mT group was significantly increased compared to control animals after each exposure ($P < 0.001$) and was the highest after *E*3. The value observed after *E*2 was lower compared to both *E*1 and *E*3 ($P < 0.001$). Regardless of the number of exposures, the level of CP was always higher in the EMF/OF/7mT group in comparison to the values observed in the EMF/OF/1mT group ($P < 0.001$).

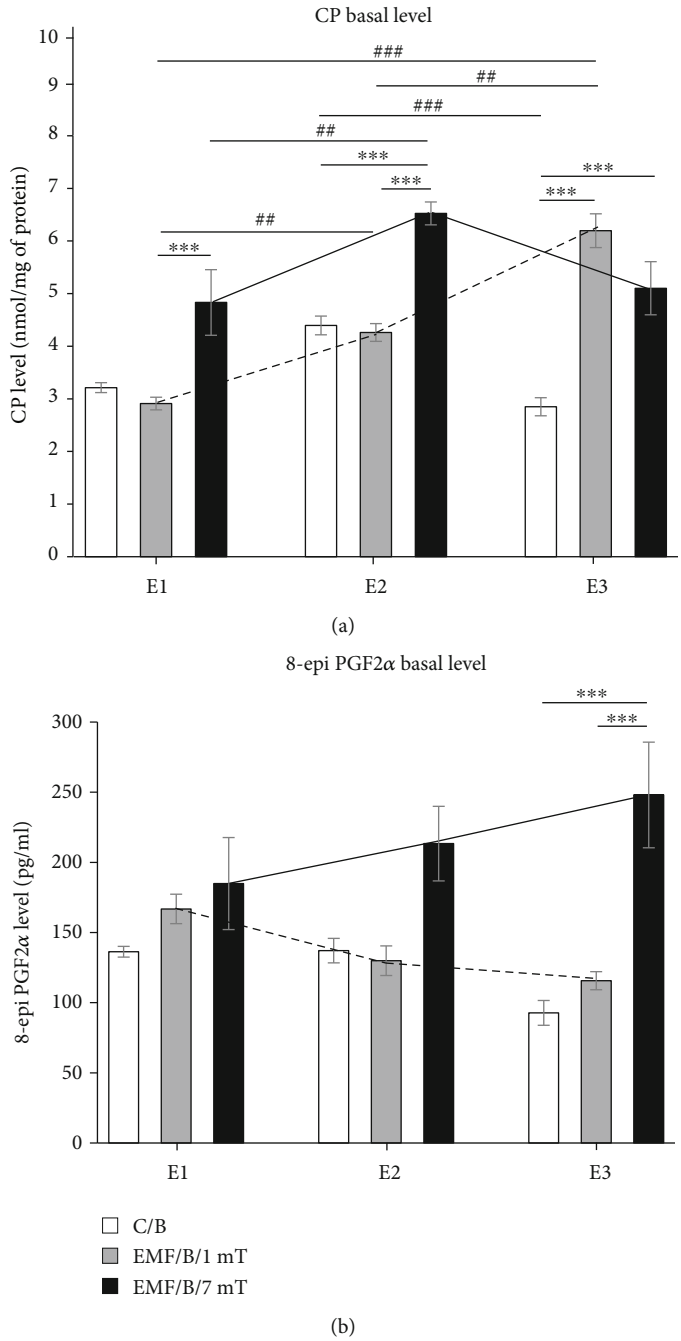


Figure 3: The basal level of oxidative stress markers: (a) CP groups and (b) 8-epi PGF2α in the prefrontal cortex of rat's brain. Animals were exposed once to three times ($E1$-$E3$) to EMF of 1 or 7 mT or control conditions. Values are presented as mean ± SEM. The lines show the direction of changes in the level of stress markers in 1 mT (dotted line) and 7 mT (solid line) groups after each subsequent exposure. Statistically significant differences between animals from the same group are denoted $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$; and these between experimental groups are denoted $^{***}P < 0.001$.

3.2.2. 8-Isoprostanes (8-epi PGF2α). The direction of OF-induced changes in the 8-epi PGF2α level was similar to that in the basal level of this marker. A significant impact of EMF intensity and the combination of two factors (EMF intensity x number of exposures) was noticed (Table 1(d); Figure 4(b)). There was no significant difference in 8-epi PGF2α level between the control group (C/OF) and animals exposed to EMF of 1 mT after $E1$; however, the level of this parameter after $E2$ was decreased compared to the control value ($P < 0.05$), and after $E3$, it returned to the control value. The decrease in 8-epi PGF2α level was also observed after $E2$ relative to $E1$ ($P < 0.05$). In the EMF/OF/7mT group, the clear increase of 8-epi PGF2α level compared to the control value was noticed only after $E3$ ($P < 0.001$); however, the tendency to increase

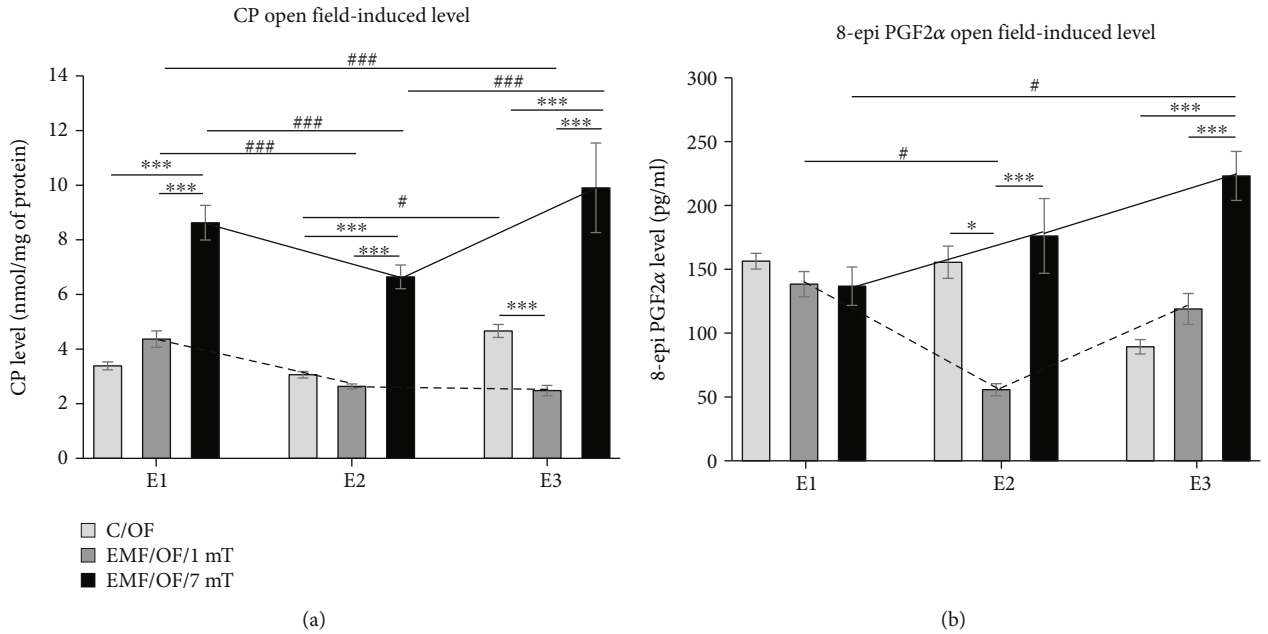


Figure 4: The open field-induced level of oxidative stress markers: (a) CP groups and (b) 8-epi PGF2α in the prefrontal cortex of rat's brain. Animals were exposed once to three times ($E1$-$E3$) to EMF of 1 or 7 mT or control conditions. Values are presented as mean ± SEM. The lines show the direction of changes in the level of stress markers in 1 mT (dotted line) and 7 mT (solid line) groups after each subsequent exposure. Statistically significant differences between animals from the same group are denoted $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$, and these between experimental groups are denoted $^{*}P < 0.05$ and $^{***}P < 0.001$.

in the level of 8-epi PGF2α was noticed with each subsequent exposure. The level of 8-epi PGF2α after $E3$ was also significantly higher compared to its value after $E1$ ($P < 0.05$). After second and third exposures to EMF of 7 mT, the significant increase of 8-epi PGF2α level relative to 1 mT was noticed ($P < 0.001$).

3.3. Comparison between Basal Level and Open Field Test-Induced Level of Oxidative Stress Markers. Table 2 presents the results of analysis comparing the basal levels of oxidative stress markers to their levels after the open field test for each group, respectively: C/B vs .C/OF, EMF/B/1mT vs. EMF/OF/1mT, and EMF/B/7mT vs. EMF/OF/7mT). The analysis showed that both the open field test and the number of exposures do not affect the CP level in the control group, only interaction of both factors had a significant influence on CP level in this group (Table 2(a)). In the group exposed to EMF of 1 mT, the CP level has been changed after open field, and the interaction between the open field test and the number of exposures also profoundly affected the CP level (Table 2(b)). In the group exposed to EMF of 7 mT, we have found the significant influence of the open field test on the increase in CP levels, and the number of exposures has no effect; however, the interaction between these factors was significant (Table 2(c)). The changes of 8-epi PGF2α level in the control group were dependent only on the number of exposures (Table 2(d)). The clearest effect of both factors as well as the interaction between them was noticed in rats exposed to EMF of 1 mT (Table 2(e)). In rats exposed to EMF of 7 mT, the 8-epi PGF2α level was not affected by any factor (Table 2(f)).

Valuable results were received when we evaluated the percentage changes in the level of stress markers in comparison to their level in the control C/B group separately after each exposure (set at 100%: reference value) (Figure 5).

CP level in the prefrontal cortex (Figure 5(a)) in group C/OF increased clearly after $E3$ (by 64%) in comparison to the C/B group. In the EMF/B/1 mT group, the level of CP was higher by 117% than that in the control group (C/B) after $E3$. Otherwise, after exposure to open field, the level of CP in rats previously exposed to EMF of 1 mT in comparison to the level in the EMF/B/1mT group was increased only after $E1$ (by 45%) and then was decreased even in comparison to both control groups (C/B and C/OF). In EMF/B/7mT, the level of CP after each subsequent exposure was higher in comparison to the value in the control group (C/B) (by 50 51 and 79%, respectively) and the open field test remarkably increased the level of the parameter after $E1$ (by 118%) and $E3$ (by 168%), except after $E2$ when the value of CP was similar to its basal level.

A similar direction of changes as in the case of CP groups level was observed in 8-epi PGF2α level in the control group as well as in groups exposed to EMF of 1 and 7 mT (Figure 5(b)). In the case of 8-epi PGF2α in animals exposed to EMF of 7 mT, the higher basal level of this marker should be noticed after each exposure in comparison to the control level (C/B) with its incredibly high level after $E3$ (267% of reference value). After OF test, the level of 8-epi PGF2α decreased slightly relative to its basal level in this group.

3.4. The Basal Level of Total Antioxidant Capacity (TAC). The intensity of EMF, as well as EMF intensity × number

Table 2: Results of statistical analysis of effects of open field stress on oxidative stress markers level in relation to its basal level in each experimental group.

	Dependent variable/group	Effect	*df*	*F*	*P*
a	CP Control group	Open field effect	1	1.964	0.168
		Number of exposures (*E*1-*E*3)	2	2.253	0.118
		Number of exposures × open field effect	2	32.078	*<0.001*
		Error	42		
b	CP EMF/1mT	Open field effect	1	52.023	*<0.001*
		Number of exposures (*E*1-*E*3)	2	3.077	0.055
		Number of exposures × open field effect	2	66.370	*<0.001*
		Error	49		
c	CP EMF/7mT	Open field effect	1	47.966	*<0.001*
		Number of exposures (*E*1-*E*3)	2	1.876	0.164
		Number of exposures × open field effect	2	12.193	*<0.001*
		Error	48		
d	8-epi PGF2α Control group	Open field effect	1	2.995	0.091
		Number of exposures (*E*1-*E*3)	2	33.444	*<0.001*
		Number of exposures × open field effect	2	1.422	0.252
		Error	44		
e	8-epi PGF2α EMF/1mT	Open field effect	1	14.228	*<0.001*
		Number of exposures (*E*1-*E*3)	2	15.181	*<0.001*
		Number of exposures × open field effect	2	6.000	*0.005*
		Error	47		
f	8-epi PGF2α EMF/7mT	Open field effect	1	2.005	0.163
		Number of exposures (*E*1-*E*3)	2	2.649	0.081
		Number of exposures × open field effect	2	0.064	0.938
		Error	48		

F: GLM; statistically significant*P*value: indicated in italic; CP: protein carbonyl groups.

of exposures interaction, had a significant influence on TAC level (Table 3(a), Figure 6(a)). The level of TAC in animals exposed to EMF of both intensities was not significantly different from that noticed in the C/B group. In the group exposed to EMF of 1 mT, only after *E*3 the tendency to decrease in the TAC level in comparison to the control value was observed. The only detectable decrease of TAC level in the EMF/B/1mT group was observed after *E*3 relative to that after *E*2 ($P < 0.05$). The TAC level in animals exposed to EMF of 7 mT was lower than that in the EMF/B/1mT group after *E*2 ($P < 0.05$). However, the tendency to decrease in the value of TAC level in animals exposed to EMF of 7 mT was observed after each subsequent exposure relative to its level in the C/B group.

3.5. The Open Field-Induced Level of Total Antioxidant Capacity (TAC). The direction of changes in the open field test-induced level of TAC in all groups was similar as in the case of its basal level. The strength of the electromagnetic field, as well as the number of exposures, significantly influenced the changes of open field-induced TAC level. The interaction between both factors was also notable (Table 3(b), Figure 6(b)). After a single exposure to EMF of 1 mT, the TAC level was higher compared to the value in the respective control group (C/OF) ($P < 0.05$). After *E*2, the value of TAC was comparable to the control level, and only after *E*3, it dropped significantly compared to the value in the control group ($P < 0.05$). In addition, in the EMF/OF/1mT group, the TAC level after *E*1 and *E*2 was clearly higher compared to that observed after *E*3 ($P < 0.001$). After first exposure to EMF of 7 mT, the level of TAC was significantly decreased compared to that in control animals exposed to OF (C/OF) ($P < 0.01$) as well as to its value in the EMF/OF/1mT group ($P < 0.001$). Moreover, in the EMF/OF/7mT group, the level of TAC was significantly decreased after *E*1 and *E*3 compared to that noticed after *E*2 ($P < 0.01$).

3.6. Comparison between Basal Level and Open Field Test-Induced Level of TAC. Any of the factors did not affect the TAC level in the control group (Table 4(a)). In the group exposed to EMF of 1 mT, the significant influence of the open field test and the number of exposures as well as their interaction on TAC level was found (Table 4(b)). In the 7 mT group, the number of exposures had a significant impact on TAC level. Moreover, the interaction between the open field test and the number of exposures was also significant (Table 4(c)).

The percentage changes in TAC level (Figure 7) in the control group after the open field test was clear. The value of antioxidant's level marker was increased in comparison

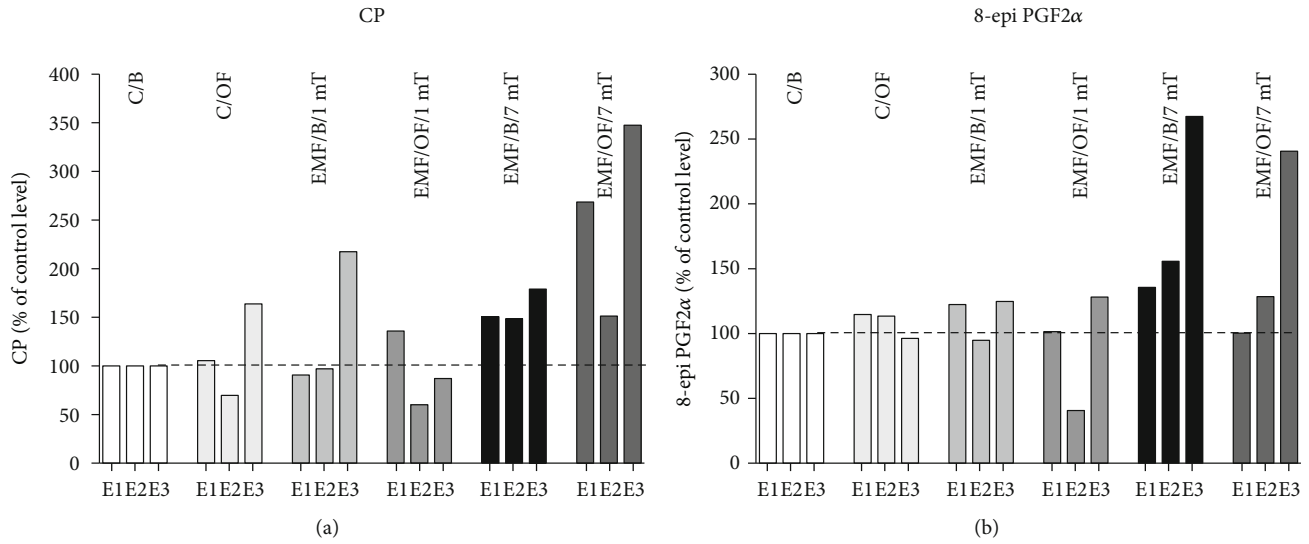


Figure 5: Percentage changes in the basal level as well as open field-induced level of oxidative stress markers: (a) CP groups and (b) 8-epi PGF2α in each experimental group in relation to their level in the control C/B group set at 100% after each subsequent exposure (*E*1-*E*3).

Table 3: Results of statistical analysis of the TAC level in the prefrontal cortex.

	Dependent variable	Effect	*df*	*F*	*P*
a	TAC basal level	Intensity of the electromagnetic field (mT)	2	5.088	*0.008*
		Number of exposures (*E*1-*E*3)	2	2.209	0.117
		(mT) × (*E*1-*E*3)	4	3.163	*0.018*
		Error	79		
b	Open field-induced TAC level	Intensity of the electromagnetic field (mT)	2	11.803	*<0.001*
		Number of exposures (E1-E3)	2	27.075	*<0.001*
		(mT) x (E1-E3)	4	11.086	*<0.001*
		Error	78		

F: GLM; statistically significant*P*value: indicated in italic; TAC: total antioxidant capacity.

to its basal level after *E*1 by 17%. In the EMF/OF/1mT group, the TAC amount was higher than that in the EMF/B/1mT group after *E*1 (by 45%) and *E*2 (by 15%), while after *E*3, the TAC level was close to its basal level. In rats from the EMF/B/7mT group after all exposures, the basal level of TAC was lower than that in the C/B group (by 13, 20, 3%, respectively). In the EMF/OF/7mT group, the noticed value of TAC after *E*1 was similar to the basal control value; after *E*2, the level of TAC was increased (by 24%); and then, after *E*3, the OF-induced level of TAC was decreased by 15% in comparison to values in EMF/B/7mT.

4. Discussion

Our experiments have shown that repeated exposure to the extremely low-frequency electromagnetic field (EMF) profoundly changes the oxidative/antioxidative status in the prefrontal cortex of rats in the EMF intensity- and number of exposure-dependent manner. The level of oxidative stress markers and antioxidants in rats exposed to EMF of 1 mT was not very different from the control value. In rats exposed to EMF of 1 mT, a significantly increased protein carbonyl groups level was noticed only after third exposure compared to the control group. In the case of 8-isoprostanes, the exposure to EMF of 1 mT did not significantly affect their level; however, the tendency to decrease with each following exposure was noticed. Moreover, in the 1 mT EMF-exposed group, the profound change in the basal TAC level was not found. The level of TAC was a little, not significantly increased after the first and second exposures.

Generally, most research confirmed the antioxidative effects of EMF of ≤ 1 mT. Patruno et al. [12] observed elevated catalase (CAT) activity in cell culture (myelogenous leukemia cells: K562) after exposure to 50 Hz 1 mT EMF concomitantly with a decrease in the activity of inducible nitric oxide synthase (iNOS). In an animal model of Huntington's disease EMF (60 Hz and 0.7 mT), exposure reduced levels of oxidative stress biomarkers [27]. In C2C12 muscle cells, no change in reactive oxygen species (ROS) production was observed after exposure to EMF of 1 mT [11]. The research indicating that even low levels (≤ 1 mT) of a single EMF exposure can cause an increase in

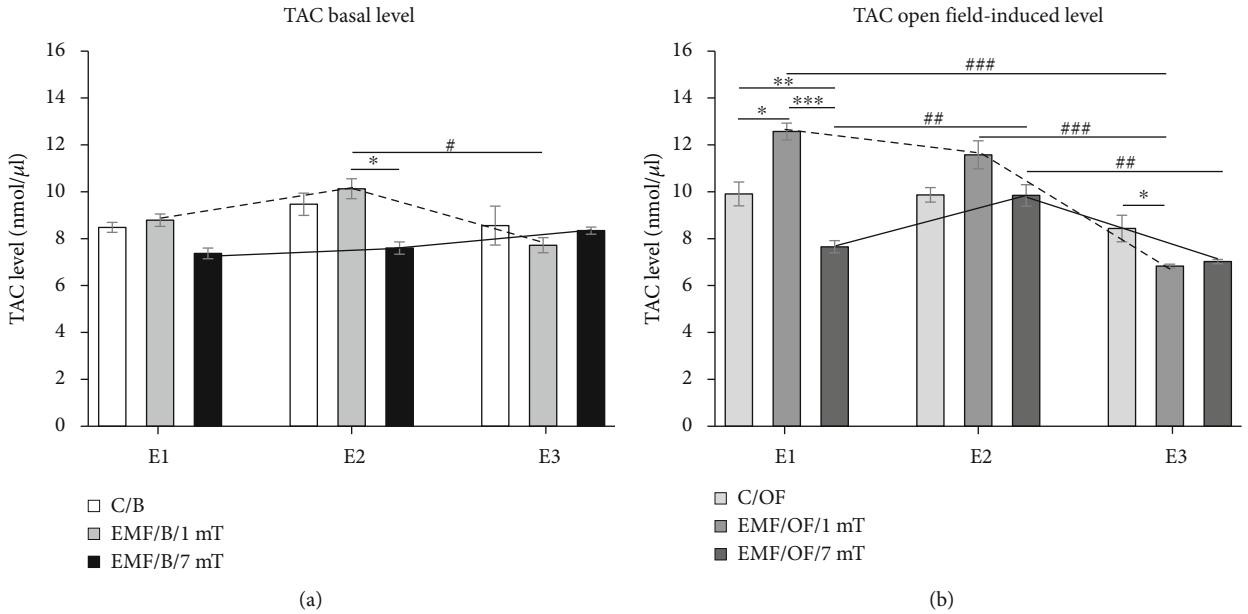


Figure 6: The basal (a) and open field-induced level of TAC (b) in the prefrontal cortex of the rat's brain. Animals were exposed once to three times (*E*1-*E*3) to EMF of 1 or 7 mT or control conditions. Values are presented as mean ± SEM. The lines show the direction of changes in the level of TAC in 1 mT (dotted line) and 7 mT (solid line) groups after each subsequent exposure. Statistically significant differences between animals from the same group are denoted $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$, and these between experimental groups are denoted $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Table 4: Results of statistical analysis of effects of open field stress on TAC level in relation to its basal level in each experimental group.

	Dependent variable/group	Effect	*df*	*F*	*P*
a	TAC Control group	Open field effect	1	1.375	0.246
		Number of exposures (*E*1-*E*3)	2	0.568	0.570
		Number of exposures × open field effect	2	0.905	0.411
		Error	52		
b	TAC EMF/1mT	Open field effect	1	12.290	*<0.001*
		Number of exposures (*E*1-*E*3)	2	39.426	*<0.001*
		Number of exposures × open field effect	2	12.297	*<0.001*
		Error	52		
c	TAC EMF/7mT	Open field effect	1	2.301	0.135
		Number of exposures (*E*1-*E*3)	2	7.958	*<0.001*
		Number of exposures × open field effect	2	13.983	*<0.001*
		Error	53		

F: GLM; statistically significant*P*value: indicated in italic; TAC: total antioxidant capacity.

oxidative stress [10, 28] can also be found. However, in the mentioned in vitro studies, the cells were treated with EMF continuously from 30 min to 24 h, or in animals, the procedure included the one, 21-day lasting period with EMF exposure 4 h/day. It is also important that the duration of exposure also determines the effect of EMF. In Caco 2 cells treated with 50 Hz EMF of 1 mT for 24 h, 48 h, or 72 h, the longer the exposure time, the greater level of oxidative stress was found [29]. It has been also shown that the long-term exposure to the extremely low-frequency magnetic field (100 and 500 μT) 2 h/day for 10 months caused a decrease in the activity of the antioxidant enzyme catalase (CAT). However, the TAC level was lower in the group exposed to 500 μT, and at the same time, in this group, the levels of oxidative stress markers, MDA and MPO (myeloperoxidase), as well as values of total oxidant status (TOS) and oxidative stress index (OSI), were significantly higher [30]. Concerning the risk of development of neurodegenerative disorders after EMF exposure, it is also important that neither 100 nor 500 μT extremely low-frequency magnetic field altered beta-amyloid protein level significantly [31]. It confirms the dose-dependent action of EMF.

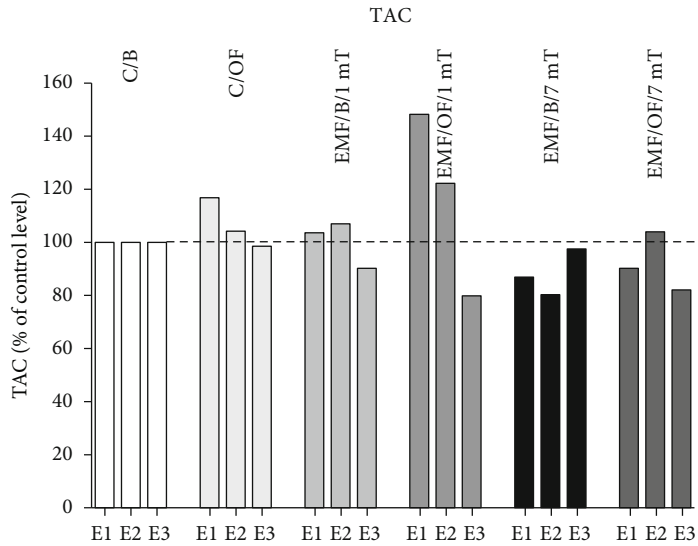


Figure 7: Percentage changes in the basal level as well as open field-induced level of TAC in each experimental group in relation to their level in control C/B group set at 100% after each subsequent exposure (*E*1-*E*3).

Our results showed that EMF of 1 mT creates weak changes in oxidative status in the rat's brain. Many studies showed that stress induces the disruption in homeostasis [15, 32], and as a consequence, an overcompensation response is triggered to reestablish homeostasis, and such effect was seen in response to EMF of 1 mT after two first exposures. The compensatory mechanisms driven during first exposure to EMF seem to be able to maintain the oxidative equilibrium even after the second exposure, only after the third exposure the cumulative effect of all exposures as an increase in oxidative stress simultaneously with a discrete decrease of antioxidants was visible.

In animals exposed to EMF of 7 mT, the characteristic of changes in oxidative stress and antioxidative defence markers was quite different. The clear increase of CP groups was visible earlier than in the group exposed to EMF of 1 mT— already after the second exposure and remained high after the third exposure. Moreover, the tendency to increase in the level of 8-epi PGF2α with each subsequent exposure was noticed, and eventually, after *E*3, the 8-epi PGF2α level was significantly higher (more than 2.5 times) than that in the control group. In animals exposed to EMF of 7 mT, the tendency to decrease in the TAC level was observed after each subsequent exposure relative to its level in the control group. The present results indicate that the changes in TAC level were accompanied by a parallel increase in oxidative stress markers. The other research also showed that exposure to EMF (40-50 Hz) of intensities close to this used in our research ($\geq$ 6 mT) alters oxidative stress and antioxidant defense parameters. In the serum of ICR mice, the level of MDA was significantly higher after exposure to EMF of 6, 8, and 10 mT, and simultaneously, the level of antioxidant enzyme superoxide dismutase (SOD) activity was decreased [8]. Similarly, in mouse brains subjected to EMF (8 mT), the levels of MDA, ROS, nitrogen oxide (NO), and nitric oxide synthase (NOS) were increased, whereas activities of antioxidants enzymes: SOD, CAT, and glutathione peroxidase (GPx), were decreased [33]. The disturbance of oxidative status was also found in testes of rats (diabetic model) exposed to EMF (8.2 mT) in the form of elevated MDA and NO levels and diminished glutathione (GSH) level was found [34]. The measurements of oxidative stress markers in the rat's heart and plasma after exposure to EMF of 7 mT showed a significant increase in thiobarbituric acid reactive substances (TBARS) and H_2O_2 concentration and the diminished antioxidant defense decreased TAC, GSH, and total free thiol groups level [20]. In vitro studies confirmed that after exposure to electromagnetic field (8 mT), a decrease in the viability and morphological changes of the rat hippocampal neurons were observed with an increase in the level of MDA and ROS and a decrease in the activity of SOD [22]. Otherwise, opposite results have also been received, e.g., poststroke patients after EMF therapy (7 mT) showed improved enzymatic antioxidant activity [13]. As in the case of studies on the effects of EMF of lower intensities $\leq$ 1 mT, the abovementioned results are the consequence of continuous exposure for 14-28 days (day by day, 15 min-4 h/day) or as in the case of in vitro studies— one 90-min lasting treatment. The present results indicate that in such challenging stress situations as repeated exposure to EMF of 7 mT, the balance is disturbed in the direction of a higher level of oxidative stress. It also suggests that not all homotypic stressors cause response habituation. Responses to more "severe" stressors are maintained over time, perhaps due to the higher costs required to adapt to the particular situational demand [16]. Thus, the cumulative effect of repeated exposure to EMF of high intensity resulted in increased oxidative stress.

In our research, we identified the directions and dynamics of some mechanisms that may be brought into play in EMF-provoked changes. Although moderate stimulation by EMF (1 mT) even repeated might be not demanding for the organism, if strong (7 mT), it can lead to deleterious effects due to the increase in oxidative stress. The integrative

processes between all "players" determining oxidative/antioxidative balance appear to determine the final effect of EMF [2, 15]. As a result, even subtle changes in the brain can change the function of the neuronal circuits. An undisturbed oxidative/antioxidative balance is essential for the normal function of the organism. Unbalanced concentrations of oxidative processes products decrease the chance of overcompensation and increase brain vulnerability to other potentially dangerous events.

We have found that the exposure of rats to EMF of 1 mT influenced the oxidative/antioxidative status evolved in response to subsequent (heterogenous in relation to EMF) stress factor—open field test. EMF of 1 mT diminished the response to another stressor as the small elevation of open field-induced CP concentration was visible only after the first exposure to EMF, each next period of EMF-treatment decreased the level of the marker. In the case of 8-isoprostanes, their level was diminished after two first exposures, and after E3 received a similar value as in the respective group not-exposed to OF. In 1 mT EMF-treated rats, the augmented antioxidant defense in response to a new stress factor was visible after first exposure; then, the adaptation as the decrease in TAC level was observed. The changes in the TAC level can partly explain the reduction in oxidative stress in this group. Our results allowed us to conclude that the subtle changes in the level of oxidative status in animals repeatedly exposed to EMF of 1 mT were enough to change the profile of oxidative processes after exposure to another kind of stress factor— open field test. It suggests that it can be a kind of habituation, when one stressor diminishes the response to the second one [16].

The EMF of 7 mT disturbs the oxidative/antioxidative balance as the changes in oxidative stress markers as well as antioxidant capacity level were still clear after each subsequent exposure to the open field. The pattern and size of the changes in 8-epi PGF2α level after subsequent exposures to open field in rats previously exposed to EMF of 7 mT were close to the changes in basal level. The open field-induced CP level in rats exposed to EMF of 7 mT has been increasing with each subsequent exposure and was definitely higher than that in respective control as well as in the 1 mT EMF-exposed group. The response to first contact with a new stressor was also the significant decrease in TAC level, which then increased to the value not different from that in the respective control group. Thus, we suggest that the exposure to one stressor (EMF) sensitizes the organism to a second stressor (open field), resulting in a faster onset of oxidative stress and its higher level in 7 mT exposed animals in comparison to the values in 1 mT EMF-treated rats. Djordjevic et al. [35] have also found that in rats exposed to open field after EMF exposure (50 Hz, 10 mT, 24 h for 7 days) the levels of superoxide anion and nitrites in the hypothalamus were increased compared to the control group; however, the observed changes are the synergic effect of both factors, as the oxidative status of rats after EMF exposure was not evaluated in this research. Our results suggest that a high level of magnetic flux density (7 mT) of EMF is able to disturb the brain oxidative/antioxidative status, and to shift its set-point in the direction of increase of oxidative processes and as a consequence can augment the oxidative processes in response to the next stress events.

5. Conclusions

Summarizing, as we hypothesized, the level of EMF appears to be essential for direction and dynamics of the stress response: changes in oxidative/antioxidative parameters after exposure to 1 mT EMF were observed at a lower level than these after exposure to 7 mT EMF; moreover, the character of changes was also different. Our data confirmed that the exposure to EMF of 1 mT can establish a new "set-point" for cellular oxidative processes and may initiate cellular adaptation by activation of intrinsic signaling pathways directed into the decrease of oxidative stress, although the cumulative effect of repeated exposure cannot be definitely excluded. Otherwise, in the case of a stronger electromagnetic field (7 mT), the adaptive processes are not sufficient to counteract its detrimental effects. Consequently, EMF can change the vulnerability of the organism to subsequent stress factors and thus to diseases, mainly related to the nervous system. Our research for the first time showed the different "mode of action" of EMF in relation to oxidative/antioxidative status in the brain dependently on its magnetic flux density value. In addition, what is even more important, we proved that the effects of EMF can be permanent and influence the response of the organism to other stress events and in this way modulate the vulnerability to the diseases. We are convinced that the results of our research extended the knowledge on mechanisms of EMF's impact on human health. Further, the elucidation of the EMF-induced changes in the oxidative/antioxidative status is necessary to a reliable assessment of the influence of EMF on the brain. The obtained results can also provide a new view on possible therapeutic properties of the magnetic field as well as a new direction in the risk assessment of EMF exposure. As the 1 mT seems to have a potentially protective impact on the brain, the studies are worth continuing.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

J.R. conceived the project and got funding. A.K., J.R., A.N., H.K., and J.W. prepared experimental protocols. A.K., A.N., H.K., J.M., and M.J. conducted the experiments. A.K. and H.K. analyzed the results. A.K. and J.R. drafted the manuscript. A.N., H.K., J.M., M.J., and J.W. reviewed the manuscript. A.K. prepared data visualization. J.R. and L.P. supervised.

Acknowledgments

We would like to thank Prof. Maria Stankiewicz (Nicolaus Copernicus University) for valuable comments on the manuscript and MSc Agnieszka Siejka and MSc Maciej Klimiuk (Nicolaus Copernicus University) for technical support. This study was supported by the National Science Centre, Poland (grant no. 2017/25/B/NZ7/00638) and project no. POWR.03.05.00-00-Z302/17 "Universitas Copernicana Thoruniensis In Futuro," cofinanced by the European Social Fund–the Operational Programme Knowledge Education Development, Module 5, Interdisciplinary PhD School "Academia Copernicana."

References

[1] G. Thut, C. Miniussi, and J. Gross, "The functional importance of rhythmic activity in the brain," *Current Biology*, vol. 22, no. 16, pp. R658–R663, 2012.

[2] Y. Touitou and B. Selmaoui, "The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system," *Dialogues in Clinical Neuroscience*, vol. 14, no. 4, pp. 381–399, 2012.

[3] P. Bieńkowski and J. Wyszkowska, "Technical aspects of exposure to magnetic fields of extremely low frequencies (ELF) in biomedical research," *Medycyna Pracy*, vol. 66, no. 2, pp. 185–197, 2015.

[4] C. N. Black, M. Bot, P. G. Scheffer, P. Cuijpers, and B. W. Penninx, "Is depression associated with increased oxidative stress? A systematic review and meta-analysis," *Psychoneuroendocrinology*, vol. 51, pp. 164–175, 2015.

[5] J. N. Cobley, M. L. Fiorello, and D. M. Bailey, "13 reasons why the brain is susceptible to oxidative stress," *Redox Biology*, vol. 15, pp. 490–503, 2018.

[6] S. Salim, "Oxidative stress and the central nervous system," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 360, no. 1, pp. 201–205, 2017.

[7] M. El-Helaly and E. Abu-Hashem, "Oxidative stress, melatonin level, and sleep insufficiency among electronic equipment repairers," *Indian journal of occupational and environmental medicine*, vol. 14, no. 3, pp. 66–70, 2010.

[8] X. Luo, M. Chen, Y. Duan et al., "Chemoprotective action of lotus seedpod procyanidins on oxidative stress in mice induced by extremely low-frequency electromagnetic field exposure," *Biomedicine & Pharmacotherapy*, vol. 82, pp. 640–648, 2016.

[9] Y. Sun, Z. Shi, Y. Wang et al., "Coupling of oxidative stress responses to tricarboxylic acid cycle and prostaglandin E2 alterations in *Caenorhabditis* elegans under extremely low-frequency electromagnetic field," *International Journal of Radiation Biology*, vol. 94, no. 12, pp. 1159–1166, 2018.

[10] J. Frahm, M. O. Mattsson, and M. Simkó, "Exposure to ELF magnetic fields modulate redox related protein expression in mouse macrophages," *Toxicology Letters*, vol. 192, no. 3, pp. 330–336, 2010.

[11] C. Morabito, F. Rovetta, M. Bizzarri, G. Mazzoleni, G. Fanò, and M. A. Mariggiò, "Modulation of redox status and calcium handling by extremely low frequency electromagnetic fields in C2C12 muscle cells: a real-time, single-cell approach," *Free Radical Biology & Medicine*, vol. 48, no. 4, pp. 579–589, 2010.

[12] A. Patruno, S. Tabrez, M. Pesce, S. Shakil, M. A. Kamal, and M. Reale, "Effects of extremely low frequency electromagnetic field (ELF-EMF) on catalase, cytochrome P450 and nitric oxide synthase in erythro-leukemic cells," *Life Sciences*, vol. 121, pp. 117–123, 2015.

[13] N. Cichoń, M. Bijak, E. Miller, and J. Saluk, "Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients," *Bioelectromagnetics*, vol. 38, no. 5, pp. 386–396, 2017.

[14] R. Szemerszky, D. Zelena, I. Barna, and G. Bárdos, "Stress-related endocrinological and psychopathological effects of short- and long-term 50 Hz electromagnetic field exposure in rats," *Brain Research Bulletin*, vol. 81, no. 1, pp. 92–99, 2010.

[15] A. Klimek and J. Rogalska, "Extremely low-frequency magnetic field as a stress factor-really detrimental?-insight into literature from the last decade," *Brain sciences*, vol. 11, no. 2, p. 174, 2021.

[16] J. P. Herman, J. M. McKlveen, S. Ghosal et al., "Regulation of the hypothalamic-pituitary-adrenocortical stress response," *Comprehensive Physiology*, vol. 6, no. 2, pp. 603–621, 2016.

[17] E. J. Calabrese, "Overcompensation stimulation: a mechanism for hormetic effects," *Critical Reviews in Toxicology*, vol. 31, no. 4-5, pp. 425–470, 2001.

[18] J. W. Gawryluk, J. F. Wang, A. C. Andreazza, L. Shao, and L. T. Young, "Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders," *The International Journal of Neuropsychopharmacology*, vol. 14, no. 1, pp. 123–130, 2011.

[19] *Directive 2013/35/EU of the European Parliament and of the Council of 26 June 2013 on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (electromagnetic fields)*, 2013.

[20] A. Goraca, E. Ciejka, and A. Piechota, "Effects of extremely low frequency magnetic field on the parameters of oxidative stress in heart," *Journal of Physiology and Pharmacology*, vol. 61, no. 3, pp. 333–338, 2010.

[21] E. Ciejka, P. Kleniewska, B. Skibska, and A. Goraca, "Effects of extremely low frequency magnetic field on oxidative balance in brain of rats," *Journal of Physiology and Pharmacology*, vol. 62, no. 6, pp. 657–661, 2011.

[22] C. Yin, X. Luo, Y. Duan et al., "Neuroprotective effects of lotus seedpod procyanidins on extremely low frequency electromagnetic field-induced neurotoxicity in primary cultured hippocampal neurons," *Biomedicine & Pharmacotherapy*, vol. 82, pp. 628–639, 2016.

[23] A. C. Calvo and M. J. Azanza, "Synaptic neurone activity under applied 50 Hz alternating magnetic fields," *Comparative Biochemistry and Physiology. Part C, Pharmacology, Toxicology & Endocrinology*, vol. 124, no. 1, pp. 99–107, 1999.

[24] T. M. Maynard, L. Sikich, J. A. Lieberman, and A. S. LaMantia, "Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia," *Schizophrenia Bulletin*, vol. 27, no. 3, pp. 457–476, 2001.

[25] *Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes*, 2010.

[26] T. Trawiński, M. Szczygieł, J. Wyszkowska, and K. Kluszczyński, "Analysis of magnetic field distribution and

mechanical vibration of magnetic field exciter under different voltage supply," *Information Technologies in Biomedicine*, vol. 69, pp. 613–622, 2010.

[27] I. Tasset, F. J. Medina, I. Jimena et al., "Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: effects on neurotrophic factors and neuronal density," *Neuroscience*, vol. 209, pp. 54–63, 2012.

[28] C. Merla, M. Liberti, C. Consales et al., "Evidences of plasma membrane-mediated ROS generation upon ELF exposure in neuroblastoma cells supported by a computational multiscale approach," *Biochimica et Biophysica Acta - Biomembranes*, vol. 1861, no. 8, pp. 1446–1457, 2019.

[29] A. M. Eleuteri, M. Amici, L. Bonfili et al., "50 Hz Extremely Low Frequency Electromagnetic Fields Enhance Protein Carbonyl Groups Content in Cancer Cells: Effects on Proteasomal Systems," *Journal of Biomedicine & Biotechnology*, vol. 2009, Article ID 834239, 10 pages, 2009.

[30] M. Z. Akdag, S. Dasdag, E. Ulukaya, A. K. Uzunlar, M. A. Kurt, and A. Taşkin, "Effects of extremely low-frequency magnetic field on caspase activities and oxidative stress values in rat brain," *Biological Trace Element Research*, vol. 138, no. 1-3, pp. 238–249, 2010.

[31] M. Z. Akdag, S. Dasdag, D. U. Cakir, B. Yokus, G. Kizil, and M. Kizil, "Do 100- and 500-μT ELF magnetic fields alter beta-amyloid protein, protein carbonyl and malondialdehyde in rat brains?," *Electromagnetic Biology and Medicine*, vol. 32, no. 3, pp. 363–372, 2013.

[32] A. Zimmermann, M. A. Bauer, G. Kroemer, F. Madeo, and D. Carmona-Gutierrez, "When less is more: hormesis against stress and disease," *Microbial Cell*, vol. 1, no. 5, pp. 150–153, 2014.

[33] Y. Duan, Z. Wang, H. Zhang et al., "The preventive effect of lotus seedpod procyanidins on cognitive impairment and oxidative damage induced by extremely low frequency electromagnetic field exposure," *Food & Function*, vol. 4, no. 8, pp. 1252–1262, 2013.

[34] D. Kuzay, C. Ozer, B. Sirav, A. G. Canseven, and N. Seyhan, "Oxidative effects of extremely low frequency magnetic field and radio frequency radiation on testes tissues of diabetic and healthy rats," *Bratislavské Lekárske Listy*, vol. 118, no. 5, pp. 278–282, 2017.

[35] N. Z. Djordjevic, M. G. Paunović, and A. S. Peulić, "Anxiety-like behavioural effects of extremely low-frequency electromagnetic field in rats," *Environmental Science and Pollution Research International*, vol. 24, no. 27, pp. 21693–21699, 2017.

Neuroendocrinology

Research Article

Neuroendocrinology 2023;113:423–441
DOI: 10.1159/000527878

Received: April 4, 2022
Accepted: October 26, 2022
Published online: November 2, 2022

New View on the Impact of the Low-Frequency Electromagnetic Field (50 Hz) on Stress Responses: Hormesis Effect

Angelika Klimek[a] Hanna Kletkiewicz[a] Agnieszka Siejka[a] Joanna Wyszkowska[a] Justyna Maliszewska[a] Maciej Klimiuk[a] Milena Jankowska[a] Jonathan Seckl[b] Justyna Rogalska[a]

[a]Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences Nicolaus Copernicus University in Torun, Toruń, Poland; [b]Centre for Cardiovascular Science, QMRI, University of Edinburgh, Edinburgh, UK

Keywords
Low-frequency electromagnetic field · Stress response · Hypothalamic-pituitary-adrenal axis · Open-field test · Mineralocorticoid receptor

Abstract
Introduction: Low-frequency electromagnetic field (50 Hz) (EMF) can modify crucial neuronal processes. Existing data indicate that exposure to EMF may represent a mild stressor and contribute to disturbances of the hypothalamic-pituitary-adrenal (HPA) axis. The important regulatory pathways controlling HPA axis activity include two types of corticosteroid receptors: mineralocorticoid receptors (MRs) and glucocorticoid receptors. They are particularly abundant in the hippocampus, a key locus of HPA axis feedback control. The research aimed at determining whether (1) EMF exhibits hormesis, it means bidirectional action depending on EMF intensity (1 or 7 mT) and (2) repeated EMF exposure changes stress response to subsequent stress factors. ***Methods:*** The exposure (7 days, 1 h/day) of adult rats to EMF (1 mT and 7 mT) was repeated 3 times. HPA axis hormones and their receptors were analysed after each following exposure. Moreover, the impact of EMF exposure on hormonal and behavioural responses to subsequent stress factor – open-field test was evaluated. ***Results:*** Our data suggest that exposure to EMF can establish a new "set-point" for HPA axis activity. The direction and dynamics of this process depend on the intensity of EMF and the number of exposures. EMF of 1 mT induced an adaptive stress response, but 7 mT EMF caused sensitization. Consequently, EMF changed the vulnerability of the organism to a subsequent stress factor. We have also shown the increase in MR mRNA abundance in the hippocampus of 1 mT EMF-exposed rats, which can represent the possible neuroprotective response and suggest therapeutic properties of EMFs.

Introduction

Exposure to electromagnetic field (EMF) has been suggested to adversely impact mammalian biology. EMF is pervasive in the contemporary environment, especially at extremely low frequencies (30–300 Hz) such as those emitted by electrical appliances and overhead power lines [1]. There is considerable interest in the health effects of EMF exposure (WHO 2007). Targets for EMF in the or-

Karger@karger.com
www.karger.com/nen

Correspondence to:
Angelika Klimek, klimek@doktorant.umk.pl
Justyna Rogalska, rogal@umk.pl

ganism include the cell membrane (e.g., its permeability, inorganic ion transport, receptor function), second messenger synthesis, chromosome structural changes and chemical changes in DNA structure, gene expression, and protein synthesis [2–10].

Although some findings indicate the deteriorating effects of EMFs on stress responses (a detrimental increase in free radical levels and radical-evoked damages in macromolecules) [11], others failed to detect any effects [1, 12–16]. Indeed, some reports have shown that EMF of low intensity exerts a neuroprotective influence (the production of protective proteins [e.g., Hsp70 or BDNF] or an increase in the activity of antioxidant enzymes) [17–21].

No explanations have addressed the potential mechanisms for these contradictory results. We postulate that the direction and nature of EMF-induced changes depend on EMF intensity (magnetic flux density).

Many studies have suggested an association between chronic EMF exposure and psychiatric disorders [22, 23]. Existing data indicate that exposure to EMF may count as a mild stressor. This could lead to disturbances of neuroendocrine stress systems, notably of the hypothalamic-pituitary-adrenal (HPA) axis [13, 14, 24–26]. The HPA axis is regulated primarily through negative feedback mechanisms. Stress induces the hypothalamus (HYP) paraventricular nucleus to secrete corticotropin-releasing hormone (CRH) which stimulates anterior pituitary corticotrophs to secrete adrenocorticotrophic hormone (ACTH) through activation of CRH type 1 receptors (CRH-R1). Systemically released ACTH acts on the adrenal glands (AG, via melanocortin receptor 2 [MC2R]) to release glucocorticoids (corticosterone in rats) [27, 28]. Corticosterone initiates physiological and behavioural responses through widely expressed nuclear receptors of two types: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). Both MRs and GRs are particularly abundant in the hippocampus (HIP), where they underpin cognitive as well as neuroendocrine feedback effects [28, 29].

In some cases, high doses of chemical or environmental factors are detrimental to biological systems, whereas low doses induce endogenous survival systems, a phenomenon known as hormesis [30–32]. The hormetic dose-response relationship can occur after an initial homoeostasis disruption, which must then be re-established. It requires gene expression and protein synthesis that progresses over time; thus, the temporal feature of hormesis determines its final effect [30]. We hypothesized that initial exposure to EMF moves up or down the set-point of stress system activity; thus, each next EMF exposure would overlap the set-point status of stress response system activity established under the previous EMF exposure. Therefore, we explored whether the effect of EMF is hormetic and results in different activation of the stress system and the direction and dynamics of which depend on the intensity of the field.

Consequently, EMF can change the vulnerability of the organism to subsequent stress factors and thus to diseases, mainly related to the nervous system, in a two-way manner: compensatory or deleterious ones. The important mediators of this phenomenon can be corticosterone receptors, MRs and GRs, known to modulate the hippocampal function and determine the plasticity processes in this area [29, 33–35].

To explore this hypothesis, we determined the directions and dynamics of EMF-induced changes in stress system activity. Specifically, we examined (1) whether any adaptation of the HPA system occurs after repeated EMF applications, (2) if there is a relationship between these processes and the intensity of the EMF, and (3) whether EMF exposure changes the HPA axis response to a subsequent physiological stressor, and (4) what possible mechanisms might account for the impacts of repeated EMF exposure.

Materials and Methods

Animals

A total of 179 3-month-old male Wistar rats weighing 300–350 g were used. The number of animals was planned in accordance with 3R principles (Replacement, Reduction, and Refinement; EU Directive 2010/63/EU) [36]. Rats were housed in acrylic cages lined with wood shavings and kept on a 12:12 h day-night schedule (lights on at 7:00 a.m.) under standard laboratory conditions (temperature: 22 ± 2°C, humidity 55 ± 10%). Standard laboratory chow and water were available ad libitum. All procedures were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decision number: 3/2018).

Experimental Design

Experiments were conducted using the applicator (coil) used in magnetotherapy as a source of EMF (Elektronika i Elektromedycyna Sp. J., Otwock, Poland). The system for EMF exposure is shown in Figure 1. An EMF with the domination of magnetic component was generated by a 20-cm-diameter coil composed of 282 turns of copper wire. A detailed description of the apparatus has been published elsewhere [37, 38]. The coil and variable autotransformer power supply produced homogeneous, sine-wave alternating EMF at 50 Hz and with intensities 1 and 7 mT. The magnetic flux density was measured before each experiment using a Gauss metre (Model GM2; AlphaLab, Inc, USA). The non-homogeneity of the EMF within the area containing the animal's

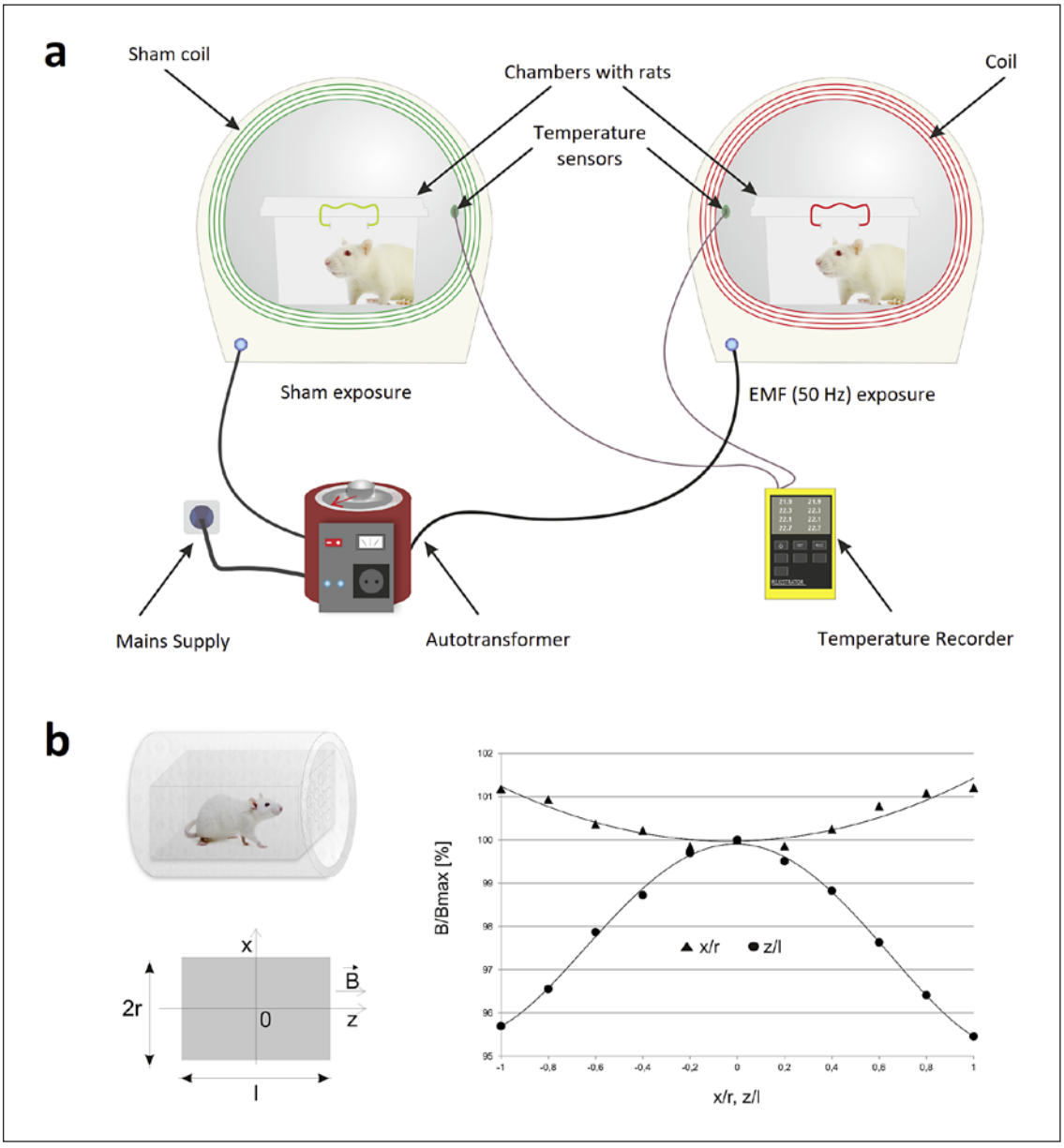


Fig. 1. EMF exposure system. **a** The scheme of the EMF treatment. **b** The scheme of the coil with the plastic box containing the rat; the coordinates system; the distribution of the EMF along the main axis of the coil within the area of the animal's cage. B, magnetic flux density vector; B/B_{max}, normalized magnetic flux density relative to the maximal value; z/l, normalized distance from the coil centre along *z*-axis; x/r, normalized distance from the solenoid centre along *x*-axis; l, coil length; r, coil radius.

cage was approximately 10%. Control groups of animals were handled in an identical manner (in the sham exposure system with similar experimental procedures) to obtain the same experimental conditions, except for the presence of EMF (<10 μT). The temperature during experiments (for both control and EMF-exposed groups) was monitored using thermocouples mounted under each exposure system and was set to 26 ± 1°C. Experiments were conducted in an isolated room (with controlled light and temperature T = 23 ± 1°C). In our study, the EMF parameters were based on European Union Directive 2013/35/EU [39], which suggests that the limitation of EMF (50 Hz) exposure to 1 mT reduces the potential for damage, while exposure to EMF >6 mT causes measurable biological effects, e.g., DNA damage [8] or the generation of neuronal networks synchrony firing [40]. Such EMF parameters are commonly applied in magnetotherapy. Rats were repeatedly exposed for 1 h/day for 7 days to an EMF (50 Hz, 1 mT, or 7 mT) in a scheme corresponding to the extensive therapeutic application (frequency lower than 100 Hz, a magnetic flux den-

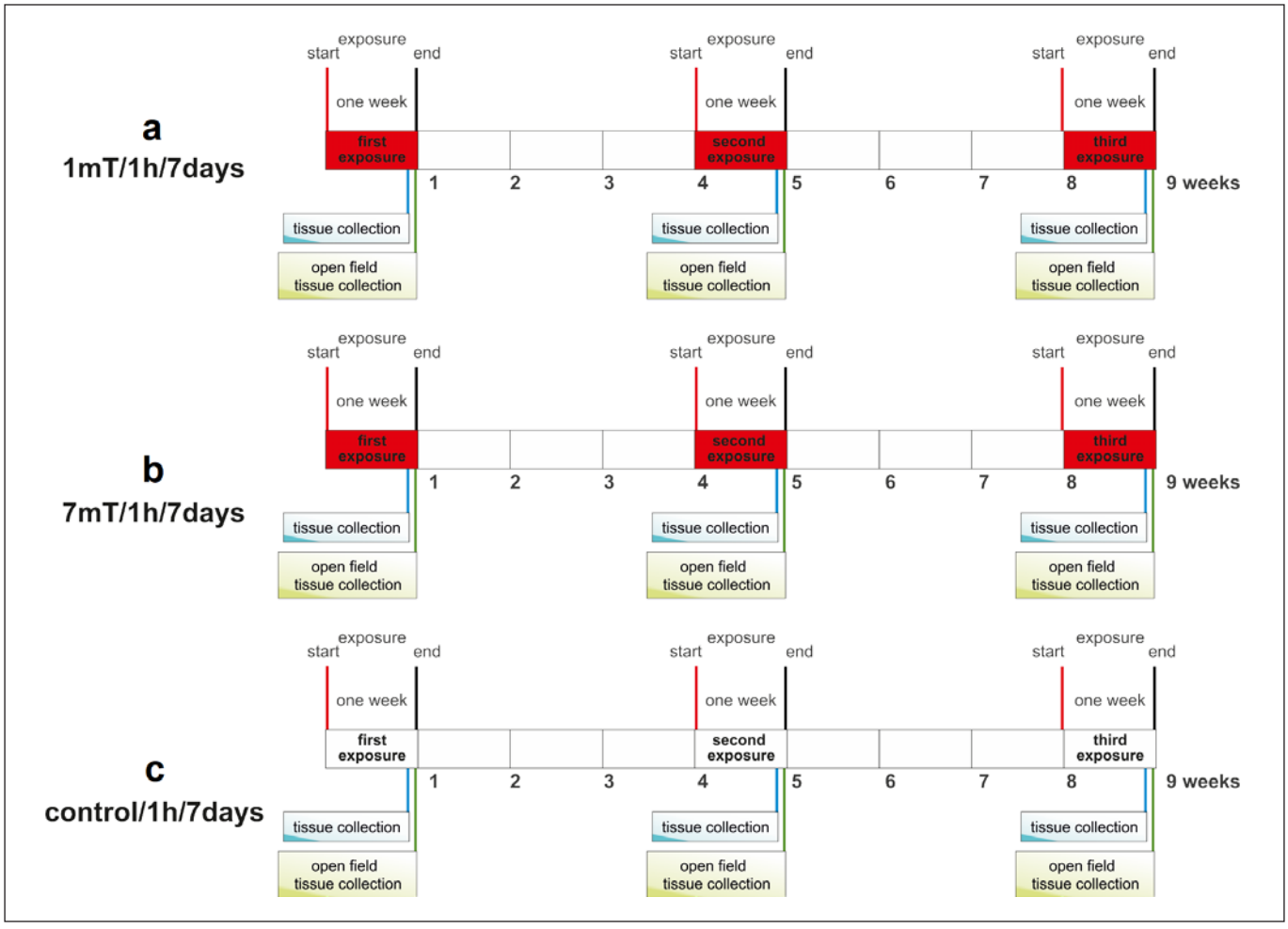


Fig. 2. Experimental design.

sity ranging from 0.1 mT to 20 mT, periods of exposure 30–60 min for 1–2 weeks) [41].

After 7 days of habituation to the laboratory environment, each rat was placed in an opaque plastic box (12 cm × 20 cm × 14 cm) with a perforated plexiglass cover and with wood shaving bedding. Randomly assigned boxes were put into the centre of the EMF coil or sham exposure system (control). Animals were able to move freely within their chambers. After exposure to EMF or control conditions, the concentrations of HPA axis hormones and relative mRNA transcript abundance of their receptors were assessed – described in this research as "basal" (B) level. The second set of experiments evaluated open-field (OF) stress-induced levels of these parameters in animals previously exposed to EMF or control conditions. All procedures were carried out between 09:00 and 12:00 to avoid circadian fluctuations.

The rats were divided into the following groups.

1. EMF/B/1mT – animals exposed to EMF (50 Hz, 1 mT),
2. EMF/OF/1mT – animals exposed to EMF (50 Hz, 1 mT) and subsequently exposed to OF test,
3. EMF/B/7mT – animals exposed to EMF (50 Hz, 7 mT),
4. EMF/OF/7mT – animals exposed to EMF (50 Hz, 7 mT) and subsequently exposed to OF test,
5. C/B – control animals subjected to the same experimental procedure as the experimental groups 1, 3, without EMF exposure,
6. C/OF – control animals subjected to the same experimental procedure as the experimental groups 2, 4, without EMF exposure.

The experimental design is shown in Figure 2. Rats from groups EMF/B/1mT, EMF/OF/1mT, EMF/B/7mT, and EMF/OF/7mT were exposed for three periods (E1, E2, E3) of low (1 mT) or high (7 mT) EMF level every 3 weeks. We have chosen such a schedule as our previous experiments showed that the effect of a single exposure to EMF was still observed 3 weeks later in the case of EMF of 1 mT and even after 3 months from the end of the exposure to 7 mT [unpublished data].

Each period included 7-day exposure, 1 h a day. After each period of exposure, a subset of rats from groups EMF/B/1mT and EMF/B/7mT was killed by decapitation to assess the effects of single or recurrent EMF exposure. Animals from groups EMF/OF/1mT and EMF/OF/7mT were tested in an OF apparatus, after

E1, E2, or E3, and then decapitated to determine stress-induced changes in parameters as a consequence of prior exposure to EMF. Control rats (C/B and C/OF) were subjected to exactly the same experimental procedure but without EMF exposure. Body weights were monitored throughout the experimental period, but no changes were found.

The influence of EMF on concentrations of HPA axis hormones and receptors' relative mRNA transcript abundance in relevant brain structures, organs, and tissues was determined: CRH in the HYP, CRH-R1 and ACTH in the pituitary gland (PG), MC2R and CORT in the AG, ACTH and CORT in plasma as well as MRs and GRs in the HIP. To evaluate the direction and dynamics of the changes in the level of stress markers, they were analysed after each period (E1–E3) of 7-day exposure to control conditions or to EMF (1 mT and 7 mT) ("basal" level of parameters) and in animals from EMF-exposed groups and control group tested in OF apparatus (OF-induced level of parameters).

Sample Collection

Following decapitation, blood samples were collected via cardiac puncture in a solution of ethylenediaminetetraacetic acid disodium salt (Na2EDTA; Sigma-Aldrich, Germany). After centrifugation (20 min, 2,000 rpm), the resulting plasma was stored at −20°C until future ELISA analysis.

The organs (brains and AG) were quickly removed. From the brain, the following areas were dissected: the HYP, HIP, and PG. Each of the collected structures was weighed and immediately frozen in liquid nitrogen and stored at −80°C until further reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and ELISA analyses.

Determination of HPA Axis Hormone Concentrations: CORT, ACTH, CRH

Tissues were homogenized on ice in a pre-cooled PBS buffer containing a proteinase inhibitor cocktail (Roche Cat. No. 11836145001) (100 mg of tissue per millilitre). Rat Corticoliberin, Rat Corticotropin, and General Corticosterone ELISA kits were used to determine the concentrations of the hormones (EIAab Cat. No. E0835r, E0626r, E0540Ge, respectively). All procedures were conducted according to standard guidelines provided by the manufacturers. Each sample was measured in duplicate. Colourimetric changes in the assay were detected using a multi-mode microplate reader, Epoch 2 (BioTek Instruments, Inc., Winooski, UT, USA). The optical density was measured at 450 nm, setting the optical density of the blank well to zero. A standard curve was created by reducing the data using Gen5 Software, generating a four-parameter logistic curve-fit.

Assay of mRNA Encoding Receptors: GRs, MRs, MC2R, CRH-R1

RT-qPCR was performed to determine the relative mRNA transcript abundance of GRs, MRs, MC2R, CRH-R1 in collected organs. Total RNA was extracted using an HP RNA tissue kit RNA (Roche Cat. No. 11828665001), and concentration was measured by absorbance at 260 nm (A260/280) using a NanoDrop 2000 (Thermo Fisher). Approximately 1 μg of total RNA was used to generate complementary DNA (cDNA) by reverse transcription using EvoScript Universal cDNA Master (Roche Cat. No. 07 912 455 001) and random hexamer primers according to manufacturer's instructions. Next, 10 ng of cDNA was used to amplify the target gene by real-time qPCR using 0.2 μM specific primers (Sigma-Aldrich) and SYBR Green PCR Master Mix (Roche Cat. No. 12239264001) on LightCycler® 96 System (Roche) (online suppl. Table S1; see www.karger.com/doi/10.1159/000527878 for all online suppl. material). Initial denaturation was performed at 95°C for 10 min, followed by 50 cycles of 95°C for 15 s primer annealing (58–64°C). β-Actin and GAPDH were evaluated as housekeeping genes using the BestKeeper approach to determine the stability of gene transcript abundance under experimental conditions [42]. β-Actin was selected as the housekeeping gene for calculations (stability coefficient was 0.963 for β-actin vs. 2.0 for GAPDH). To determine the PCR efficiencies, standard curves for both target and control genes were obtained using a series of cDNA dilutions as a template. The relative gene transcript abundance was calculated according to the Pfaffl et al. [42] method using REST software. Results were reported as the fold change in gene transcript abundance.

OF Test

An OF test was used to evaluate the responsiveness of the animals to a stressful novel environment. The OF apparatus was a grey square box (100 cm × 100 cm), enclosed by walls (50 cm height) with a 100 W halogen bulb suspended 30 cm above the centre as the only source of light. To measure the thigmotaxis; the behaviour of locomotion close to the walls, avoiding the central area and the non-thigmotactic behaviour; locomotion in the central exposed area away from the walls, the arena for analysis was divided into zones (online suppl. Fig. S1): the border zone including corners, inner zone, and central zone. The following variables of behaviour were analysed (1) in the whole arena: latency to first movement (s); time of motor activity (s), distance moved (cm), and mean velocity – the distance moved per unit of time (cm/s); (2) in the border zone: total time spent in the zone (s), total time spent in corners (s); (3) in the central zone: total time spent in the zone (s), number of entries into the central zone. The OF apparatus was placed in a sound-isolated room with lighting conditions and environmental cues held constant throughout testing. Each trial lasted 5 min, and the box was cleaned with Mediseptol H Neutral (Alpinus Chemia, Poland). All behavioural sessions were recorded and analysed with the EthoVision 11 software (Noldus, Wageningen, The Netherlands).

Data Analysis

To analyse the effect of repeated exposure to EMF on stress responses, we applied 2-way general linear models (GLMs), separately for each hormone. To test the impact of EMF on behavioural stress responses, we used multivariate analysis of variance. The data were transformed when necessary to meet the GLM and multivariate analysis of variance assumptions (normality was tested with a Shapiro-Wilk test and homoscedasticity with a Levene test). Significant terms were further explored using the Bonferroni-corrected post hoc test adjusted to the design of particular experiments. Part of the data did not meet the criteria for parametric tests, even after transformations (natural logarithm, decimal logarithm, square root). For such data, we used a non-parametric analysis of variance (Kruskal-Wallis test with Bonferroni correction). Differences between compared data were considered significant when $p < 0.05$. The analyses were carried out using SPSS 25.0 package (IBM Inc.). The figures in the Results section present only significant effects of categorical factors displayed in the analysis (see online suppl. Tables S2–S5).

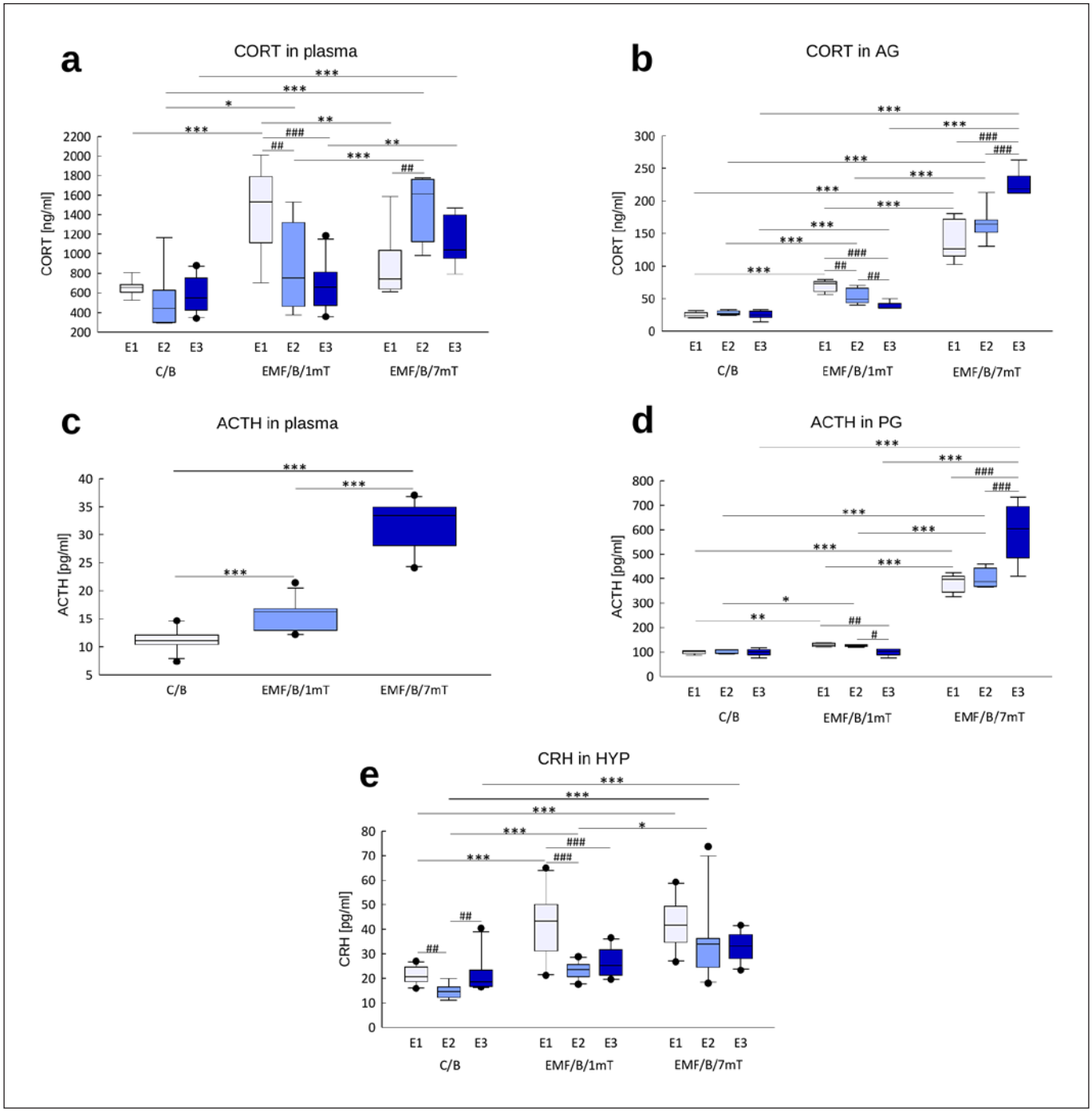


Fig. 3. "Basal" concentrations of HPA axis hormones: CORT in plasma ($n = 77$; 8–10 in each group for each exposure) (**a**), CORT in AG ($n = 56$; 5–7 in each group for each exposure) (**b**), ACTH in plasma ($n = 86$; 8–10 in each group for each exposure) (**c**), ACTH in PG ($n = 45$; 5 in each group for each exposure) (**d**), and CRH in the HYP ($n = 89$; 9–10 in each group for each exposure) (**e**) in rats exposed once to three times (E1–E3) to EMF of 1 or 7 mT or control conditions. Presented values are predicted by the GLM model for a significant intensity of the EMF (mT) × number of exposures (E1–E3) interaction (**a**, **b**, **d**, **e**) or significant effect of intensity of the EMF (mT) (**c**). Boxes indicate the lower quartile, median, and upper quartile, and whiskers indicate the range of changes (minimum to maximum). Statistically significant differences between animals from the same group are denoted $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ and these between experimental groups are denoted $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Note different scales on each plot.

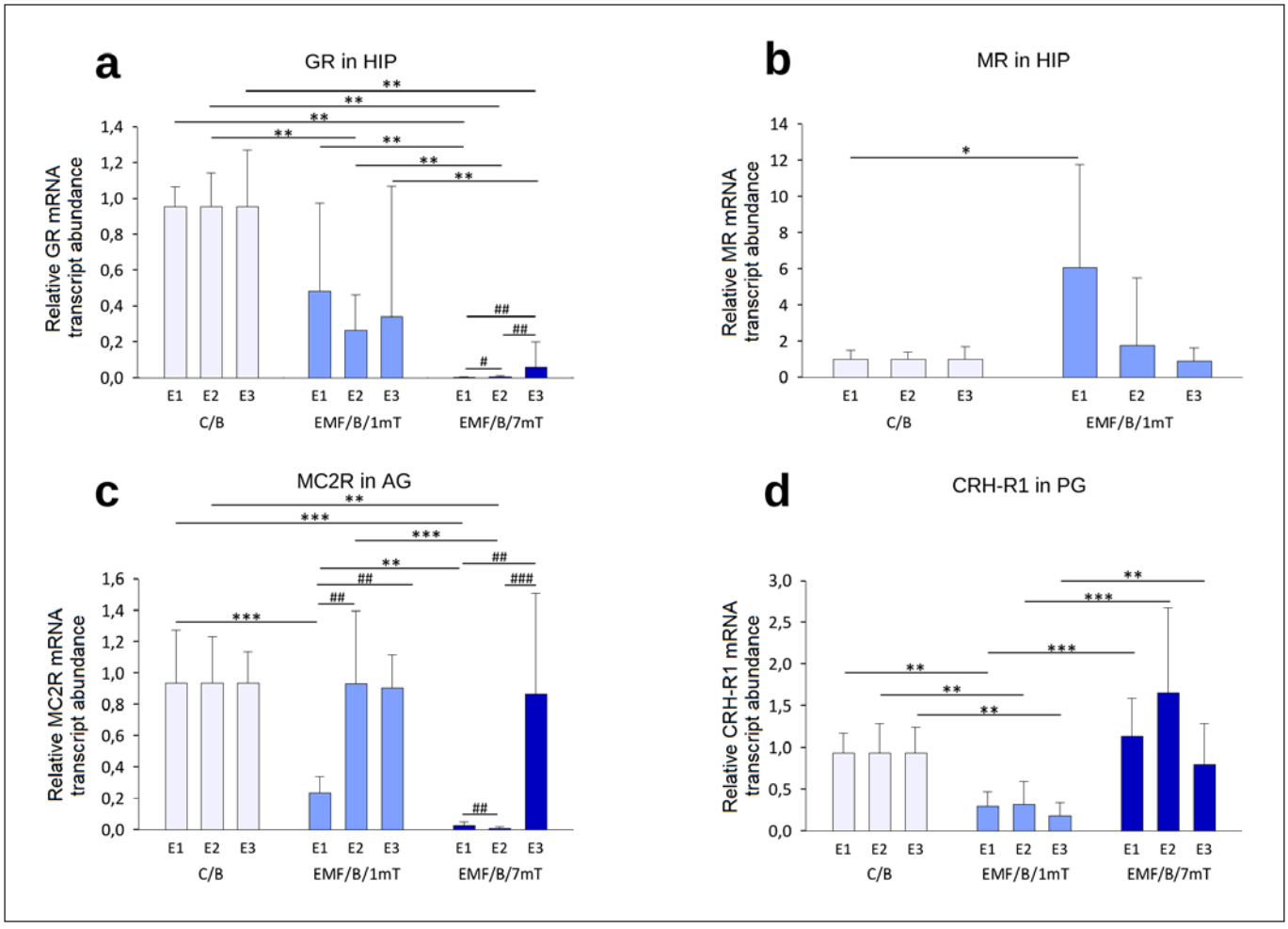


Fig. 4. Receptor genes' "basal" relative mRNA transcript abundance (fold change vs. β-actin) GR (**a**) and MR (**b**) in the HIP, MC2R in AG (**c**), CRH-R1 in the PG (**d**) in rats exposed once to three times (E1–E3) to EMF of 1 or 7 mT or control conditions. Values are presented as mean ± SEM. Statistically significant differences between animals from the same group are denoted, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ and these between experimental groups are denoted $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ ($n = 54$; 6 in each group for each exposure). Note different scales on each plot.

Results

EMF-Modified HPA Axis Hormone Concentrations and Receptor mRNA Abundance ("Basal")

Overall, the data showed that EMF exposure activated HPA axis variables in a dose-related manner.

Hormones

The profile of changes in "basal" plasma CORT concentrations (Fig. 3a) was similar to that of adrenal CORT concentrations (Fig. 3b). Under control circumstances, "basal" concentrations of corticosterone were low and were unaltered by single or repeated exposure to the apparatus, suggesting the set-up was not stressful per se. There was a significant effect of EMF dose, with the higher level of EMF producing greater elevation of CORT in adrenals and plasma ($p < 0.001$, online suppl. Table S2a, b). A single period (E1) of exposure to low 1 mT EMF significantly increased adrenal and plasma CORT concentrations ($p < 0.001$, Fig. 3a, b). Although there was no effect of the number of exposures (online suppl. Table S2a, b), a trend for lesser responses to repeated EMF exposure was found. Conversely, with the 7 mT EMF, there was no decline in CORT with repeated exposure.

As expected, ACTH concentrations in the pituitary and plasma parallelled the changes in CORT (online suppl. Table S2c, d). The higher EMF led to greater induction of ACTH (Fig. 3c, d). There was a significant effect of

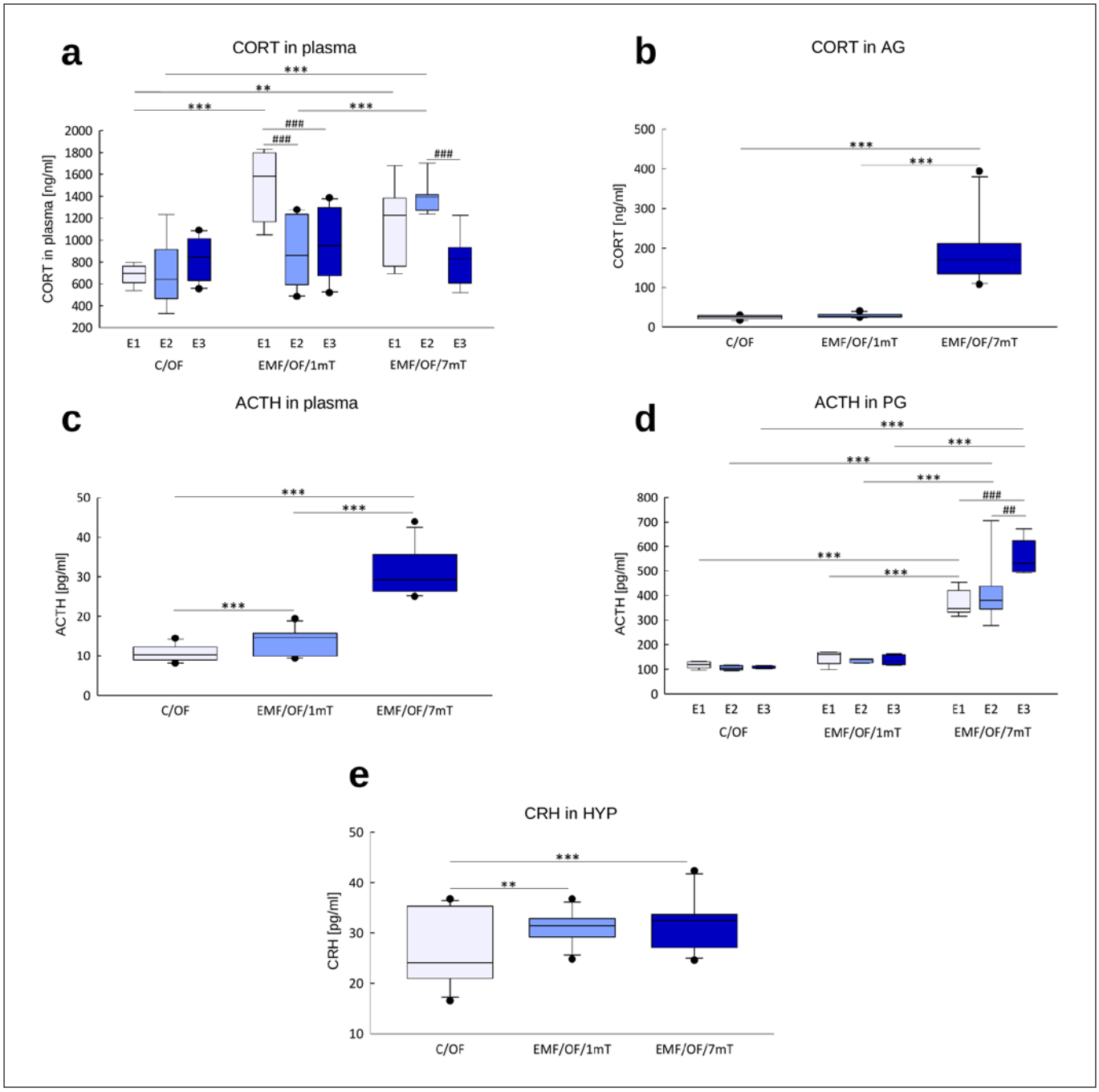


Fig. 5. The OF-induced concentrations of HPA axis hormones: CORT in plasma (n = 78; 7–10 in each group for each exposure) (**a**), CORT in AG (n = 57; 5–7 in each group for each exposure) (**b**), ACTH in plasma (n = 90; 10 in each group for each exposure) (**c**), ACTH in the PG (n = 51; 5–7 in each group for each exposure) (**d**), and CRH in the HYP (n = 90; 10 in each group for each exposure) (**e**) in rats exposed once to three times (E1–E3) to EMF of 1 or 7 mT or control conditions. Presented values are predicted by the GLM model for a significant intensity of the EMF (mT) × number of exposures (E1–E3) interaction (**a**, **d**) or significant effect of intensity of the EMF (mT) (**b**, **c**, **e**). Boxes indicate the lower quartile, median, and upper quartile, and whiskers indicate the range of changes (minimum to maximum). Statistically significant differences between animals from the same group are denoted, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ and these between experimental groups are denoted $^{**}p < 0.01$ and $^{***}p < 0.001$. Note different scales on each plot.

EMF intensity on ACTH concentration in plasma, with the higher level of EMF causing a greater elevation of hormone concentration ($p < 0.001$, Fig. 3c). In the EMF/B/1mT group, the ACTH concentrations in the pituitary (Fig. 3d) were significantly increased after E1 and E2. However, after the third exposure, there was attenuation of ACTH induction, and its concentration came back to the control value. In rats exposed to EMF of 7 mT, the concentration of ACTH in PG increased with each subsequent exposure.

CRH concentrations in the HYP increased with greater intensity of EMF and were further augmented with each subsequent exposure to EMF (online suppl. Table S2e; Fig. 3e). Single exposure (E1) to EMF of 1 mT significantly increased CRH compared to control ($p < 0.001$). After second exposure (E2), CRH was still elevated to receive the value not significantly different from control, perhaps suggesting habituation after E3. In the EMF/B/7mT group, a statistically significant increase in CRH concentration relative to the control group was noted after each subsequent exposure.

Receptors

Figure 4 presents intergroup comparisons of the "basal" relative mRNA transcript abundance of ("mRNA") encoding HPA axis receptors (expressed as fold change from an unrelated reference gene). Overall, the GR mRNA abundance in the HIP (Fig. 4a) of EMF-exposed groups was lower than control values, with a dose-response effect. However, the significant decrease of GR mRNA in the EMF/B/1mT group was observed only after E2 relative to the control group, while in the EMF/B/7mT group, GR mRNA was remarkably lower after all exposures to EMF in comparison to both: control and EMF/B/1mT groups. In animals from the EMF/B/1mT group, significantly higher hippocampal MR mRNA (Fig. 4b) was observed after E1; after E2, the receptor mRNA still tended to be elevated to receive the level not clearly different from the control value after E3. In rats exposed to EMF of 7 mT, the MR mRNA in the HIP was under the detectable threshold.

The profile of changes of MC2R mRNA in AG (Fig. 4c) in EMF-exposed groups was different. In EMF/B/1mT, the lower of MC2R mRNA relative to that in the control group was noticed only after a single exposure (E1) to EMF ($p < 0.001$). The lower of MC2R mRNA was found in the EMF/B/7mT group in comparison to C/B and EMF/B/1mT after E1 and E2, which normalized to control level after E3.

Overall, CRH-R1 mRNA in the PG (Fig. 4d) in EMF/B/1mT was lower after each exposure to EMF in comparison to both: control and EMF/B/7mT groups. In rats exposed to EMF of 7 mT, the CRH-R1 mRNA was not significantly different relative to control group.

OF-Modified HPA Axis Hormone Concentrations and Their Receptors' Relative mRNA Transcript Abundance

Hormones

Generally, the direction of changes in hormone concentrations in animals exposed to EMF of 1 mT and 7 mT and then exposed to subsequent stress factor – OF test (EMF/OF/1mT and EMF/OF/7mT groups) – coincided with their "basal" concentrations observed only after exposure to EMF (EMF/B/1mT and EMF/B/7mT groups) (online suppl. Table S3; Fig. 5). CORT concentration in plasma in animals exposed to OF test was influenced by EMF intensity as well as by the number of exposures (online suppl. Table S3a; Fig. 5a). The concentration of CORT in EMF/OF/1mT was higher after E1 relative to the control group to reach the value typical for control animals already after E2. In EMF/OF/7mT, we observed a higher concentration of the hormone after E1, which was maintained after E2, and then after E3, a clear decrease in the CORT concentration was noticed. The adrenal concentration of CORT was enhanced only in animals exposed to EMF of 7 mT (Fig. 5b).

In animals exposed to OF test, ACTH concentration in plasma and PG was influenced by EMF intensity (online suppl. Table S3c, d). The pituitary concentration of ACTH in EMF/OF/1mT did not differ from the value noticed in the control group, while in animals exposed to EMF of 7 mT, the concentration of ACTH was significantly enhanced in comparison to both control and EMF/OF/1mT groups at all steps of the experiment (Fig. 5d). In plasma, the hormone concentration increased with higher EMF intensity (Fig. 5c). CRH concentration in the HYP (Fig. 5e) was higher in both groups of rats exposed to EMF (1 and 7 mT) relative to control.

Receptors

The level and direction of changes in all receptors' relative mRNA transcript abundance (GRs, MRs, MC2R, and CRH-R1) in all groups after the OF test were not very different from their "basal" level (Fig. 6). There was an increase in MR mRNA in EMF/OF/1mT in comparison to the C/OF group, detected not only after E1 but also after E2 (Fig. 6b). Similarly, as in the EMF/B/7mT group,

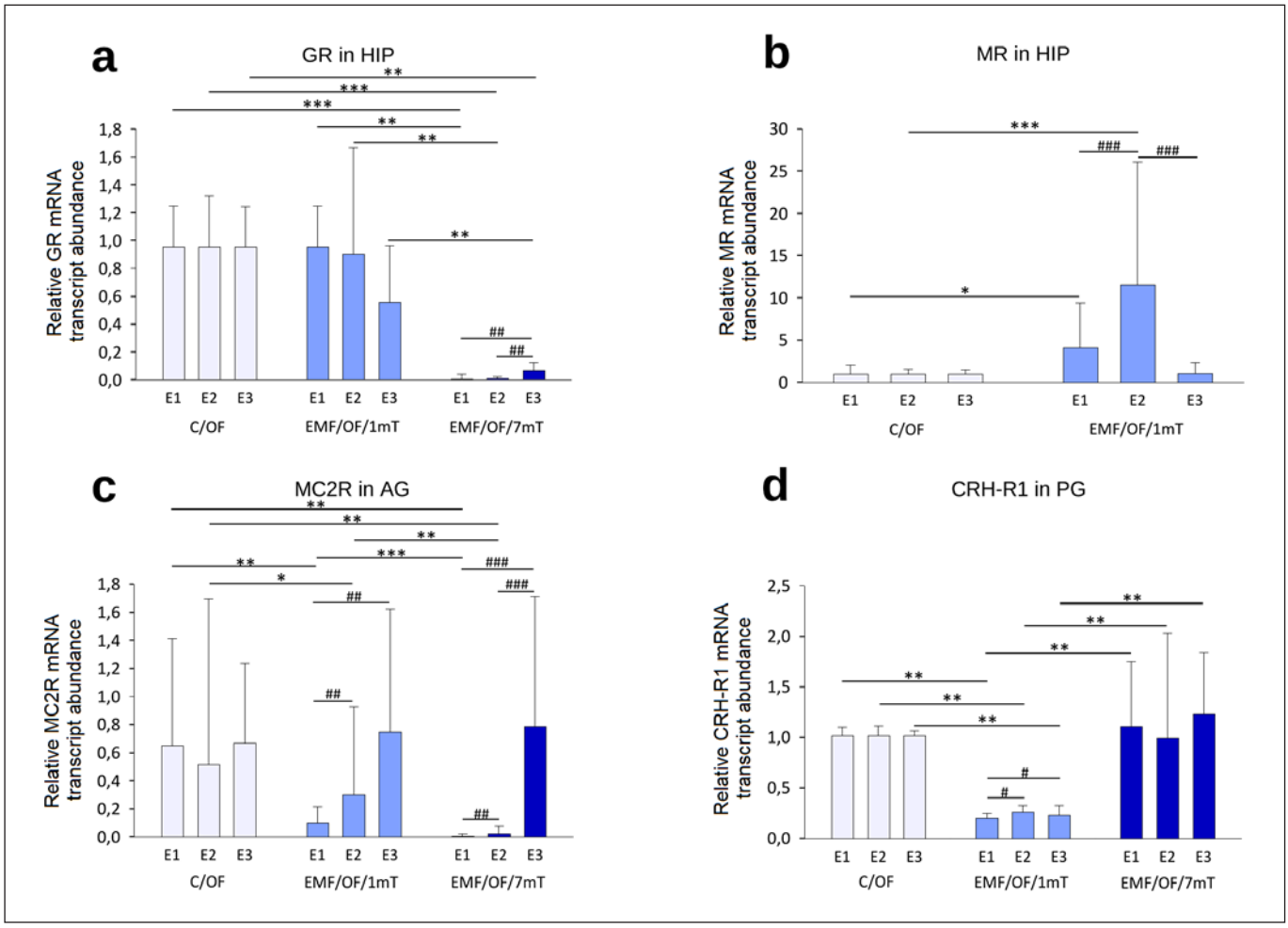


Fig. 6. Receptor genes' relative mRNA transcript abundance after OF test (fold change vs. β-actin) GR (**a**) and MR (**b**) in the HIP, MC2R in AG (**c**), CRH-R1 in the PG (**d**) in rats exposed once to three times (E1–E3) to EMF of 1 or 7 mT or control conditions. Values are presented as mean ± SEM. Statistically significant differences between animals from the same group are denoted $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ and these between experimental groups are denoted $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ ($n = 54$; 6 in each group for each exposure). Note different scales on each plot.

in the EMF/OF/7mT group, MR mRNA was not detectable.

Comparison between "Basal" and OF Test-Induced Concentrations of HPA Axis Hormones and Their Receptors' Relative mRNA Transcript Abundance

Hormones

We compared the "basal" concentrations of HPA hormones to the concentrations of these hormones after the OF test for each group: C/B versus C/OF; EMF/B/1mT versus EMF/OF/1mT; and EMF/B/7mT versus EMF/OF/7mT (online suppl. Table S4). In the control group, the increase in concentrations of CRH in the HYP, ACTH in the PG, and CORT in plasma after exposure to OF test was significant, which confirmed the stressogenic effect of this kind of stress. The exposure to subsequent stress factor profoundly decreased the ACTH in PG and CORT in AG in rats exposed to EMF of 1 mT. In animals exposed to EMF of 7 mT, the analysis did not reveal a significant effect of OF test on hormone concentrations.

Interesting results have been received when we evaluated the percentage changes in the concentrations of hormones in comparison to their concentrations in the control (C/B) group separately after each exposure (set at 100% – reference value) (Fig. 7).

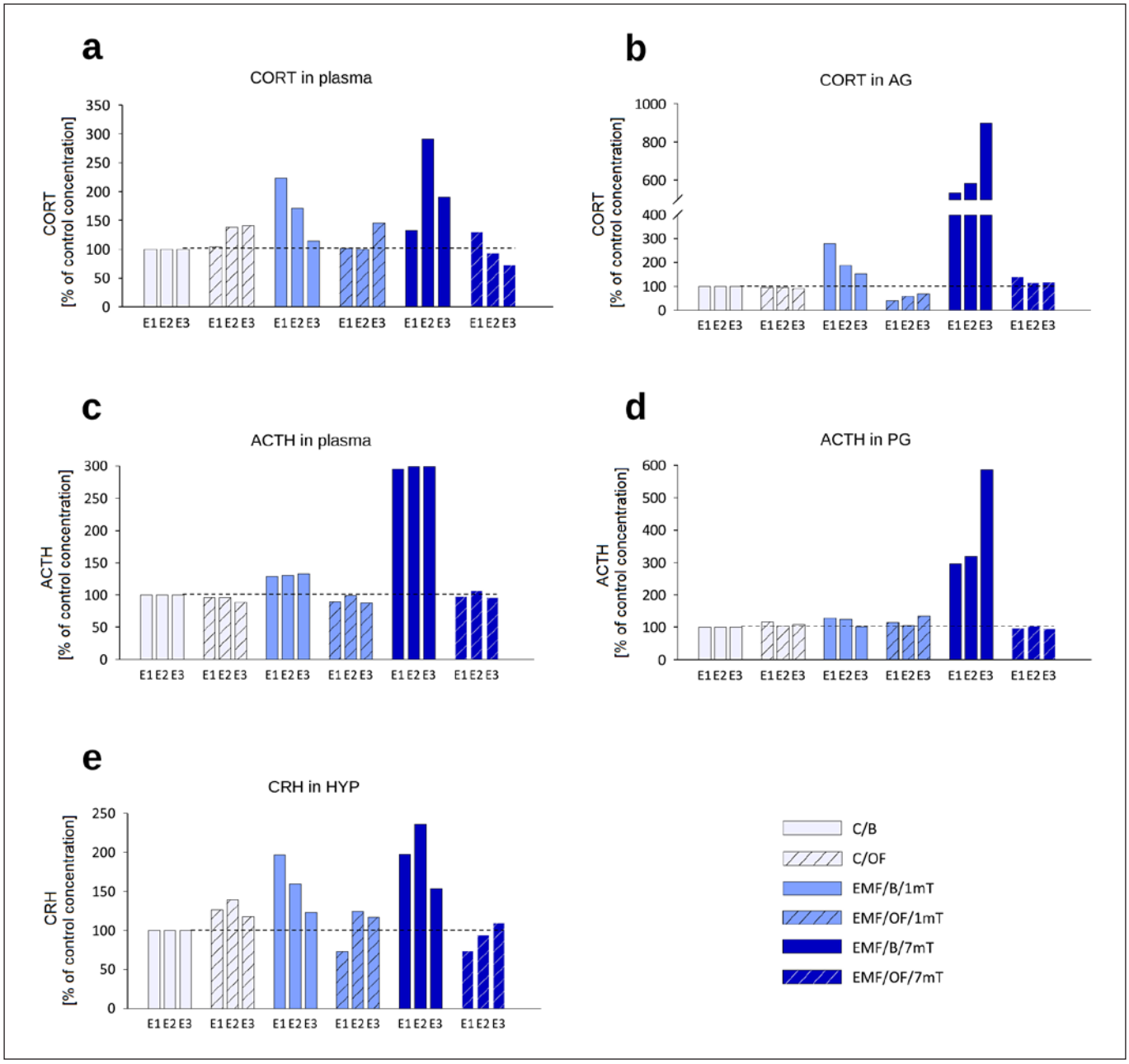


Fig. 7. Percentage changes in the "basal" concentrations as well as OF-induced concentrations of hormones in each experimental group in relation to their level in control C/B group set at 100% after each subsequent exposure (E1–E3): CORT in plasma (**a**), CORT in AG (**b**), ACTH in plasma (**c**), ACTH in the PG (**d**) and CRH in the HYP (**e**) in rats exposed once to three times (E1–E3) to EMF of 1 or 7 mT or control conditions. Note different scales on each plot.

In the EMF/OF/1mT group, CORT concentrations in plasma (Fig. 7a) were lower than in EMF/B/1mT group after E1 and E2; however, after E3 when the "basal" concentration returned to a level close to the control one, we observed increased CORT concentrations after OF. In rats exposed to EMF of 7 mT and then to OF after E1, CORT was similar to the respective "basal" change ~30% of the reference value. Then after E2 and E3, when the

"basal" value increased to 292 and 190%, respectively, the OF-induced concentrations were decreased to 94 and 73% of C/B value, respectively. The "basal" concentration of CORT in AG (Fig. 7b) of animals exposed to EMF of 7 mT after each subsequent exposure was incredibly high (536, 585, 901%, respectively), while OF did not further increase CORT concentrations. In EMF/OF/1mT, similarly, the "basal" concentration of the hormone was higher than the reference value in the C/B group, (although the increase is not as high as in the EMF/B/7mT group), and again after OF test, the CORT concentration in AG was not further elevated.

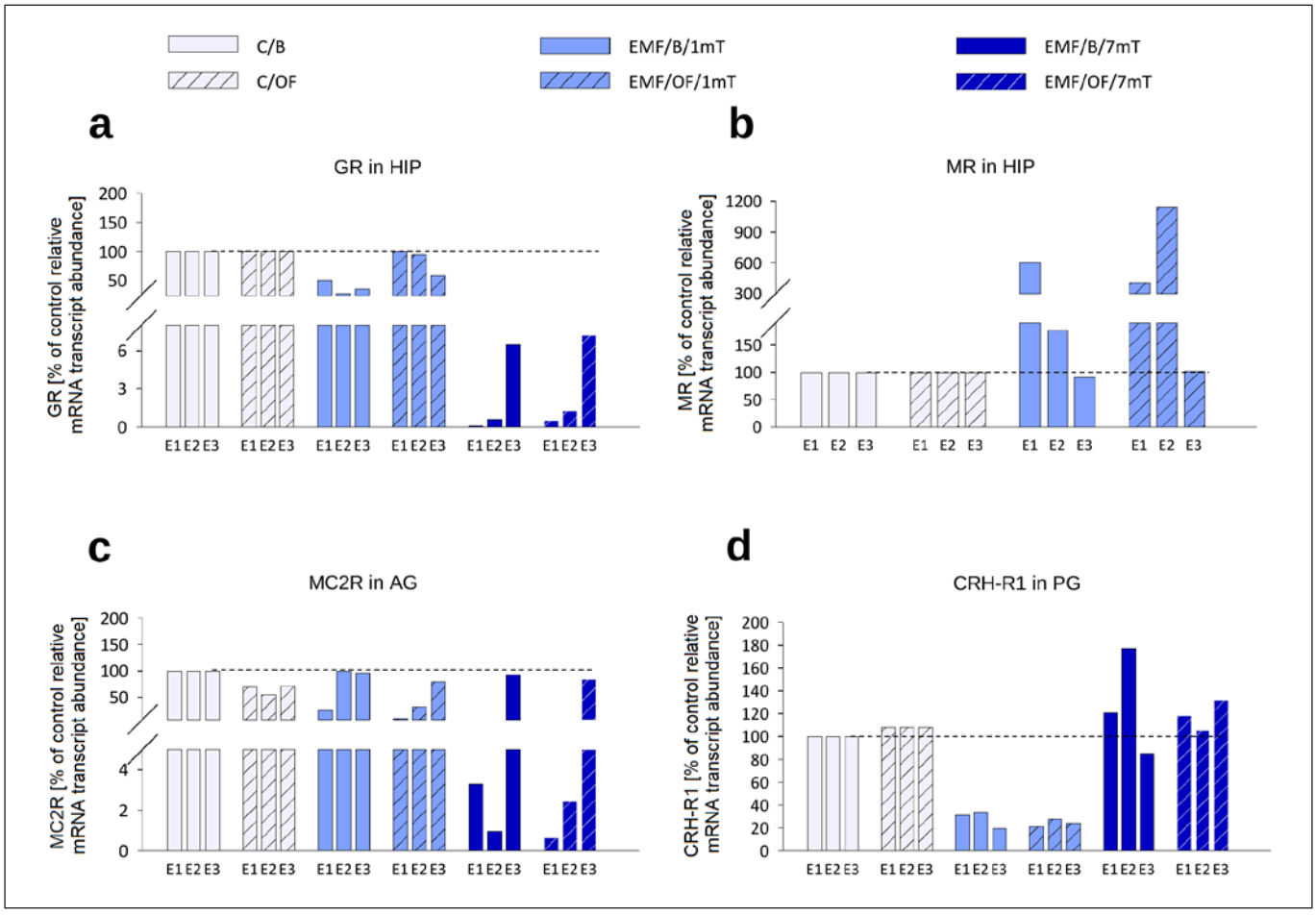
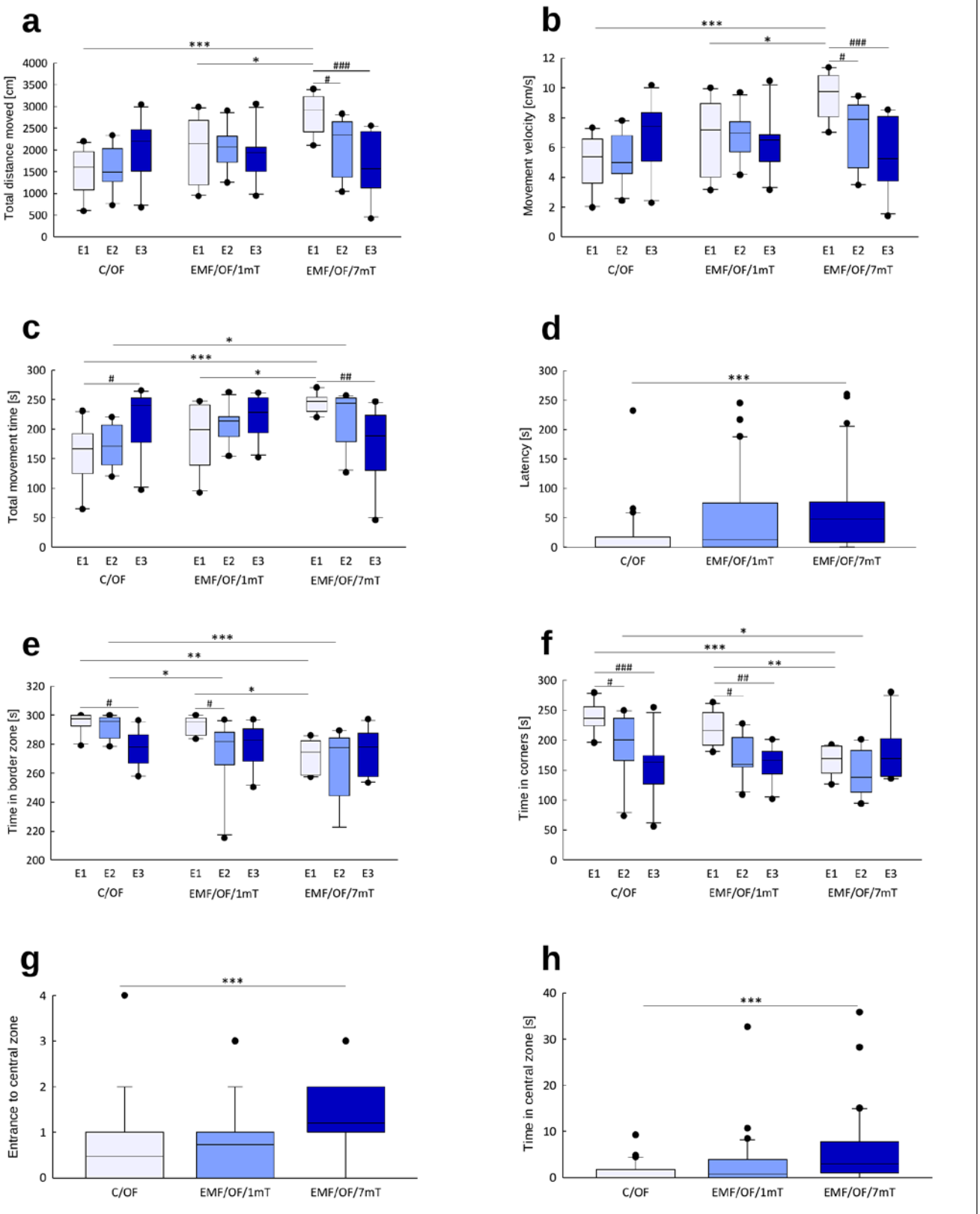


Fig. 8. Percentage changes in the "basal" as well as OF-induced relative mRNA transcript abundance of receptor genes in each experimental group in relation to their mRNA transcript abundance in control C/B group set at 100% after each subsequent exposure (E1–E3): GR (**a**) and MR in the HIP (**b**), MC2R in AG (**c**), CRH-R1 in the PG (**d**) in rats exposed once to three times (E1–E3) to EMF of 1 or 7 mT or control conditions. Note different scales on each plot.

Fig. 9. Behavioural changes in OF test: total distance moved (cm) (**a**), movement velocity (cm/s) (**b**), total movement time (s) (**c**), latency (s) (**d**), time in border zone (s) (**e**), time in corners (s) (**f**), entrance to central zone (**g**), time in central zone (s) (**h**) in rats exposed once to three times (E1–E3) to EMF of 1 or 7 mT or control conditions. Presented values are predicted by the GLM model for a significant intensity of the EMF (mT) x number of exposures (E1–E3) interaction (**a–c**, **e**, **f**) or significant effect of intensity of the EMF (mT) (**d**, **g**, **h**). Boxes indicate the lower quartile, median, and upper quartile, and whiskers indicate the range of changes (minimum to maximum). Statistically significant differences between animals from the same group are denoted $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ and these between experimental groups are denoted $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (n = 90; 10 in each group for each exposure). Note different scales on each plot.

(For figure see next page.)



It should be noticed the c.a. triple increase of the "basal" concentration of ACTH in plasma in EMF/B/7mT after each subsequent exposure in comparison to all other groups and the strikingly reduced concentrations of the hormone after OF test, which was similar to the control level (Fig. 7c). In the pituitary, the OF-induced changes in ACTH concentrations were similar (Fig. 7d). In both groups exposed to EMF (1 mT and 7 mT), OF test did not induce an increase in CRH concentrations (Fig. 7e).

Receptors

We evaluated the percentage changes in the relative mRNA transcript abundance of receptors (GR, MR, MC2R, and CRH-R1) after OF test in comparison to their mRNA transcript abundance in the control C/B group after each exposure (set at 100% – reference value) (Fig. 8). After the OF test, the hormone receptor mRNA did not differ from its "basal" abundance. The only important change concerned the MR and GR mRNA in the HIP in rats exposed to EMF of 1 mT. The OF-induced increase of MR mRNA was visible not only after the first exposure to EMF but also after the second one. We also noticed the restoration of GRs to the control level in this group.

Behavioural Analysis

Overall, the activity level in rats exposed to EMF of 7 mT was higher relative to both control and animals exposed to EMF of 1 mT (online suppl. Table S5; Fig. 9).

Whole Arena

The distance moved (Fig. 9a) by control animals was at a similar level after all exposures (E1–E3) with a slight increase after the last exposure. In the EMF/OF/1mT group, the distance moved during all subsequent tests did not differ from values noticed in C/OF group. The longest distance in OF was travelled by rats from EMF/OF/7mT after the first exposure (E1) to the EMF. After subsequent exposures to EMF (E2 and E3), the decrease in the value of this parameter was observed.

In all groups, the profile of changes in movement velocity (Fig. 9b) and total movement time (Fig. 9c) was similar to that observed in the case of distance moved as the parameters are related to each other. Rats exposed to EMF of 7 mT showed significantly shorter latency to start the movement after placement in OF than that noticed in control and exposed to 1 mT EMF rats (Fig. 9d).

Border Zone

In the control group, the time spent in the border zone (Fig. 9e) after E1 and E2 remained at a similar level and was significantly reduced after E3. In the EMF/OF/1mT group, the significant reduction in time spent in the border was observed already after E2. Rats exposed to EMF of 7 mT moved significantly less in the border zone already after E1; after E2, the value of the parameter was still decreased to receive the control value after E3.

The time spent in corners (Fig. 9f) did not differ between control and EMF/OF/1mT groups. However, in both groups, a significant gradual reduction in time spent in the corners after subsequent exposures was observed. Rats exposed to EMF of 7 mT spent significantly less time in the corners relative to other groups.

Central Zone

Rats exposed to EMF of 7 mT made more entries to the central zone (Fig. 9g) and spent a relatively greater proportion of time in the central zone of OF (Fig. 9h) than that noticed in control and exposed to 1 mT EMF rats.

Discussion

Our research has shown that the impact of repeated exposure to EMF on the HPA axis activity depends on the intensity of EMF. The increase of HPA axis hormone concentrations in rats exposed to EMF of 1 mT was maximal after the first exposure to EMF and was attenuated with each subsequent exposure. The initial increase in CRH induced the compensatory reaction – the reduction of relative mRNA transcript abundance of CRH-R1 receptors after each subsequent exposure to EMF. This may reflect an adaptive process aiming at diminishing the HPA axis activity in response to moderate stress [43]. The EMF-induced increase of ACTH release was accompanied by a parallel decline in MC2R mRNA transcript abundance only after the first exposure. Data suggest negative feedback on MC2R expression imposed by both its ligand (ACTH) and glucocorticoids [44]. The profile of corticosterone release from AG in the 1 mT EMF group appears to be a summated effect of several processes at higher levels of the HPA axis: the initial increase and then the decrease of hormone concentrations with each subsequent exposure. However, it should be stressed that the CORT concentrations were higher than that in control animals after each subsequent exposure. The results suggest that even a low intensity of EMF (1 mT) is a challenge, causing an HPA axis stress response, if relatively weak and temporary. Similarly, most other research also indicated that a low level of EMF (≤1 mT) causes an increase of HPA axis hormone concentrations [12, 13, 15].

In the group exposed to high EMF intensity (7 mT), the concentrations of HPA axis hormones were significantly higher than values recorded in both control and low-dose EMF (1 mT), and there was no apparent habituation with repeated exposure. Up-regulation of CRH predicts an enhanced capacity for stress excitation and is purported to reflect a "chronic stress-recruited" pathway [45], a phenomenon that can explain the high activity of the HPA axis in the 7 mT EMF-exposed group. The lack of down-regulation of CRH-R1 in this group can be the result of the stimulating role of a much higher concentration of CORT on the mRNA transcript abundance of the receptors in rats exposed to 7 mT than in 1 mT exposed ones. The ACTH concentration in the PG increased 4 times after first exposure and becomes even higher with each next exposure. Although we observed a decrease in relative MC2R mRNA transcript abundance after the 1st and 2nd exposure to EMF of 7 mT, the CORT release was not limited. The decrease in MC2R receptors may represent the adaptive response aimed to diminish the HPA axis activity; however, in such challenging stress situation, it seems that the regulatory mechanisms became insufficient or the HPA axis activity is regulated outside the main short-loop feedback. As a consequence of the cumulative effect of repeated severe stress, the glucocorticoid responses to a given stressor can be amplified [46]. A much stronger influence of the high-density EMF on the activation of stress systems was also demonstrated in other studies, e.g., increase in corticosterone secretion after chronic exposure to EMFs (3 mT) [14].

Apart from the negative feedback operating between components of the HPA axis, the important role in the regulation of HPA axis activity is played by GRs and MRs in the HIP. MRs play a crucial role at the onset of the stress reaction and increase neuronal excitability [35]. Finally, mineralocorticoid signalling is believed to play a major role in inhibiting HPA axis tone. GRs maintain the initiated stress response and then dampen neuron excitation, normalizing brain activity and promoting recovery [28].

MR-mediated corticosteroid negative feedback during stress may be an important mechanism that helps minimize the exposure of target tissue to corticosteroids, especially in the context of repeated stress [47–49]. Thus, increased MR mRNA might culminate in decreased HPA axis activity [50, 51] observed in our experiments with each subsequent exposure to 1 mT EMF. In contrast, hippocampal MR and GR down-regulation promotes CORT hypersecretion. The degree of such down-regulation varies with the nature and duration of the stressor applied, but in general, the stronger the stress, the more profound the MR and GR down-regulation [52, 53]. The increase in circulating CORT with each subsequent exposure to EMF of 7 mT may thus be related to loss of glucocorticoid feedback control of the HPA axis associated with decreased GR mRNA transcript abundance and even more with profound down-regulation of MR mRNA in the HIP. This phenomenon is also observed with other kinds of stress. In chronically stressed mice, the decrease in the relative mRNA transcript abundance of hippocampal MR and GR was accompanied with the increase of corticosterone concentration [54]. Also, changes in MR/GR balances have been found in patients with psychiatric disorders [55], emphasizing the importance of both CORT receptors, as particularly noted with high-intensity EMF exposure. Any implications for psychopathology are now important to determine.

Our results are consistent with the studies that confirmed the differences in the course of repeated exposure to some kind of stressor depending on stressor stimulus intensity [46, 56]. One weak/moderate challenge to the organism results in the inhibition of the HPA axis activity to the next second stimulus, as a consequence of feedback inhibition, characterized by a decreasing glucocorticoid response over time [57]. It was suggested that MR blockade impairs the ability to habituate to stress [48]. Our results showed that repeated exposure to EMF of 1 mT engenders a weak and temporary stress response. The decrease in CORT release with each exposure to 1 mT EMF may be partly explained by increased MR expression in the HIP. However, if stress stimuli are sufficiently intense, facilitation can override feedback inhibition, resulting in higher peak glucocorticoid concentrations to successive stimuli [56, 58]. The increasing activation of the HPA axis with each subsequent exposure to 7 mT EMF may reflect this phenomenon.

Our study is the first to show a hormetic, bidirectional effect of EMF (50 Hz) in vertebrates in vivo: adaptation at low EMF but sensitization at a higher magnetic flux density. A hormetic effect of radiofrequency EMF (1,800 MHz) on genomic DNA has been also reported in vitro in mouse embryonic fibroblasts [59]. In case of low-frequency EMF (50 Hz), the hormetic effect to our knowledge, has only been shown till now in *Drosophila melanogaster* [60]. Our results allowed us to conclude that repeated exposure to EMF of 1 mT shifts the set-point of stress systems activity, leading to adaptation to this kind of stress. Conversely, in animals exposed to EMF of 7 mT, the shift of the set-point of stress systems activity into augmented vulnerability was noticed. In consequence,

the shifted set-point of HPA axis can influence the response to the subsequent heterotypic stress – the OF. It has been found that the novel stress-induced activation of the HPA axis after the repeated exposure to homotypic stress may cause increased glucocorticoid release, the shut-off of the stress response or in extreme cases hyporesponsiveness driven by adrenal exhaustion [61]. E.g., the strong social stress in mice finally caused them to cross the line from enhanced adrenal reactivity to adrenal exhaustion, a condition which was associated with substantial physical morbidity [62].

The profile and dynamics of OF-induced changes in hormone concentrations and their receptors' mRNA transcript abundance almost completely covered their basal levels – observed just after subsequent exposures to EMF or control conditions. However, if we evaluated the percentage changes in specific hormone concentrations, it became clear that in groups exposed to EMF, the release of hormones stimulated by another stress was diminished. This was most evident in the 7 mT EMF group, where OF-induced concentrations of all hormones of the HPA axis were severalfold lower than their respective "basal" concentrations. This may express a "ceiling effect" because "basal" CORT concentration was 5, 7, and 9 times higher than the value in control animals. Thus, it is likely that the further increase of CORT concentration in response to the next stressor was limited which reflects the adrenal exhaustion phenomenon. In animals exposed to 1 mT EMF, HPA hormonal responses to the OF challenge were attenuated. As levels of stress hormones were lower than after 7 mT EMF exposure, this is not likely to be adrenal exhaustion but is more probably an adaptation to heterotypic stressors. Interestingly, this coincided with the restoration of GR mRNA. Moreover, OF-induced increase in hippocampal MR mRNA appeared not only after the first exposure to EMF but was even higher after the second one. This suggests that previous EMF exposure acts as preconditioning which facilitates an adaptive response to subsequent stress events. A common observation in studies of hormesis is that exposure to low levels of one type of hormetic agent induces pathways responsible for brain plasticity to protect cells and organisms against subsequent heterotypic stress [33].

An association between EMF exposure and emotional behaviour has been indicated in many but not all studies. Behavioural effects of the EMF depend on the length, frequency, and intensity of exposure [15, 63]. Some reports noted reduced activity of animals after EMF exposure, and an anxiety-like behaviour, others observed anxiolytic effect or did not observe any changes. It is reasonable to speculate that EMF-induced behavioural changes may be attributed in part to the effect on HPA axis [33]. In our study, rats exposed to EMF of 7 mT showed less anxiety-related behaviour than that noticed in control and exposed to 1 mT EMF rats. This anxiolytic behaviour in the OF test may be due to substantive corticosterone release reduction. Moreover, the lower MR mRNA in the HIP, following 7 mT EMF exposure, could also underlie increased activity in OF. Indeed, MR antagonism decreases anxiety behaviour in rats [64].

We did not observe the difference in behaviour in OF tests in animals previously experienced with 1 mT EMF in comparison to control animals. We suggest that the stress system reaction evoked by the low-stress factor – 1 mT EMF – was not strong enough to change their behaviour. Similarly, mice prenatally exposed to EMF (50 Hz, 1 mT) did not show changes in anxiety-like behaviour [65].

The effects of exposure to EMF are particularly prevalent in the HIP [17, 19]. Corticosterone exerts a bidirectional, dose-dependent effect on hippocampal function. Whereas the highly elevated corticosterone concentrations potentiate excitotoxicity and disturb HPA axis function, low concentrations of corticosterone protect against excitotoxic neuronal damage and promote brain plasticity (hormesis-like effect) [29, 47]. High-density (8 mT) EMF causes memory impairment and cell death [8, 24]. On the other hand, low EMF (1 mT or less) stimulates hippocampal plasticity [17–20]. Glucocorticoids impact the survival of hippocampal neurons and neuroplasticity via activation of MR [27, 29, 47]. The finding that EMF of 1 mT specifically induces MR mRNA in the HIP is in line with the previous findings that sub-lethal challenge can increase neuronal MR expression and may serve as a compensatory mechanism designed to induce neuronal plasticity. To summarize, the bidirectional effects of low- and high-density EMF exposure might be related to different MR mRNA abundance as they influence the HPA axis activity and also because of their neuroprotective value.

Conclusion

Our study suggests that the EMF intensity is the crucial factor determining the bidirectional influence of low-frequency EMF on the brain: positive – promoting brain plasticity that improves the neuroadaptation to subsequent stress exposures or negative – responsible for the disturbance of stress response and increasing sensitivity to subsequent stressors and perhaps the risk of stress-in-

duced disorders. Thus, the proposed study is a pioneer because for the first time, we suggest such "hormetic mode of action" of EMF (50 Hz) in vertebrates (verified on rat model). The results can contribute to explaining the fundamental mechanisms of the bidirectional responses to EMF and provide a new view on possible therapeutic properties of the magnetic field as well as a new direction in the risk assessment of EMF exposure. We have shown for the first time the induction of hippocampal neuronal MR mRNA in response to EMF of 1 mT in rat brain. Although the consequences of increased MR expression under these circumstances are not precisely known, this phenomenon may represent an endogenous response to protect the brain from subsequent injury. It would be of interest to explore the long-lasting effects of EMF-induced MR changes. As the consequences of permanent changes in the stress response can be multidimensional, future research should further address the EMF-induced mechanisms, mainly related to their therapeutic properties. We are also convinced that the results of our research will help clarify the mechanisms of EMF impact on health – which is very important for humanity – living in more and more full of EMF environment.

Acknowledgments

We would like to thank Prof. Maria Stankiewicz (Nicolaus Copernicus University) for her valuable comments on the manuscript.

Statement of Ethics

This study protocol was reviewed and approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland; approval number 3/2018.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was supported by National Science Centre, Poland (Grant No. 2017/25/B/NZ7/00638) and project No. POWR.03.05.00-00-Z302/17 "Universitas Copernicana Thoruniensis In Futuro," co-financed by the European Social Fund – the Operational Programme Knowledge Education Development. Module 5. Interdisciplinary PhD School "Academia Copernicana."

Author Contributions

Justyna Rogalska conceived the project and got funding; Angelika Klimek, Hanna Kletkiewicz, and Joanna Wyszkowska prepared experimental protocols; Angelika Klimek, Hanna Kletkiewicz, Maciej Klimiuk, Agnieszka Siejka, Justyna Maliszewska, and Milena Jankowska conducted the experiments; Angelika Klimek and Hanna Kletkiewicz analysed the results; Angelika Klimek and Justyna Rogalska drafted the manuscript; Hanna Kletkiewicz, Justyna Maliszewska, Milena Jankowska, and Joanna Wyszkowska reviewed the manuscript; Angelika Klimek and Agnieszka Siejka prepared data visualization; Justyna Rogalska and Jonathan Seckl supervised.

Data Availability Statement

The data used to support the findings of this study are included in the article and its online supplementary material file. Further enquiries can be directed to the corresponding authors.

References

1 Touitou Y, Selmaoui B. The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system. Dialogues Clin Neurosci. 2012;14(4):381–99.
2 Johansson O. Disturbance of the immune system by electromagnetic fields: a potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment. Pathophysiology. 2009;16(2–3):157–77.
3 Corallo C, Battisti E, Albanese A, Vannoni D, Leoncini R, Landi G, et al. Proteomics of human primary osteoarthritic chondrocytes exposed to extremely low-frequency electromagnetic fields (ELF EMFs) and to therapeutic application of musically modulated electromagnetic fields (TAMMEF). Electromagn Biol Med. 2013;33(1):3–10.
4 Li SS, Zhang ZY, Yang CJ, Lian HY, Cai P. Gene expression and reproductive abilities of male Drosophila melanogaster subjected to ELF-EMF exposure. Mutat Res Genet Toxicol Environ Mutagen. 2013;758(1–2):95–103.
5 Merla C, Liberti M, Consales C, Denzi A, Apollonio F, Marino C, et al. Evidences of plasma membrane-mediated ROS generation upon ELF exposure in neuroblastoma cells supported by a computational multiscale approach. Biochim Biophys Acta Biomembr. 2019;1861(8):1446–57.
6 Tiwari R, Lakshmi NK, Bhargava SC, Ahuja YR. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations. Electromagn Biol Med. 2014;34(1):56–62.
7 Duan W, Liu C, Zhang L, He M, Xu S, Chen C, et al. Comparison of the genotoxic effects induced by 50 Hz extremely low-frequency electromagnetic fields and 1800 MHz radiofrequency electromagnetic fields in GC-2 cells. Radiat Res. 2015;183(3):305–14.

8 Yin C, Luo X, Duan Y, Duan W, Zhang H, He Y, et al. Neuroprotective effects of lotus seedpod procyanidins on extremely low frequency electromagnetic field-induced neurotoxicity in primary cultured hippocampal neurons. Biomed Pharmacother. 2016;82:628–39.
9 Ferroni L, Tocco I, De Pieri A, Menarin M, Fermi E, Piattelli A, et al. Pulsed magnetic therapy increases osteogenic differentiation of mesenchymal stem cells only if they are pre-committed. Life Sci. 2016;152:44–51.
10 Haghighat N, Abdolmaleki P, Parnian J, Behmanesh M. The expression of pluripotency and neuronal differentiation markers under the influence of electromagnetic field and nitric oxide. Mol Cell Neurosci. 2017;85:19–28.
11 Blank M, Goodman R. Electromagnetic fields stress living cells. Pathophysiology. 2009;16(2–3):71–8.
12 Ebadi A, Sedghi H, Zare S, Hayatgeibi H, Alivandi S. Effects of 50 HZ magnetic field on some factors of immune system in the male Guinea pigs. Am J Immunol. 2005;1(1):37–41.
13 Szemerszky R, Zelena D, Barna I, Bárdos G. Stress-related endocrinological and psychopathological effects of short- and long-term 50Hz electromagnetic field exposure in rats. Brain Res Bull. 2010;81(1):92–9.
14 Kitaoka K, Kitamura M, Aoi S, Shimizu N, Yoshizaki K. Chronic exposure to an extremely low-frequency magnetic field induces depression-like behavior and corticosterone secretion without enhancement of the hypothalamic-pituitary-adrenal axis in mice. Bioelectromagnetics. 2012;34(1):43–51.
15 Mahdavi SM, Sahraei H, Yaghmaei P, Tavakoli H. Effects of electromagnetic radiation exposure on stress-related behaviors and stress hormones in male Wistar rats. Biomol Ther. 2014;22(6):570–6.
16 Martínez-Sámano J, Flores-Poblano A, Verdugo-Díaz L, Juárez-Oropeza MA, Torres-Durán PV. Extremely low frequency electromagnetic field exposure and restraint stress induce changes on the brain lipid profile of Wistar rats. BMC Neurosci. 2018;19(1):31.
17 Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, et al. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol. 2010;226(1):173–82.
18 Tasset I, Medina FJ, Jimena I, Agüera E, Gascón F, Feijóo M, et al. Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: effects on neurotrophic factors and neuronal density. Neuroscience. 2012;209:54–63.
19 Komaki A, Khalili A, Salehi I, Shahidi S, Sarihi A. Effects of exposure to an extremely low frequency electromagnetic field on hippocampal long-term potentiation in rat. Brain Res. 2014;1564:1–8.
20 Sakhaie MH, Soleimani M, Pourheydar B, Majd Z, Atefimanesh P, Asl SS, et al. Effects of extremely low-frequency electromagnetic fields on neurogenesis and cognitive behavior in an experimental model of hippocampal injury. Behav Neurol. 2017;2017:9194261.
21 Cichoń N, Rzeźnicka P, Bijak M, Miller E, Miller S, Saluk J. Extremely low frequency electromagnetic field reduces oxidative stress during the rehabilitation of post-acute stroke patients. Adv Clin Exp Med. 2018;27(9):1285–93.
22 Bagheri Hosseinabadi M, Khanjani N, Ebrahimi MH, Haji B, Abdolahfard M. The effect of chronic exposure to extremely low-frequency electromagnetic fields on sleep quality, stress, depression and anxiety. Electromagn Biol Med. 2019;38(1):96–101.
23 Klimek A, Rogalska J. Extremely low-frequency magnetic field as a stress factor-really detrimental?-insight into literature from the last decade. Brain Sci. 2021;11(2):174.
24 Jadidi M, Firoozabadi SM, Rashidy-Pour A, Sajadi AA, Sadeghi H, Taherian AA. Acute exposure to a 50 Hz magnetic field impairs consolidation of spatial memory in rats. Neurobiol Learn Mem. 2007;88(4):387–92.
25 Korpinar MA, Kalkan MT, Tuncel H. The 50 Hz (10 mT) sinusoidal magnetic field: effects on stress-related behavior of rats. Bratisl Lek Listy. 2012;113(09):521–4.
26 de Kleijn S, Ferwerda G, Wiese M, Trentelman J, Cuppen J, Kozicz T, et al. A short-term extremely low frequency electromagnetic field exposure increases circulating leukocyte numbers and affects HPA-axis signaling in mice: ELF-EMF effect on stress regulation in mice. Bioelectromagnetics. 2016;37(7):433–43.
27 Krugers HJ, Karst H, Joels M. Interactions between noradrenaline and corticosteroids in the brain: from electrical activity to cognitive performance. Front Cell Neurosci. 2012;6:15.
28 Arnett MG, Muglia LM, Laryea G, Muglia LJ. Genetic approaches to hypothalamic-pituitary-adrenal axis regulation. Neuropsychopharmacol. 2016;41(1):245–60.
29 Rogalska J. Mineralocorticoid and glucocorticoid receptors in hippocampus: their impact on neurons survival and behavioral impairment after neonatal brain injury. Vitam Horm. 2010;82:391–419.
30 Calabrese EJ. Overcompensation stimulation: a mechanism for hormetic effects. Crit Rev Toxicol. 2001;31(4–5):425–70.
31 Mushak P. Temporal stability of chemical hormesis (CH): is CH just a temporary stop on the road to thresholds and toxic responses? Sci Total Environ. 2016;569–570:1446–56.
32 Calabrese EJ, Mattson MP. How does hormesis impact biology, toxicology, and medicine? NPJ Aging Mech Dis. 2017;3(1):13.
33 Cirulli F, Alleva E. The NGF saga: from animal models of psychosocial stress to stress-related psychopathology. Front Neuroendocrinol. 2009;30(3):379–95.
34 Datson NA, van den Oever JM, Korobko OB, Magarinos AM, de Kloet ER, McEwen BS. Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus. Endocrinology. 2013;154(9):3261–72.
35 Koning A-SCAM, Buurstede JC, van Weert LTCM, Meijer OC. Glucocorticoid and mineralocorticoid receptors in the brain: a transcriptional perspective. J Endocr Soc. 2019;3(10):1917–30.
36 Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.
37 Bieńkowski P, Wyszkowska J. Techniczne aspekty ekspozycji na pole magnetyczne ekstremalnie niskich częstotliwości (ELF) w badaniach biomedycznych [Technical aspects of exposure to magnetic fields of extremely low frequencies (ELF) in biomedical research]. Med Pr. 2015;66(2):185–97.
38 Trawiński T, Szczygieł M, Wyszkowska J, Kluszczyński K. Analysis of magnetic field distribution and mechanical vibration of magnetic field exciter under different voltage supply. Inf Technol Biomed. 2010;69:613–22.
39 Directive 2013/35/EU of the European Parliament and of the Council of 26 June 2013 on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (electromagnetic fields).
40 Calvo AC, Azanza MJ. Synaptic neurone activity under applied 50 Hz alternating magnetic fields. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 1999;124(1):99–107.
41 Hug K, Röösli M. Therapeutic effects of whole-body devices applying pulsed electromagnetic fields (PEMF): a systematic literature review. Bioelectromagnetics. 2012;33(2):95–105.
42 Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST(C)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 2002;30(9):e36.
43 Ochedalski T, Rabadan-Diehl C, Aguilera G. Interaction between glucocorticoids and corticotropin releasing hormone (CRH) in the regulation of the pituitary CRH receptor in vivo in the rat. J Neuroendocrinol. 1998;10(5):363–9.
44 Agulleiro MJ, Sánchez E, Leal E, Cortés R, Fernández-Durán B, Guillot R, et al. Molecular characterization and functional regulation of melanocortin 2 receptor (MC2R) in the sea bass. A putative role in the adaptation to stress. PLoS One. 2013;8(5):e65450.
45 Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, et al. Chronic stress and obesity: a new view of "comfort food. Proc Natl Acad Sci U S A. 2003;100(20):11696–701.

46 Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am J Physiol Endocrinol Metab. 2006; 291(5):E965–73.
47 de Kloet ER, Vreugdenhil ER, Oitzl E, Joëls MS. Brain corticosteroid receptor balance in health and disease. Endocr Rev. 1998;19(3): 269–301.
48 Cole MA, Kalman BA, Pace TW, Topczewski F, Lowrey MJ, Spencer RL. Selective blockade of the mineralocorticoid receptor impairs hypothalamic-pituitary-adrenal axis expression of habituation: mineralocorticoid receptor antagonists block habituation expression. J Neuroendocrinol. 2001;12(10):1034–42.
49 Gądek-Michalska A, Spyrka J, Rachwalska P, Tadeusz J, Bugajski J. Influence of chronic stress on brain corticosteroid receptors and HPA axis activity. Pharmacol Rep. 2013; 65(5):1163–75.
50 McCullers DL, Sullivan PG, Scheff SW, Herman JP. Traumatic brain injury regulates adrenocorticosteroid receptor mRNA levels in rat hippocampus. Brain Res. 2002;947(1):41–9.
51 Rogalska J, Kang P, Wotherspoon W, Macleod MR, Lai M. Effect of hyperthermia and anoxia on glucocorticoid and mineralocorticoid receptor expression in neonatal rat hippocampus. Neurosci Lett. 2009;450(2):196–200.
52 Wang Q, Yu K, Wang J, Lin H, Wu Y, Wang W. Predator stress-induced persistent emotional arousal is associated with alterations of plasma corticosterone and hippocampal steroid receptors in rat. Behav Brain Res. 2012; 230(1):167–74.
53 Zhang L, Zhang J, Sun H, Zhu H, Liu H, Yang Y. An enriched environment elevates corticosteroid receptor levels in the hippocampus and restores cognitive function in a rat model of chronic cerebral hypoperfusion. Pharmacol Biochem Behav. 2013;103(4):693–700.
54 Sterlemann V, Ganea K, Liebl C, Harbich D, Alam S, Holsboer F, et al. Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: implications for stress-related disorders. Horm Behav. 2008;53(2):386–94.
55 Von Werne Baes C, de Carvalho Tofoli SM, Martins CMS, Juruena MF. Assessment of the hypothalamic-pituitary-adrenal axis activity: glucocorticoid receptor and mineralocorticoid receptor function in depression with early life stress – a systematic review. Acta Neuropsychiatr. 2012;24(1):4–15.
56 Rabasa C, Muñoz-Abellán C, Daviu N, Nadal R, Armario A. Repeated exposure to immobilization or two different footshock intensities reveals differential adaptation of the hypothalamic-pituitary-adrenal axis. Physiol Behav. 2011;103(2):125–33.
57 Dhabhar FS, McEwen BS, Spencer RL. Adaptation to prolonged or repeated stress--comparison between rat strains showing intrinsic differences in reactivity to acute stress. Neuroendocrinology. 1997;65(5):360–8.
58 Figueiredo HF, Bodie BL, Tauchi M, Dolgas CM, Herman JP. Stress integration after acute and chronic predator stress: differential activation of central stress circuitry and sensitization of the hypothalamo-pituitary-adrenocortical axis. Endocrinology. 2003;144(12): 5249–58.
59 Sun C, Wei X, Fei Y, Su L, Zhao X, Chen G, et al. Mobile phone signal exposure triggers a hormesis-like effect in Atm+/+ and Atm-/- mouse embryonic fibroblasts. Sci Rep. 2016; 6(1):37423.
60 Graham JH, Fletcher D, Tigue J, McDonald M. Growth and developmental stability of Drosophila melanogaster in low frequency magnetic fields. Bioelectromagnetics. 2000; 21(6):465–72.
61 Herman JP. Neural control of chronic stress adaptation. Front Behav Neurosci. 2013;7:61.
62 Reber SO, Birkeneder L, Veenema AH, Obermeier F, Falk W, Straub RH, et al. Adrenal insufficiency and colonic inflammation after a novel chronic psycho-social stress paradigm in mice: implications and mechanisms. Endocrinology. 2007;148(2):670–82.
63 Janać B, Selaković V, Rauš S, Radenović L, Zrnić M, Prolić Z. Temporal patterns of extremely low frequency magnetic field-induced motor behavior changes in Mongolian gerbils of different age. Int J Radiat Biol. 2012; 88(4):359–66.
64 Smythe JW, Murphy D, Timothy C, Costall B. Hippocampal mineralocorticoid, but not glucocorticoid, receptors modulate anxiety-like behavior in rats. Pharmacol Biochem Behav. 1997;56(3):507–13.
65 Alsaeed I, Al-Somali F, Sakhnini L, Aljarallah OS, Hamdan RMM, Bubishate SA, et al. Autism-relevant social abnormalities in mice exposed perinatally to extremely low frequency electromagnetic fields. Int J Dev Neurosci. 2014;37(1):58–64.

Supplemental Materials:

New view on the impact of the low-frequency electromagnetic field (50 Hz) on stress responses – hormesis effect

Angelika Klimek[1#], Hanna Kletkiewicz[1], Agnieszka Siejka[1], Joanna Wyszkowska[1], Justyna Maliszewska[1], Maciej Klimiuk[1], Milena Jankowska[1], Jonathan Seckl[2], Justyna Rogalska[1#]

[1] Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences Nicolaus Copernicus University in Torun, 87-100, Poland.

[2] Centre for Cardiovascular Science, QMRI, University of Edinburgh, Edinburgh, U.K.

[#]*Angelika Klimek, Justyna Rogalska*

Email: klimek@doktorant.umk.pl, rogal@umk.pl

Tel: +48 785 150 453

Supplement 1

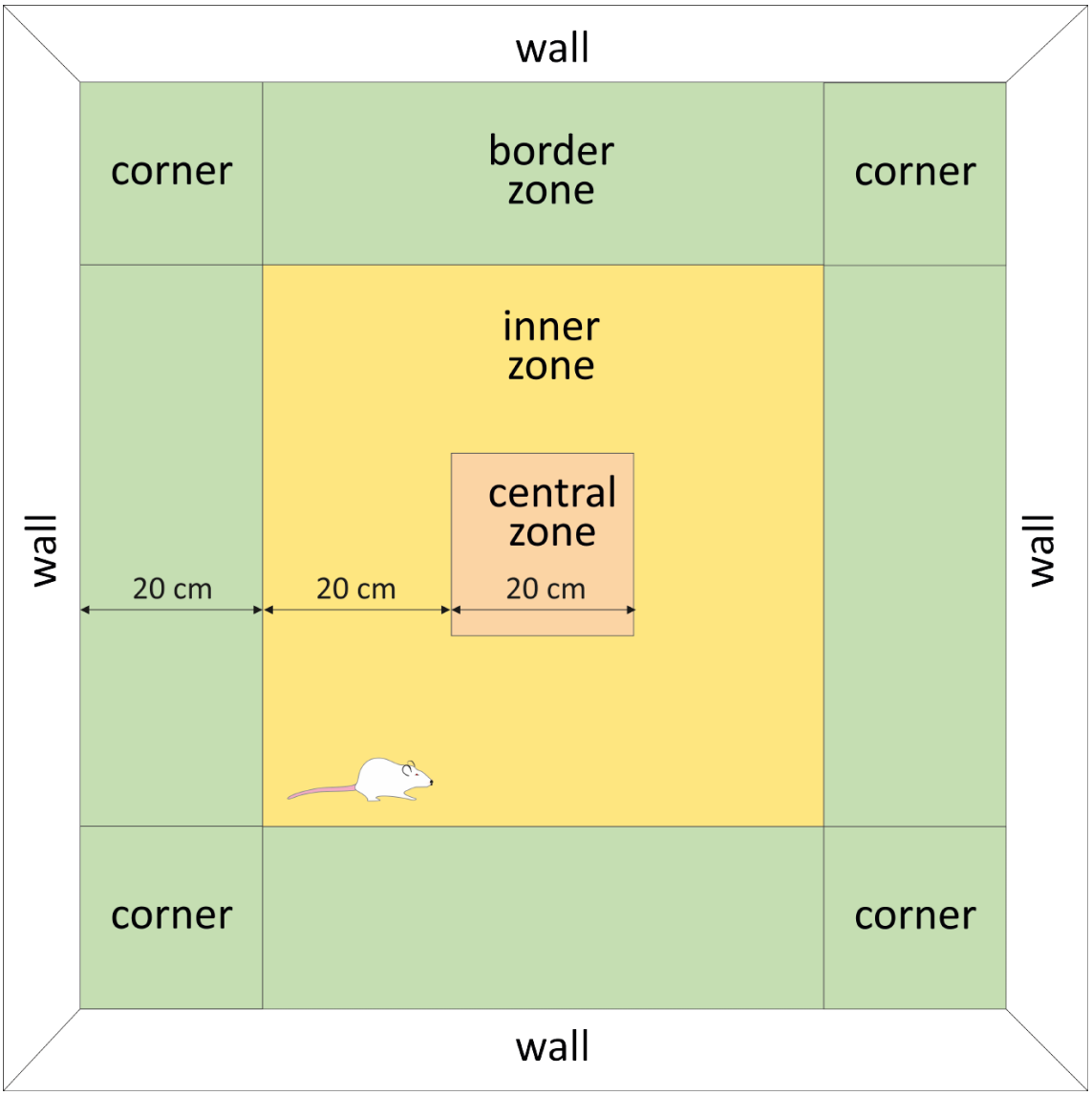


Fig. S1

Schematic representation of the open field (100x100x50cm) showing the areas in which the various parameters of behaviour were recorded.

Supplement 2

Table S1 Primers for reverse transcription-quantitative polymerase chain reaction

Gene	Forward primer	Reverse primer	Annealing
CRH-R1	TTCTGAACAGTGAGGTCCGC	AGGTGGGGATGGACATAGCT	60˚C, 30 sec
MC2R	ATCTGCAGTTTGGCCATTTC	GCAATCACAGACAGGCTGAA	64˚C, 30 sec
MR	TGCATGATCTCGTGAGTGA	AAGTTCTTCCTGGCCGGTAT	63˚C, 20 sec
GR	CACCCATGACCCTGTCAGTC	AAAGCCTCCCTCTGCTAACC	60˚C, 20 sec
GAPDH	TAAAGAACAGGCTCTTAGCACA	AGTCTTGGAAATGGATTGTCTC	59˚C, 15 sec
β-actin	CGTTGACATCCGTAAAGACCTC	TAGGAGCCAGGGCAGTAATCT	58˚C, 30 sec

CRH-R1, corticotropin releasing hormone receptor 1; MC2R, melanocortin receptor 2; MR, mineralocorticoid receptor; GR, glucocorticoid receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

Table S2 Results of statistical analysis of the 'basal' hormones concentrations in examined brain areas, adrenal glands and plasma

	Dependent variable	Effect	*df*	*F*	*P*
a	CORT in plasma	intensity of the electromagnetic field (mT)	2	26.711 (F)	**<0.001**
		number of exposures (E1-E3)	2	2.922 (F)	0.061
		(mT) x (E1-E3)	4	9.686 (F)	**<0.001**
		Error	68		
b	CORT in adrenal gland	intensity of the electromagnetic field (mT)	2	582.248 (F)	**<0.001**
		number of exposures (E1-E3)	2	0.217 (F)	0.806
		(mT) x (E1-E3)	4	17.567 (F)	**<0.001**
		Error	48		
c	ACTH in plasma	intensity of the electromagnetic field (mT)	2	327.958 (F)	**<0.001**
		number of exposures (E1-E3)	2	0.191 (F)	0.827
		(mT) x (E1-E3)	4	0.097 (F)	0.983
		Error	80		
d	ACTH in pituitary gland	intensity of the electromagnetic field (mT)	2	690.749 (F)	**<0.001**
		number of exposures (E1-E3)	2	0.832 (F)	0.443
		(mT) x (E1-E3)	4	12.135 (F)	**<0.001**
		Error	36		
e	CRH in hypothalamus	intensity of the electromagnetic field (mT)	2	50.460 (F)	**<0.001**
		number of exposures (E1-E3)	2	18.540 (F)	**<0.001**
		(mT) x (E1-E3)	4	2.549 (F)	**0.046**
		Error	80		

F - ANOVA test, statistically significant P value - indicated in bold, CORT - corticosterone, ACTH - adrenocorticotropic hormone, CRH - corticotropin-releasing hormone

Table S3 Results of statistical analysis of the OF-induced hormones concentrations in examined brain areas, adrenal glands and plasma

	Dependent variable	Effect	*df*	*F/H*	*P*
a	CORT in plasma	intensity of the electromagnetic field (mT)	2	15.613 (F)	**<0.001**
		number of exposures (E1-E3)	2	4.239 (F)	**0.018**
		(mT) x (E1-E3)	4	8.621 (F)	**<0.001**
		Error	69		
b	CORT in adrenal gland	intensity of the electromagnetic field (mT)	2	359.091 (F)	**<0.001**
		number of exposures (E1-E3)	2	0.467 (F)	0.630
		(mT) x (E1-E3)	4	2.424 (F)	0.061
		Error	48		
c	ACTH in plasma	intensity of the electromagnetic field (mT)	2	67.874 (H)	**<0.001**
		number of exposures (E1-E3)	2	980 (H)	0.613
d	ACTH in pituitary gland	intensity of the electromagnetic field (mT)	2	381.977 (F)	**<0.001**
		number of exposures (E1-E3)	2	2.853 (F)	0.069
		(mT) x (E1-E3)	4	4.997 (F)	**0.002**
		Error	42		
e	CRH in hypothalamus	intensity of the electromagnetic field (mT)	2	19.883 (H)	**<0.001**
		number of exposures (E1-E3)	2	3.810 (H)	0.145

F - ANOVA test, H - Kruskal-Wallis test, statistically significant P value - indicated in bold, CORT - corticosterone, ACTH - adrenocorticotropic hormone, CRH - corticotropin-releasing hormone

Table S4 Results of statistical analysis of effects of open-field stress on hormones concentrations in relation to their 'basal' concentrations in each experimental group

	Dependent variable/group	Effect	*df*	*F*	*P*
a	CORT in plasma	open field effect	1	5.783	**0.020**
	Control group	number of exposures (E1-E3)	2	2.215	0.121
		number of exposures x open field effect	1	1.199	0.311
		Error	46		
b	CORT in plasma	open field effect	1	1.581	0.215
	EMF/1 mT	number of exposures (E1-E3)	2	20.034	**<0.001**
		number of exposures x open field effect	1	0.841	0.438
		Error	49		
c	CORT in plasma	open field effect	1	0.318	0.576
	EMF/7 mT	number of exposures (E1-E3)	2	13.978	**<0.001**
		number of exposures x open field effect	1	4.246	**0.021**
		Error	42		
d	CORT in adrenal	open field effect	1	0.383	0.542
	gland	number of exposures (E1-E3)	2	5.030	**0.015**
	control group	number of exposures x open field effect	2	0.318	0.578
		Error	25		
e	CORT in adrenal	open field effect	1	87.208	**<0.001**
	gland	number of exposures (E1-E3)	2	12.840	**<0.001**
	EMF/1 mT	number of exposures x open field effect	2	10.580	**<0.001**
		Error	30		
f	CORT in adrenal	open field effect	2	3.972	0.054
	gland	number of exposures (E1-E3)	1	11.238	**<0.001**
	EMF/7 mT	number of exposures x open field effect	1	0.354	0.704
		Error	36		
g	ACTH in plasma	open field effect	1	1.678	0.201
	control group	number of exposures (E1-E3)	2	0.054	0.947
		number of exposures x open field effect	1	0.230	0.796
		Error	53		
h	ACTH in plasma	open field effect	1	3.233	0.078
	EMF/1 mT	number of exposures (E1-E3)	2	0.713	0.495
		number of exposures x open field effect	1	0.634	0.534
		Error	54		
i	ACTH in plasma	open field effect	1	0.011	0.916
	EMF/7 mT	number of exposures (E1-E3)	2	1.591	0.213
		number of exposures x open field effect	1	0.778	0.464
		Error	54		
j	ACTH in pituitary	open field effect	1	8.492	**0.008**
	gland	number of exposures (E1-E3)	2	0.925	0.410
	control group	number of exposures x open field effect	2	1.002	0.382
		Error	24		
k	ACTH in pituitary	open field effect	1	12.681	**0.002**
	gland	number of exposures (E1-E3)	2	3.596	**0.043**
	EMF/1 mT	number of exposures x open field effect	2	1.784	0.190
		Error	24		
l	ACTH in pituitary	open field effect	1	0.435	0.515
	gland	number of exposures (E1-E3)	2	6.820	**0.004**
	EMF/7 mT	number of exposures x open field effect	1	0.431	0.517
		Error	25		

m	CRH in hypothalamus control group	open field effect	1	14.574	**<0.001**
		number of exposures (E1-E3)	2	11.258	**<0.001**
		number of exposures x open field effect	2	0.575	0.566
		Error	52		
n	CRH in hypothalamus EMF/1 mT	open field effect	1	0.036	0.850
		number of exposures (E1-E3)	2	8.430	**<0.001**
		number of exposures x open field effect	2	4.238	**0.020**
		Error	54		
o	CRH in hypothalamus EMF/7 mT	open field effect	1	1.385	0.244
		number of exposures (E1-E3)	2	1.213	0.305
		number of exposures x open field effect	2	3.213	**0.048**
		Error	54		

F - ANOVA test, statistically significant P value - indicated in bold, CORT - corticosterone, ACTH - adrenocorticotropic hormone, CRH - corticotropin-releasing hormone

Table S5 Results of statistical analysis of behavioural variables in open field test

	Dependent variable	Effect	*df*	*F/H*	*P*
a	Total distance moved (cm)	intensity of the electromagnetic field (mT)	2	4.545 (F)	**0.013**
		number of exposures (E1-E3)	2	1.947 (F)	0.149
		(mT) x (E1-E3)	4	5.013 (F)	**0.001**
		Error	81		
b	Movement Velocity (cm/s)	intensity of the electromagnetic field (mT)	2	4.545 (F)	**0.013**
		number of exposures (E1-E3)	2	1.909 (F)	0.155
		(mT) x (E1-E3)	4	5.141 (F)	**0.001**
		Error	81		
c	Total movement time (s)	intensity of the electromagnetic field (mT)	2	3.907 (F)	**0.024**
		number of exposures (E1-E3)	2	0.111 (F)	0.895
		(mT) x (E1-E3)	4	5.858 (F)	**<0.001**
		Error	81		
d	Latency (s)	intensity of the electromagnetic field (mT)	2	15.355 (H)	**<0.001**
		number of exposures (E1-E3)	2	2.590 (H)	0.274
e	Time in border zone (s)	intensity of the electromagnetic field (mT)	2	9.584 (F)	**<0.001**
		number of exposures (E1-E3)	2	4.197 (F)	**0.018**
		(mT) x (E1-E3)	4	2.572 (F)	**0.044**
		Error	81		
f	Time in corners (s)	intensity of the electromagnetic field (mT)	2	5.352 (F)	**0.007**
		number of exposures (E1-E3)	2	11.091 (F)	**<0.001**
		(mT) x (E1-E3)	4	4.415 (F)	**0.003**
		Error	81		
g	Entrance to central zone	intensity of the electromagnetic field (mT)	2	14.526 (H)	**0.001**
		number of exposures (E1-E3)	2	2.218 (H)	0.330
h	Time in central zone (s)	intensity of the electromagnetic field (mT)	2	15.082 (H)	**0.001**
		number of exposures (E1-E3)	2	5.295 (H)	0.071

F - ANOVA test, H - Kruskal-Wallis test, statistically significant P value - indicated in bold

Research Article

The electromagnetic field (50 Hz) can establish a new "set-point" for the activity of the locus coeruleus–noradrenergic (LC-NA) system in rat

Angelika Klimek[1], Hanna Kletkiewicz[1], Agnieszka Siejka[1] , Joanna Wyszkowska[1], Justyna Maliszewska[1], Maciej Klimiuk[1], Milena Jankowska[1], Justyna Rogalska[1]

[1] Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences Nicolaus Copernicus University in Torun, 87-100, Poland.

Short Title: Dose-dependent effect of electromagnetic field on LC-NA system activity

Corresponding Authors:

Angelika Klimek, Justyna Rogalska

Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences
Nicolaus Copernicus University in Torun
Lwowska 1
Torun, 87-100, Poland
Tel: +48 785 150 453
E-mail: klimek@doktorant.umk.pl; rogal@umk.pl

Keywords: low-frequency electromagnetic field, stress response, locus coeruleus, noradrenaline, β2-adrenergic receptor, hippocampus

Abstract

Exposure of organisms to extremely low-frequency electromagnetic field (EMF; 50 Hz) has been increasing in recent decades, which is connected with dynamic technological development. EMF is considered as a stress factor and its effects on organisms are still being investigated. We aimed to determine its impact on the locus coeruleus–noradrenergic (LC-NA) system enabling adaptation to stressful conditions. For this purpose, we exposed rats to 50 Hz EMF of 1 and 7 mT, 1 h/day for 7 days. The procedure was repeated three times to examine the organism's adaptive capabilities. Subsequently, the concentration of adrenaline, noradrenaline and its metabolite MHPG as well as the expression of the β2-adrenergic receptor were assessed. After the end of each exposure, part of the animals were subjected to a behavioural test to assess the influence of repeated EMF exposure on stress response to subsequent stress factors.

Our research proved that mechanisms underlying the effects of EMF on stress response include LC-NA system. EMF of 1 mT induced adaptive changes in the NA-LC system. However, exposure to 7 mT caused increased activity of stress system which resulted in sensitization to subsequent, heterotypic stress factor. As EMF of 7 mT caused profound decrease in β2-AR would strongly inhibit the potential for neuroplastic processes in the hippocampus. Moreover, rats exposed to EMF of 7 mT showed moderately increased anxiety-related behavior.

Disturbances in NA-LC transmission may underlie the development of some neurodegenerative and psychiatric diseases which indicates the possible involvement of EMF in the pathogenesis of these disorders.

Highlights

1) The effect of the low-frequency electromagnetic field effect on LC-NA activity is hormetic

2) High dose of ELF-EMF (7 mT) causes hyperreactivity of the adrenergic system

3) The repeated exposure to EMF change the response to another stress factor

4) Rats exposed to EMF of 7 mT showed disturbed anxiety-related behaviour

5) 7 mT EMF caused a decrease in β2-AR thus can inhibit the neuroplastic processes in the hippocampus

1. Introduction

Society is temporarily or in some cases permanently exposed to electromagnetic fields (EMF), which as was proved have an impact on the function of the organism. The rapid development of science and technology has resulted in the introduction of many new devices and technologies in industry, agriculture, and everyday life (Touitou & Selmaoui 2012). Electrical appliances and overhead power lines emit electromagnetic fields at extremely low frequencies (1–300 Hz) (Touitou and Selmaoui, 2012). The important question which arises is whether our organism can adapt to this environmental factor or whether its impact is permanent.

According to the Directive 2013/35/EU, the limitation of EMF (50 Hz) exposure level to the value of 1 mT reduces the potential for damage. On the other hand, 50 Hz EMF of intensity higher than 6 mT causes clear biological effects, e.g. apoptosis, DNA damage or oxidative stress (Duan et al., 2013; Yin et al., 2016; Klimek et. al., 2022).

It is accepted, that EMF exposure may count as a stress situation (Sedghi et al., 2005; Mahdavi et al. 2014; Ebrahimzadeh Abarghoee et al., 2022; Huang et al., 2023; Klimek et al., 2023) and may activate a wide spectrum of interacting neuronal, molecular and neurochemical systems that underpin behavioural and physiological responses (Szemerszky et al., 2010; Kitaoka et al., 2013). Numerous studies have suggested an association between chronic EMF exposure and psychiatric disorders (Hosseinabadi et al., 2019; Klimek and Rogalska, 2021). The mechanisms underlying the purported impact of EMF could involve disturbances in stress systems.

External and internal stimuli are channelled via the nervous system to the hypothalamus. Once a stressor has been perceived, the sympatho-adrenal-medullary (SAM) axis becomes activated to help the organism in adapting (Krugers et al. 2012). SAM is regulated primarily by neurons of the locus coeruleus (LC), the main source of noradrenaline (NA) in the brain (Morris et al., 2020).

The locus coeruleus –noradrenergic system (LC-NA) exerts a widespread influence on neuronal circuits. Dysregulation of the LC–NA system may be associated with numerous cognitive and affective disorders including stress-related disorders (Berridge and Waterhouse, 2003; McCall et al., 2015). The LC projects to various structures, including the hippocampus, and the hypothalamus. Stress-induced noradrenaline release acting via specific adrenergic receptors leads to an enhanced state of arousal, which is critical for adaptive responses to stress (Tafet and Bernardini 2003; Morris et al., 2020). Noradrenaline (NA) can modify the neuronal plasticity in the hippocampus mainly via β2-adrenoreceptors (β2-AR) (Bacon et al., 2020). NA acting on β2-AR is a key determinant of stress-related gene expression (Aloyz et al. 1999; Roszkowski et al. 2016; Privitera et al., 2023) and underlies the hippocampal synaptic plasticity (Marien et al. 2004; Maity et al., 2022).

Electromagnetic fields accompany the life of the organisms and probably, to a certain extent modify some crucial neuronal processes (Touitou and Selmaoui, 2012), but we postulate that the impact is not definitely negative, and the direction and dynamics of EMF-induced changes depend on the EMF intensity (magnetic flux density). It is known that elevated doses of compounds or environmental factors can be harmful to biological systems, whereas lower doses prompt the activation of endogenous survival systems, a phenomenon called hormesis (Calabrese, 2001; Mushak, 2016; Calabrese and Mattson, 2017). The hormetic dose-response relationship may manifest following an initial disturbance of homeostasis, necessitating subsequent re-establishment. It involves gene expression and protein synthesis unfolding over time; hence, the temporal aspect of hormesis determines its final impact (Calabrese, 2001).

We suggest that the impact of electromagnetic fields (EMF) on the organism follows a hormetic pattern, leading to activations of stress response systems involving the LC-NA system. This implies that EMF exposure has the potential to establish a new "set-point" for stress system activity, with the direction and dynamics of this process dependent on the field's intensity. The alterations induced by EMF in NA and its receptor levels may trigger "cellular hormesis" through the activation of intrinsic signalling pathways. The initial exposure to electromagnetic fields (EMF) may modulate the set-point of stress system activity, whereby each subsequent EMF exposure would overlap the established set-point status of stress response system activity during the preceding exposure. Consequently, EMF can modify the organism's susceptibility to subsequent stress factors and, thereby, to diseases, particularly those associated with the nervous system, in a bidirectional manner: either compensatory or deleterious. Hence, the final effect of exposure to EMF can depend on the subtle interplay between all components of stress response and subsequent cellular processes. Our previous research has confirmed that the impact of the electromagnetic field on one of the key regulatory systems of the stress response - the hypothalamic-pituitary-adrenal axis (HPA), aligns with the principles of hormesis. The impact of repeated exposure to EMF on the HPA axis activity is bidirectional and depends on the intensity of EMF (Klimek et al., 2023).

The aim of the research was the verification of the impact of EMF on LC-NA system activity. Thus, we decided to test the hypotheses:

1. ELF-MF-induced changes in LC-NA system response and their direction and dynamics depend on the strength of the field (hypothesis I)
2. ELF-MF modifies the activity of LC-NA system in permanent way, and thus changes the stress response to subsequent stress factors (hypothesis II)

2. Materials and Methods

Animals

The experiments were performed on a total number of 180 3-month-old male Wistar rats (weighing between 300 and 350 g). Animals were kept in acrylic cages lined with wood shavings in the room with a 12h:12h day-night cycle (lights on at 7:00 a.m.). Standard conditions were ensured in the room (temperature: 22 ± 2◦C, humidity 55±10 %). Rats received standard laboratory food and tap water ad libitum.

All experiments were planned and carried out following 3R principles (Replacement, Reduction and Refinement; EU Directive 2010/63/EU). Protocols of the experiments were approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland (decision number: 3/2018).

Electromagnetic field exposure setup

20 cm diameter coils (Elektronika i Elektromedycyna Sp. J., Otwock, Poland) composed of 282 turns of copper wire were used to generate an electromagnetic field (EMF) with the predominance of the magnetic component. The details of the exposure system have been described in previous publications (Bieńkowski and Wyszkowska, 2015; Trawiński et al., 2010). EMF produced by coil and Variac power supply was homogeneous and sine-wave alternating at 50 Hz and the range of the intensities 0.1 - 8 mT (Fig. S1). Two values of magnetic flux density were used in the experiments: 1 and 7 mT. Gauss meter (Model GM2, AlphaLab, Inc, USA) was used to measure the magnetic flux

density before each exposure. EMF within the area containing the animal's cage was non-homogeneous at the level of 10 %. Animals in control groups were subjected to the same experimental procedure (sham exposure system) without the influence of EMF (<10 µT). During exposures, the temperature in coils was monitored using thermocouples mounted under each exposure system.

Experimental design

Animals were habituated to laboratory conditions for 7 days. Afterwards, exposures were performed. The rats were assigned to the following groups:
1) EMF/B/1mT – animals exposed to EMF (50 Hz, 1 mT),
2) EMF/PM/1mT – animals exposed to EMF (50 Hz, 1 mT), and subsequently exposed to plus maze test,
3) EMF/B/7mT – animals exposed to EMF (50 Hz, 7 mT),
4) EMF/PM/7mT – animals exposed to EMF (50 Hz, 7 mT), and subsequently exposed to plus maze test,
5) C/B – control animals subjected to the same experimental procedure as the experimental groups 1, 3; without EMF exposure,
6) C/PM – control animals subjected to the same experimental procedure as the experimental groups 2, and 4; without EMF exposure.

The experimental design is shown in Fig. 1. Each experimental group included 10 animals. The series of 7-day exposures to EMF of 1 and 7 mT (1 h per day, exposures started at 9 am), as well as sham exposures, were performed once (E1; n=10), two (E2; n=10) and three (E3; n=10) times with a three-week break between consecutive series. Rats were put into plastic boxes (12 cm × 20 cm × 14 cm) with drilled holes ensuring air access. Boxes were placed in the centre of coils generating EMF (exposed groups) or in sham coils (control groups). Animals were able to move freely inside the boxes. The day after the end of the last exposure from the series, the part of animals was decapitated. The brain and blood were collected for further analysis of the "basal level" (B) of parameters (EMF/B/1mT, EMF/B/7mT). In the second variant of the experiment, the rats were exposed to EMF as previously described and tested in an elevated plus maze (PM) the day after the end of the last exposure from the series (EMF/PM/1mT, EMF/PM/7mT). The scheme of PM is shown in Fig. S2. Immediately after the completion of the PM test rats were decapitated. The tissues were collected and stored to analyze the "PM-induced level" of parameters. All procedures were carried out between 09:00 and 12:00 to avoid circadian fluctuations.

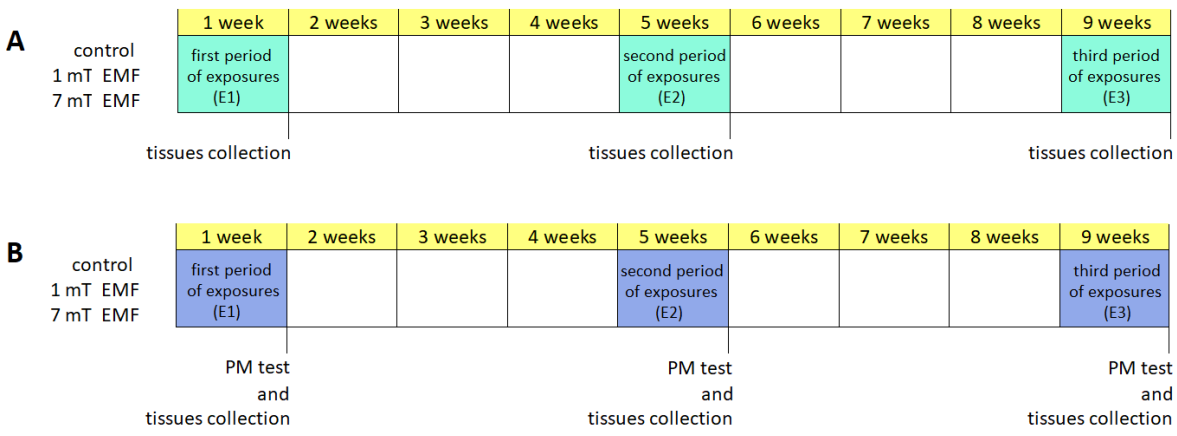


Fig. 1

Experimental design. A) First set of experiments: assessment of "basal" level of parameters. B) Second set of experiments: assessment of "plus maze induced" level of parameters.

The influence of EMF on the concentrations of noradrenaline (NA) and its metabolite - 3-methoxy-4-hydroxyphenylglycol (MHPG) was determined in brain structures: locus coeruleus (LC) and hypothalamus (HPT) as well as in plasma. Moreover, the relative mRNA transcript abundance of β2-adrenergic receptor (β2-AR) was measured in the hippocampus. The levels of parameters were analyzed after each exposure period (E1-E3) in groups exposed to EMF of 1 and 7 mT as well as in animals remaining in control conditions ("basal level" and "PM-induced level") to evaluate the direction and dynamics of the changes. Body weights were monitored throughout the experimental period, but no changes were found.

Sample collection

Blood was collected via cardiac puncture in a solution of ethylenediaminetetraacetic acid disodium salt (Na_2EDTA, Sigma-Aldrich) directly after decapitation. Samples were then centrifuged (2000 rpm, 20 min) and stored at -20^{o}C until future ELISA analysis. The following brain structures were collected: locus coeruleus (LC) hypothalamus (HPT) and hippocampus (HIP). Next, all structures were weighed and immersed in liquid nitrogen until freeze. Samples were stored at -80^{o}C until further ELISA and RT-qPCR analyses.

Determination of concentrations of NA and MHPG

Tissues were homogenized on ice in a pre-cooled PBS buffer containing a proteinase inhibitor cocktail (Roche Cat. No. 11836145001) (100 mg of tissue per millilitre). Noradrenaline Research ELISA and MHPG (3-Methoxy-4-hydroxyphenylglycol) ELISA Kits were used to determine the concentrations of NA and its metabolite (Demeditec Cat. No. DEE5200R, Wuhan Fine Biotech Cat. No. EU2593, respectively). All procedures were conducted according to the manufacturer's instructions. Each sample was measured in duplicate. A multi-mode microplate reader Epoch 2 (BioTek Instruments, Inc., Winooski, UT, USA) was used to detect colourimetric changes in the assay. The optical density (OD) was measured at 450 nm and the OD of the blank well was set to zero. A standard curve was created using Gen5 Software generating a four-parameter logistic (4-PL) curve fit.

Assay of mRNA encoding β2-adrenergic receptors

The EXTRACT ME RNA & DNA KIT (Cytogen Cat. No. EM15) was used to extract total RNA from tissue samples according to manufacturer instructions. RNA quantification quantity (ng/µL) and the quality (260/280 ratio) were done using a NanoDrop One Spectrophotometer (Thermo Fisher Scientific).

The relative levels of specific mRNAs were determined by reverse transcription (RT)—quantitative PCR (qPCR) based on SybrGreen detection chemistry. The cDNA was synthesized from equal amounts of RNA per sample, using the EvoScript Universal cDNA Master (Roche Cat. No. 07912455001) according to the manufacturer's protocol.

β-actin and GAPDH were evaluated as housekeeping genes using the BestKeeper approach to determine the stability of gene transcript abundance under experimental conditions (Pfaffl, 2002). Sequences of the primers used in qPCR were: β2-adrenergic receptor (β2-AR): 5'-CTCCTTAACTGGTTGGGCTATG-3'(F); 5'-CCTGGAAGGCAATCCTGAAA-3' (R); β-actin (5'-CGTTGACATCCGTAAAGACCTC-3' (F); 5'- TAGGAGCCAGGGCAGTAATCT-3' (R)) and GAPDH (5'-TAAAGAACAGGCTCTTAGCACA-3' (F); 5'- AGTCTTGGAAATGGATTGTCTC-3' (R) (Table S1).

β2-AR and housekeeping genes PCRs were carried out in separate runs with 10 ng of cDNA per reaction, 500 nM of each primer in a final volume of 10 µl containing 5 µl of SYBR Green PCR Master Mix (Roche Cat. No. 04707516001). PCR conditions for the β2-AR primers were 10 min at 95 °C followed by 40 cycles of 10 sec at 95 °C, 20 sec at 56 °C and 20 sec at 72 °C. For β-actin and GAPDH, PCR conditions were 10 min at 95 °C followed by 40 cycles of 10 sec at 95 °C, 20 sec at 61 °C and 20 sec at 72 °C. A final melting step was included in each run with the temperature ramping from 65 °C to 95 °C in 1 °C steps to check for target specificity via unimodal melt dissociation peaks. A negative (no-template) control was also included in each run. RT-qPCR was performed using LightCycler® 96 System (Roche). To determine the PCR efficiencies, standard curves for both target and control genes were obtained using a series of cDNA dilutions as a template. Relative mRNA expression was calculated employing the ΔΔCt method using β-actin as the housekeeping gene (stability coefficient was 0.963 for β-actin vs. 2.0 for GAPDH).

Elevated plus maze test

The elevated plus maze test (PM) was used to evaluate the level of anxiety in animals. The EPM test arena was black painted and consisted of four 40-cm-long and 10-cm-wide cross-like arms—two closed, surrounded by walls, and two open - limited only by transparent plexiglass (40 cm high). All arms were extended from a central square, 10 × 10 cm. The maze was elevated 50 cm above the floor surface.

A source of light was placed above the PM. The intensity of the lighting in the centre of the maze was set at 60 lx, in closed arms- 40 lx and in open arms- 100 lx. Each animal was in the PM for 5 minutes. After each test, the area of the maze was disinfected with Mediseptol H Neutral (Alpinus Chemia). Trials were recorded and analyzed using video tracking software EthoVision XT 11 by Noldus. Detection of the centre-point of the animal was used to locate the rat's movement in the area of PM. The analyzed parameters were: total distance moved (cm), movement velocity (cm/s), movement duration (s), time spent in open arms (s), and number of entrances into open arms.

Data analysis

The combination of methods (biochemical and behavioural) used in the research confirming and/or complementing each other was used as necessary for the reliable detection of the effects of EMF on the stress response. 2-way General Linear Models (GLM) were used to analyze the effect of EMF on hormones and receptor level of the locus coeruleus–noradrenergic system. Behavioural response to stress factor- PM was investigated using multivariate analysis of variance (MANOVA). When data did not meet the requirements for the GLM and MANOVA assumptions (normality was tested with a Shapiro-Wilk test and homoscedasticity with a Levene test) they were transformed (natural logarithm, decimal logarithm, square root). Post-hoc test with Bonferroni correction adjusted to the design of particular experiments was used to assess significant terms. Some of the data did not

show a normal distribution even after transformation. In such cases, we applied a non-parametric analysis of variance (Kruskal-Wallis test with Bonferroni correction). The significance of differences between the compared data was accepted when $P<0.05$. All analyses were carried out using the SPSS 25.0 package (IBM Inc.). The figures in the *Results* section present only significant effects of categorical factors displayed in the analysis. To increase the clarity of the text, only a portion of the results of the statistical analyses are in the text itself. The results of the entire statistical analysis can be found in the Supplementary materials.

3. Results

EMF-modified noradrenaline and its metabolite (MHPG) concentrations and MHPG/NA ratio level ('basal')

The profile of changes in 'basal' NA and its metabolite MHPG concentrations was similar in LC (Fig. 2A, B), HPT (Fig. 2C, D), and plasma (Fig. 2E, F).

Noradrenaline (Fig.2 A,C,E, Tab. S2 A,D,G)

Under control circumstances, 'basal' concentrations of NA in LC and HPT were unaltered with each subsequent exposure to the sham apparatus (Fig.2A, C). Only in plasma (Fig.2E), a decrease in the NA level after E2 and E3 was observed. The results suggest that the set-up was not stressful per se.

There was a significant effect of EMF dose, with the higher level of EMF producing greater elevation of NA in LC, HPT and plasma ($P<0.001$, Tab. S2A, D and G). The interaction between EMF intensity and the number of exposures was also significant ($P<0.001$, $P=0.013$, $P=0.001$; respectively). A single period (E1) of exposure to low 1 mT EMF significantly increased NA concentration compared to the control group only in HPT (Fig. 2C), then the decline with each subsequent exposure was found, suggesting habituation. In LC in the EMF/B/1mT group NA level was not different from the control value, however the tendency to attenuation with each next exposure was also observed (Fig. 2A). Conversely, in the EMF/B/7mT group the NA level was significantly higher compared to the control and 1mT EMF groups starting from the first exposure (E1) in LC and HPT (Fig.2A, C) and from the second one (E2) in plasma (Fig. 2E). There was also no decline in NA level with repeated exposures with the highest level of NA observed after E3.

MHPG *(Fig.2 B,D,F, Tab. S2 B,E,H)*

The opposite direction in changes of MHPG concentrations was observed in both analysed brain structures and plasma (Fig.2 B, D and F). MHPG levels were influenced by EMF intensity ($P<0.001$) (Table S2 B, E, H). The interaction between EMF intensity and the number of exposures was also significant in LC and plasma ($P<0.001$). In the EMF/B/1mT group, the MHPG concentrations were not significantly different from the control ones.

The higher EMF intensity (7 mT) led to a significant decrease in MHPG level, just after the first exposure (E1) in LC and after the second one (E2) in plasma; after subsequent exposures, the attenuation of MHPG level was profound (Fig. 2B, F). In HPT (Fig. 2D) the effect of the number of

exposures was not significant (Tab. S2E). In the EMF/B/7mT group, the hormone level was significantly lower compared to other groups.

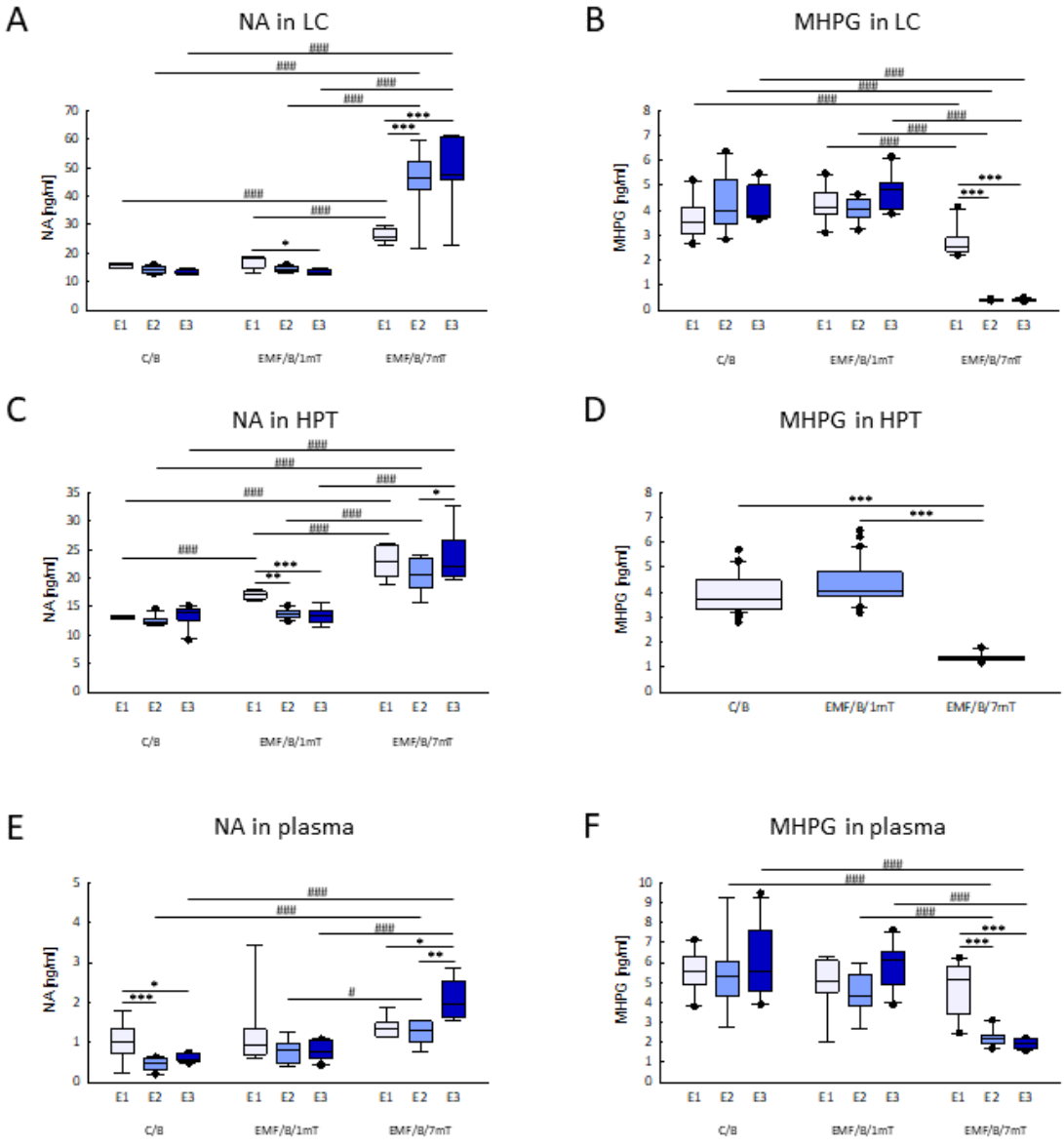


Fig. 2

"Basal" concentrations of NA and MHPG: A) NA in locus coeruleus (n= 72, 5-10 in each group for each exposure), B) MHPG in locus coeruleus (n= 90, 10 in each group for each exposure), C) NA in hypothalamus (n= 74, 5-10 in each group for each exposure), D) MHPG in hypothalamus (n= 90, 10 in each group for each exposure), E) NA in plasma (n= 78, 8-10 in each group for each exposure), F) MHPG in plasma (n= 86, 8-10 in each group for each exposure) in rats exposed once to three times (E1-E3) to EMF of 1 or 7 mT or control conditions. Presented values are predicted by the GLM model for a significant intensity of the electromagnetic field (mT) x number of exposures (E1-E3) interaction (Fig. 2A, B, C, E and F) or significant effect of intensity of the electromagnetic field (mT) (Fig. 2D). Boxes indicate the lower quartile, median, and upper quartile, and whiskers indicate the range of changes (minimum to maximum). Statistically significant differences between animals from the same group are

denoted *p <0.05, **p < 0.01, and ***p < 0.001; and these between experimental groups are denoted #p < 0.05 and ###p < 0.001. Note different scales on each plot.

MHPG/NA ratio *(Fig.4 A, B, C, Tab. S2 C, F, I)*

MHPG/NA ratio level attenuated with greater intensity of EMF (P<0.001, Tab. *S2 C, F, I)* and the effect was further augmented with each subsequent exposure to EMF in LC and plasma (P<0.001, P=0.024, Tab. *S2 C, I)*. MHPG/NA ratio level in LC (Fig. 4A) in the control and 1 mT group increased with each exposure, however, the index value was not different between these groups.

In the EMF/B/7mT group, a statistically significant decrease in MHPG/NA level relative to both the control and EMF/B/1mT group was noted after each subsequent exposure in LC (Fig. 4A). The clear attenuation of the index in 7mT group was also found after E2 and E3 in comparison to its value after E1 (Fig. 4A).

In plasma the index value in the 7 mT group after E1 was lower (Fig.4C) than that in the control group, and then was further diminished (after E2 and E3) receiving the decreased value relative to both control and 1 mT groups. In the HPT, the value of the index in the EMF/B/7mT group was significantly lower than in the other groups, regardless of the number of exposures. (Fig. 4B).

Plus maze-modified noradrenaline, MHPG concentrations and MHPG/NA ratio

Generally, the direction of changes in NA, MHPG and MHPG/NA levels in rats exposed to subsequent stress factor – plus maze test coincided with their 'basal' concentrations observed only after exposure to electromagnetic field (Tab. S3, Fig.3 and 4 D, E, F).

Noradrenaline (Fig.3 A,C,E, Tab. S3 A,D,G)

NA concentration in LC, HPT and plasma in animals exposed to the plus maze test was influenced only by EMF intensity (P<0.001, Tab. S3 A, D, G). In all cases, the NA level in the EMF/PM/1mT group was not different from the control one and the concentration of NA in EMF/PM/7mT was higher relative to the both control and 1 mT group (Fig.3 A, C, E).

MHPG *(Fig.3 B,D,F, Tab. S3 B,E,H)*

In animals exposed to plus maze test MHPG concentration in LC and HPT was affected only by EMF intensity (P<0.001, Tab. S3 B, E). No difference was found between the control and 1 mT group, while in the 7 mT group, the metabolite level was clearly lower in comparison to both other groups (Fig.3 B, D). The plasma concentration of MHPG was changed with EMF intensity as well as with the number of exposures; the interaction between the factors was also found (P<0.001, Tab. S3 H). In EMF/PM/1mT MHPG did not differ from the value noticed in the control group, while in animals exposed to EMF of 7 mT, the concentration of MHPG in comparison to both control and EMF/PM/1mT groups was not different only after E1, but was significantly decreased after E2 and E3 suggesting sensitization (Fig. 3F).

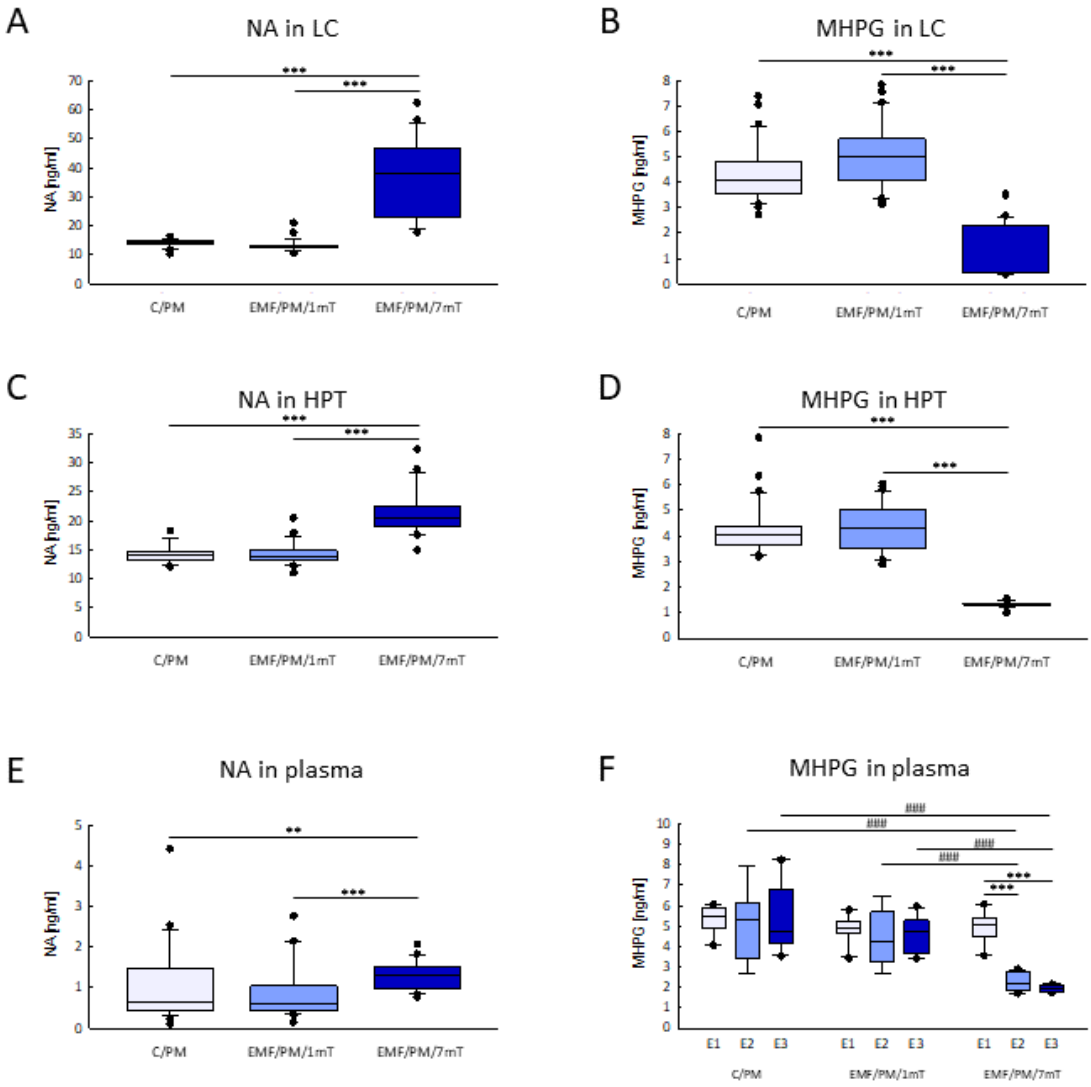


Fig. 3

"Plus maze induced" concentrations of NA and MHPG: A) NA in locus coeruleus (n= 75, 5-10 in each group for each exposure), B) MHPG in locus coeruleus (n= 90, 10 in each group for each exposure), C) NA in hypothalamus (n= 75, 5-10 in each group for each exposure), D) MHPG in hypothalamus (n= 90, 10 in each group for each exposure), E) NA in plasma (n= 79, 8-10 in each group for each exposure), F) MHPG in plasma (n= 88, 9-10 in each group for each exposure) in rats exposed once to three times (E1-E3) to EMF of 1 or 7 mT or control conditions. Presented values are predicted by the GLM model for a significant intensity of the electromagnetic field (mT) x number of exposures (E1-E3) interaction (Fig. 3F) or significant effect of intensity of the electromagnetic field (mT) (Fig. 2A, B, C, D and E). Boxes indicate the lower quartile, median, and upper quartile, and whiskers indicate the range of changes (minimum to maximum). Statistically significant differences between animals from the same group are denoted ***$p < 0.001$; and these between experimental groups are denoted ##$p < 0.01$ and ###$p < 0.001$. Note different scales on each plot.

MHPG/NA ratio *(Fig.4 D, E, F, Tab. S3 C, F, I)*

Plus maze-induced direction of changes in the MHPG/NA ratio level almost completely covered the characteristics of changes in the 'basal' level of the index (Fig. 4D, E, F). MHPG/NA ratio level attenuated with greater intensity of EMF ($P<0.001$, Tab. S3 C, F, I), the interaction between the EMF intensity and number of exposures was observed only in LC and plasma ($P<0.001$, Tab. S3 C, I). The number of exposures affected the index value in HPT ($P=0.001$, Tab. S3 F), but there was no interaction between factors ($P=0.167$, Tab. S3 F). In LC the only difference between control and EMF/PM/1mT groups was found after E2 (Fig. 4D). The exposure to EMF of 7 mT caused the attenuation in the index value just after the first exposure (E1) in LC (Fig.4D) and after E2 in plasma (Fig.4F) in comparison to both control and 1 mT groups. In HPT of rats exposed to EMF of 7 mT the MHPG/NA ratio level was significantly lower than that in other groups (Fig.4E).

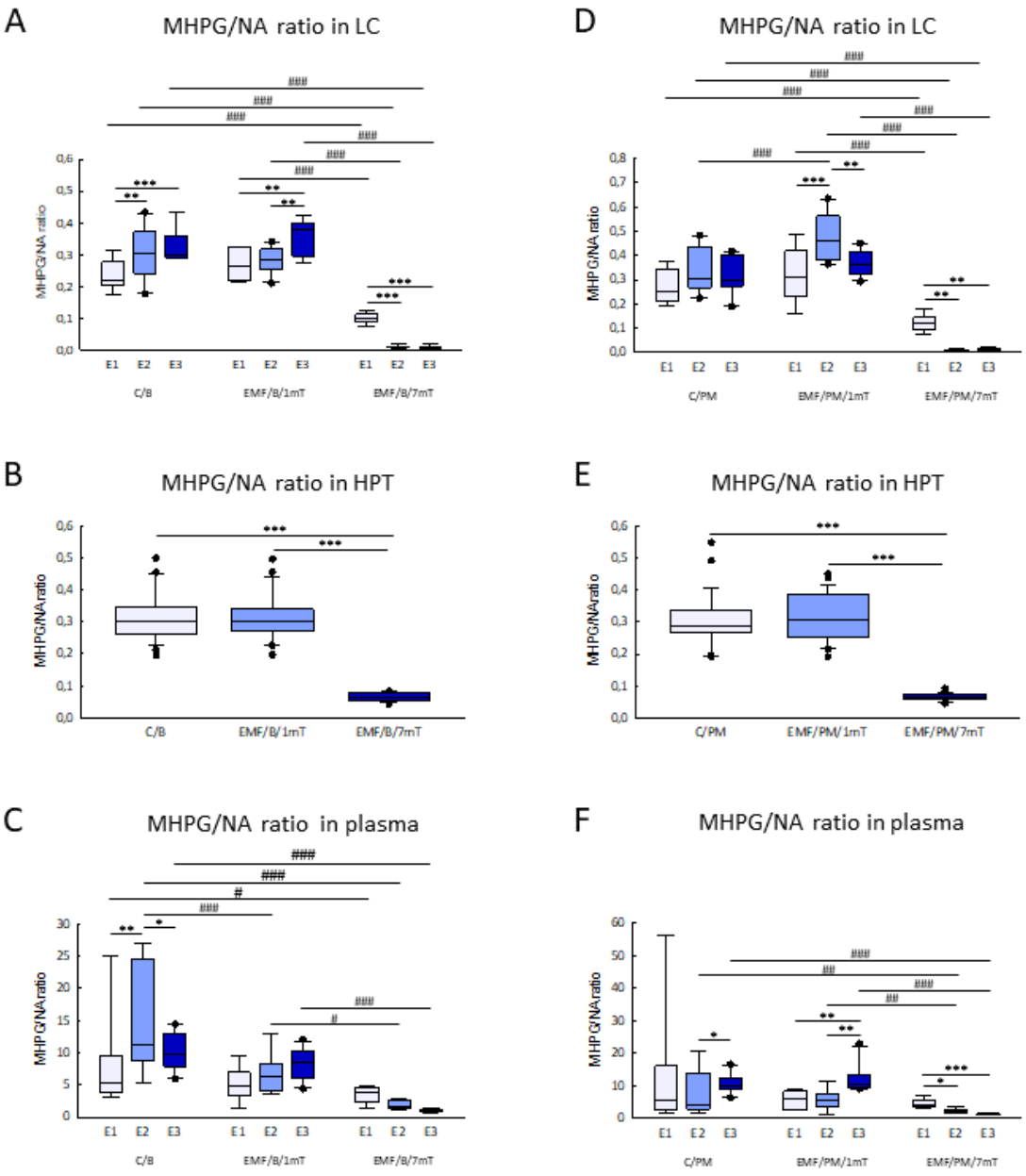


Fig. 4

Utilization index of NA as a ratio of "basal" and "plus maze induced" concentrations of MHPG to NA: A) "basal" MHPG/NA ratio in locus coeruleus (n= 72, 5-10 in each group for each exposure), B) "basal" MHPG/NA ratio in hypothalamus (n= 74, 5-10 in each group for each exposure), C) "basal" MHPG/NA ratio in plasma (n= 78, 8-10 in each group for each exposure), D) "plus maze induced" MHPG/NA ratio in locus coeruleus (n= 75, 5-10 in each group for each exposure), E) "plus maze induced" MHPG/NA ratio in hypothalamus (n= 75, 5-10 in each group for each exposure), F) "plus maze induced" MHPG/NA ratio in plasma (n= 79, 8-10 in each group for each exposure) in rats exposed once to three times (E1-E3) to EMF of 1 or 7 mT or control conditions. Presented values are predicted by the GLM model for a significant intensity of the electromagnetic field (mT) x number of exposures (E1-E3) interaction (Fig. 4A, C, D and F) or significant effect of intensity of the electromagnetic field (mT) (Fig. 2B and E). Boxes indicate the lower quartile, median, and upper quartile, and whiskers indicate the range of changes (minimum to maximum). Statistically significant differences between animals from the same group are denoted *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$; and these between experimental groups are denoted #$p < 0.05$, ##$p < 0.01$ and ###$p < 0.001$. Note different scales on each plot.

Comparison between 'basal' and plus maze test-induced levels of NA, MHPG and MHPG/NA ratio

We compared the level of analysed parameters after the plus-maze test to their 'basal' concentrations for each group: C/PM vs C/B; EMF/PM/1mT vs EMF/B/1mT and EMF/PM/7mT vs EMF/B/7mT in LC (Tab. S4) in HPT (Tab. S5) and plasma (Tab. S6).

In the control group, the increase in concentrations of NA after exposure to the plus maze was significant in HPT ($P<0.001$) (Tab.S5 A) and the tendency to increase after PM was found in plasma ($P=0.077$) (Tab.S6 A), which confirmed the stressogenic effect of this kind of stress.

The exposure to subsequent stress factor profoundly decreased the NA and increased MHPG and MHPG/NA index in LC in rats exposed to EMF of 1 mT (Tab. S4 B, E, H).

In animals exposed to EMF of 7 mT, the analysis did not reveal a significant effect of the plus-maze test on hormone concentrations in LC (Tab. S4 C). In HPT the values of hormones and their ratio did not differ between the 'basal' and plus-maze-induced levels in both EMF-exposed groups (Tab. S5 B, C). However, in plasma, the exposure of the EMF/PM/7mT group to the other stress factor resulted in a decrease in NA level, and an increase in MHPG/NA ratio (Tab. S6 C, I).

We also evaluated the percentage changes in the values of parameters in each group in comparison to their concentrations in the control group (C/B; set at 100 % - reference value) separately after each exposure (Fig. S3 and S4). The analysis confirmed the profound effect of EMF of 7 mT on the parameters level and characteristics of the changes, however, a clear effect of the plus-maze test was not found. The NA levels (both basal and PM-induced) were increased c.a. 3,5 times in LC (e.g. 372%, EMF/B/7mT, E3) and plasma (e.g. 346%, EMF/B/7mT, E3) and 2 times in HPT (e.g. 178%, EMF/B/7mT, E3). The biggest decrease in MHPG level was observed in LC - c. a. 90% (e.g. 9%, EMF/B/7mT, E3), in HPT the reduction of the hormone level was c. a. 70% (e.g. 34%, EMF/PM/7mT, E2) and in plasma c.a. 70% (e.g. 32%, EMF/B/7mT, E3). Analogously, the indexes of NA utilization (both basal and PM-induced) were clearly lower in the 7 mT group (Fig. S4). It should be noticed the progressive character of observed changes in parameters level with each subsequent exposure to EMF.

***EMF-modified* β2-adrenergic receptors relative mRNA transcript abundance in the hippocampus**

Fig. 5A presents intergroup comparisons of the 'basal' relative mRNA transcript abundance of ('mRNA') encoding β2-AR (expressed as fold change (FC) from an unrelated reference gene). Both the EMF intensity as well as the number of exposures influenced the β2-adrenergic receptors abundance. However, no interaction between the factors was found (Tab. S7 A). The β2-AR abundance in the hippocampus of the 7 mT EMF-exposed group was lower than in both: the control and EMF/B/1mT groups.

The level and direction of changes of β2-AR relative mRNA transcript abundance in all groups after the plus-maze test were not very different from their 'basal' level (Fig. 5B). β2-AR mRNA attenuated with greater intensity of EMF and the effect was further augmented with each subsequent exposure (P<0.001), the interaction between the factors was also significant (P=0.038) (Tab. *S7 B)*. No difference was found between the control and 1 mT group in the β2-AR mRNA. In EMF/PM/7mT group, a statistically significant decrease in β2-AR mRNA relative to the control group was noted after each subsequent exposure and in relative to EMF/PM/1mT after E2 and E3 (Fig. 5B).

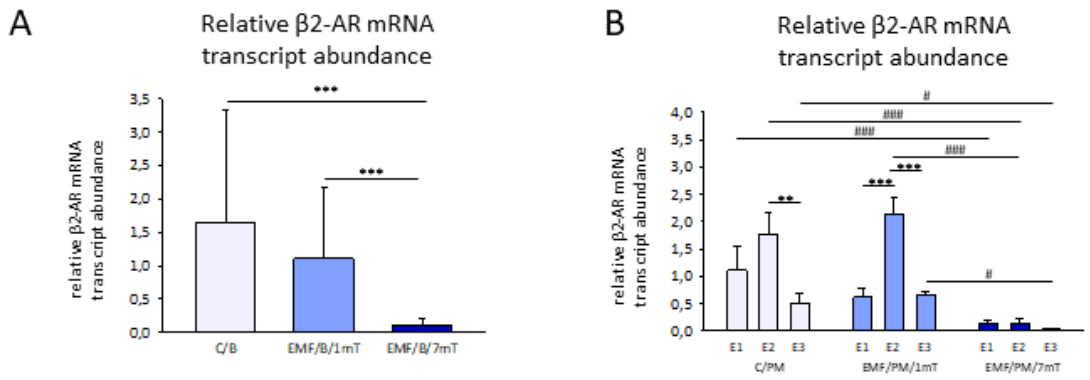


Fig. 5

Relative receptor gene mRNA transcript abundance (fold change versus β-actin): A) "basal" β2-AR (n= 45, 5 in each group for each exposure), B) "plus maze induced" β2-AR (n= 45, 5 in each group for each exposure) in rats exposed once to three times (E1-E3) to EMF of 1 or 7 mT or control conditions. Presented values are predicted by the GLM model for a significant intensity of the electromagnetic field (mT) x number of exposures (E1-E3) interaction (Fig. 5B) or significant effect of intensity of the electromagnetic field (mT) (Fig. 5A). Values are presented as mean ±SEM. Statistically significant differences between animals from the same group are denoted, ***p* < 0.01, and ****p* < 0.001; and these between experimental groups are denoted #*p* < 0.05 and ###*p* < 0.001. Note different scales on each plot.

***Comparison between 'basal' and plus maze test-induced* β2-adrenergic receptors relative mRNA transcript abundance**

The analysis did not reveal the effect of PM on β2-AR mRNA in each group (Tab. S8).
We evaluated the percentage changes in the relative β2-AR mRNA transcript abundance after the plus-maze test compared to their mRNA transcript abundance in the control C/B group after each exposure

(set at 100 % - reference value) (Fig. S5). After the plus-maze, an almost 2.5 times increase (257% after E1) in β2-AR mRNA in the control group was found. In the EMF/B/1mT group the β2-AR mRNA was slightly lower than in the C/B group, however after exposure to the second stress factor the mRNA level was higher after E1 and E2 in comparison to the basal level in this group. It must be stressed the profound decrease of both 'basal' and plus-maze-induced β2-AR mRNA in the hippocampus of rats exposed to EMF of 7mT.

Behavioural analysis

Total distance moved (Fig. 6A, Tab. S9A), movement velocity (Fig. 6B, Tab. S9B) and movement duration (Fig. 6C, Tab. S9C) in the plus maze were not affected by EMF intensity. Overall, the activity level in all groups of rats was maintained at a similar level. However, the longer time spent in closed arms (Fig. 6D, Tab. S9D) and decreased open arms frequency (Fig. 6E, Tab. S9E) in animals exposed to EMF of 7 mT relative to the control group may suggest moderate anxiety level.

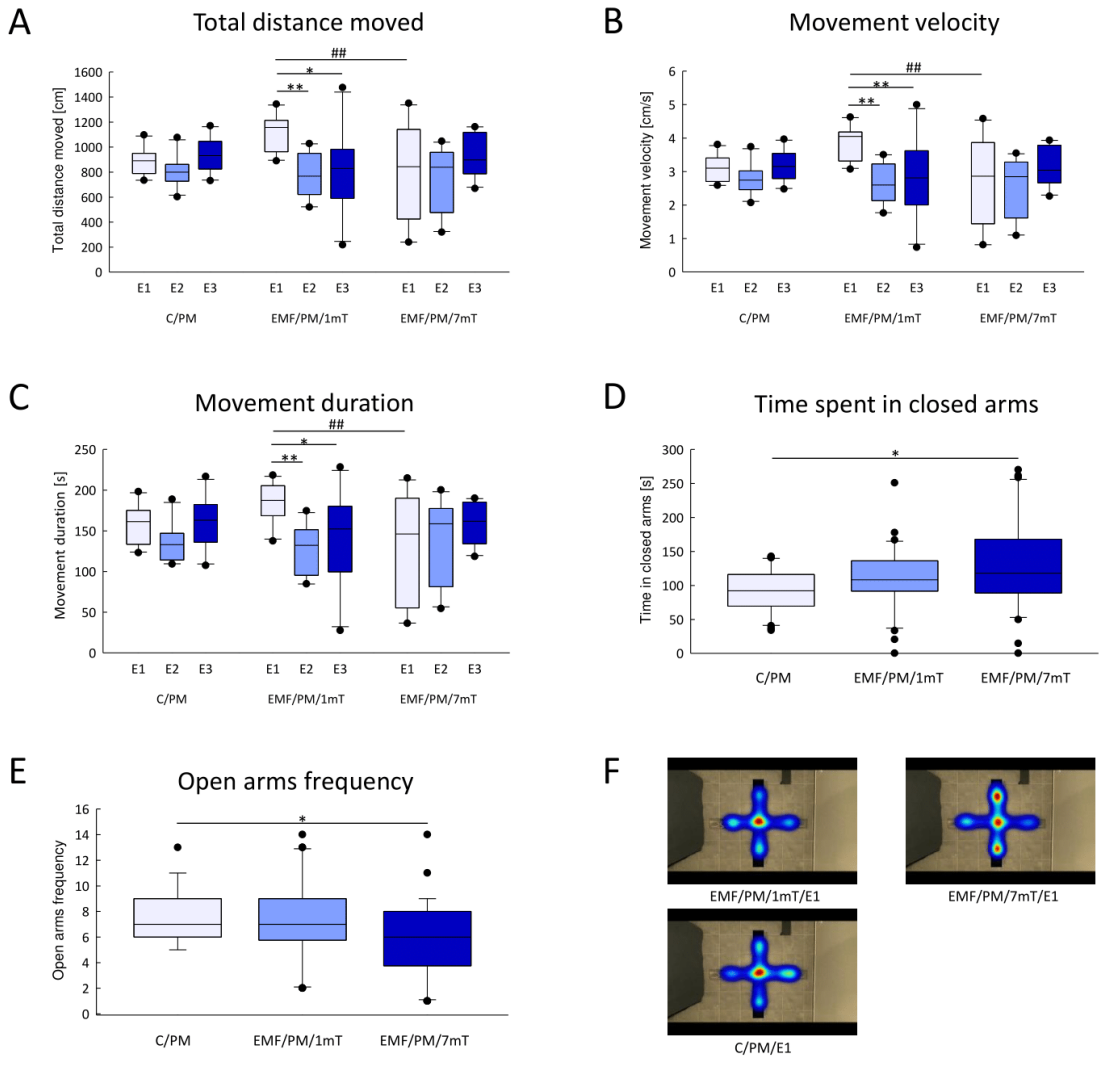


Fig. 6

Behavioural changes in plus maze test: A) total distance moved (cm), B) movement velocity (cm/s), C) movement duration (s), D) time spent in closed arms (s), E) number of the entrances into open arms (frequency) in rats

exposed once to three times (E1-E3) to EMF of 1 or 7 mT or control conditions. Presented values are predicted by the GLM model for a significant intensity of the electromagnetic field (mT) x number of exposures (E1-E3) interaction (Fig. 6A, B and C) or significant effect of intensity of the electromagnetic field (mT) (Fig. 6D and E). Boxes indicate the lower quartile, median, and upper quartile, and whiskers indicate the range of changes (minimum to maximum). Statistically significant differences between animals from the same group are denoted *p <0.05 and **p < 0.01; and these between experimental groups are denoted #p < 0.05, ##p < 0.01 (n = 90; 10 in each group for each exposure). Note different scales on each plot. F) Heat maps representing time spent by the animal in open and closed arms of plus maze after E1 from control, 1 mT EMF and 7 mT EMF groups.

4. Discussion

Our research has shown that the impact of repeated exposure to EMF on the LC-NA system activity depends on its intensity. The LC-NA system responds to each exposure to stress during repeated exposures, resulting in an observable cumulative effect (Ulrich-Lai et al., 2006). The exposure of rats to EMF of low or high magnetic density (1 mT or 7 mT) in our study was repeated three times based on the assumption that a hormetic dose-response relationship can occur after an initial disruption in homeostasis. Then the direction and dynamics of the subsequent exposures are the consequence of previous ones.

Noradrenaline concentration and MHPG and MHPG/NA ratio levels in rats exposed to EMF of 1 mT did not differ from the control value. The significant increase in NA level was observed only after E1 in HPT – the main structure coordinating stress response. It suggests that even 1 mT EMF is a stressful condition for organisms. However, then with each next exposure, a decrease in the hormone level was observed, which may reflect the adaptation to this moderate stress.

In the group exposed to higher EMF intensity (7 mT), the concentrations of NA were significantly higher than values recorded in both control or low-dose EMF (1 mT) groups and the rise in the parameter value was observed with repeated exposures, which suggests the sensitisation to this kind of homotypic stressor. The same pattern of corticosterone release (the main marker of HPA axis function) in rats exposed to 7 mT EMF was observed in our previous research (Klimek et al., 2023). Moreover, we have also found a significant increase in corticotropin releasing hormone (CRH) levels in the hypothalamus (Klimek et al., 2023). CRH may contribute to stressor-induced activation of LC (Berridge and Waterhouse, 2003; McCall et al., 2015) predicts an enhanced capacity for stress excitation and is purported to reflect a ‘chronic stress-recruited’ pathway (Ochedalski et al., 1998). The stress-induced upregulation of CRH in rats exposed to EMF of 7 mT to some extent can explain the high level of NA in this group. However, since the noradrenergic fibers originating mainly in the locus coeruleus (LC) project widely throughout the forebrain (O’Dell et al., 2015), other mechanisms underlying this phenomenon cannot be excluded. The hypothalamus receives a relatively dense noradrenergic innervation and thus LC could modify back the activity state of HPT (Giorgi et al., 2021; Holland et al., 2021). It may elucidate the most clear noradrenergic response to EMF of 7 mT in HPT. Other studies also highlight the effect of EMF exposure on NA levels. A significant increase in noradrenaline levels was found in the developing chick embryo brain exposed continuously to 50 Hz magnetic fields at varying intensities (5 and 50 μT) for up to 15 days, with a positive correlation to the magnetic density values (Rajendra et al., 2004).

In rats exposed to EMF of 7 mT, the decrease in MHPG levels was obvious. Accordingly, to the level of NA and MHPG in LC and plasma, the MHPG/NA ratio level in the 7 mT group was lower than in

other groups just after E1, and the next exposures caused the subsequent decrease. The lowered level of MHPG/NA ratio in the 7 mT group suggests a disturbed NA turnover rate, however, other steps in the neurotransmission process (e.g. signal transduction mechanisms) as possible sites of NA dysregulation cannot be excluded. There is a variety of evidence that MHPG is decreased in depression (Garvey et al., 1996; Kurita et al., 2015) and Parkinson's disease (van der Zee et al., 2018). Moreover, in Down syndrome subjects the reduced serum MHPG levels predicted the development of Alzheimer's disease (Dekker et al., 2015). The results imply that low MHPG levels in the 7 mT EMF group can emerge as a biomarker of possible nervous system dysfunction.

Our results suggest that even a low intensity of EMF (1 mT) is a stress factor causing an LC-NA system activation if relatively weak and temporary, on the other hand, the impact of 7 mT EMF was definitely stronger. The experimental studies have shown that repeated exposure to some stressors attenuates LC neuronal responsivity and thus NA release to the same (homotypic) stressor (Abercrombie and Jacobs, 1987; Nisenbaum et al., 1991). It means the tolerance of the LC activating actions to those stressors. Instead, the enhanced responsivity of LC neurons to subsequent stressors was also observed (Pavcovich et al., 1990), indicating that tolerance or sensitization to a given stressor is not obligatory and depends on the character and intensity of stress. Similarly, the HPA axis activity was moderately increased in animals exposed to EMF of 1 mT and significantly more robust elevation was observed in rats exposed to EMF of 7 mT (Klimek et al., 2023).

Noradrenaline (NA) exerting its influence on β-adrenergic receptors (β-AR) determines whether external stimuli lead to enduring synaptic plasticity within the hippocampus. In essence, it is hypothesized that β-AR constitutes a fundamental molecular mechanism determining the nature and longevity of synaptic information (O'Dell et al., 2015; Hagena et al., 2016). In the 1 mT group, the decrease in relative mRNA transcript abundance of β2-AR level in the hippocampus was nonsignificant. However, the profound downregulation of β2-AR levels in the hippocampus after each subsequent exposure was found in the 7 mT group. This may reflect a well-known adaptive process aiming at maintaining adequate noradrenergic system activity in response to moderate stress (Stone and Platt, 1982). Nevertheless, in such a challenging stress situation, this physiological adaptive response seems to be insufficient resulting in a higher peak of noradrenaline concentrations in response to successive homotypic stimuli. Consequently, the disturbed adrenergic response to a given stressor can be amplified. An influence of the EMF on the β-adrenergic receptor function was also demonstrated in other studies. The exposure of female turkeys to EMF (50 Hz, 10 μT) led to a decrease in NA-activated β-adrenoceptor function, a phenomenon associated with the development of emotional disinterest and depression (Laszlo et al., 2018).

Within the hippocampus, the activity of the noradrenergic system strongly modulates synaptic strength and neural network physiology (Bacon et al., 2020). The 7 mT EMF-induced decrease in β2-adrenergic receptors in the hippocampus can have profound consequences. The induction of plasticity-related genes is dependent upon the activity of the locus coeruleus (Marien et al., 2004; Roszkowski et al., 2016)**.** Under conditions where an arousal change is triggered (by a novel event), the noradrenaline release from the locus coeruleus acting by β2-AR changes excitability levels in the hippocampus. Thus the receptor activation may be decisive in determining the synaptic strength as well as the persistence of synaptic plasticity (Roozendaal et al., 2006; O'Dell et al., 2010) and could increase dendritic branches and spine density accompanied by the upregulation of synaptic protein levels (Chai et al., 2017). In addition, the effect of pharmacologically- or stressor-induced increases in NA neurotransmission on neuroplasticity was diminished with pretreatment of β2-AR antagonists (Bing et al., 1992; Stone et al., 1991). The significant role of β2-AR in plasticity processes underscores

that exposure to an electromagnetic field (EMF) of 7 mT would strongly inhibit the potential for neuroplastic processes in the hippocampus.

Multiple studies demonstrate that stress induces a disruption in homeostasis (Calabrese and Mattson, 2011; Mushak, 2016), prompting an overcompensation response to restore equilibrium. This process leads to the establishment of a new set-point for stress response systems. The EMF as a stress factor can modify the endocrinological regulations in response to other kinds of stressors (Sedghi et al., 2005; Klimek et al., 2023). The profile and dynamics of plus maze induced changes in noradrenergic system activity almost completely covered their basal levels found in rats exposed only to EMF or control conditions. The increase in concentrations of NA after exposure to the plus maze in the control group, confirmed the stressogenic effect of this kind of stress. In the 1 mT EMF group, the plus maze exposure caused the significant decrease of the NA and increase of MHPG and MHPG/NA index only in LC. It suggests that in group exposed to EMF of lower intensity the characteristic of response to heterotypic stressor was changed in direction to adaptation. A recurrent observation in hormesis studies is that exposure to low levels of a specific hormetic agent triggers pathways associated with brain plasticity, protecting cells and organisms against subsequent heterotypic stressors (Chauhan et al., 2015; Calabrese and Mattson, 2017).

When we evaluated the percentage changes in NA concentrations it became clear that in group exposed to EMF of 7 mT the change in hormones level evoked by plus maze test was lower in LC and plasma, without the change in MHPG level which resulted in the increase of MPHG/NA ratio. This may express a well-known “ceiling effect” because ‘basal’ NA concentration was 2-3 times higher than the value in control animals. However, it must be stressed that still after the exposure to another stress factor the level of NA concentration was significantly higher in this group than in control and 1mT EMF groups. These results proved that the effect of EMF of 7 mT EMF is permanent. The similar effect we observed concerning the change in activity of HPA axis in response to heterotypic stress in 7 mT EMF group (Klimek et al., 2023).

The relative β2-AR mRNA transcript abundance in hippocampus after the plus maze test increased after E1 in control and 1 mT exposed animals (by almost 2.5 and 0.5 times, respectively), then the decrease was observed. This effect seems to be rather the consequence only the plus maze test as it was observed in both groups exposed (1 mT group) and not exposed to EMF (control group). It is crucial to emphasize that, in rats exposed to EMF of 7mT, after exposure to heterogeneous stress. a significant reduction in β2-AR mRNA levels in the hippocampus comparable to the 'basal' level was observed. This means that changes in the activity of the LC-NA system induced by exposure to electromagnetic fields are maintained at a similar level after the exposure to another type of stress.

No discernible differences in behaviour were noted in plus maze test among animals previously exposed to 1 mT EMF compared to control animals. This lack of variation suggests that the stress system change triggered by the low-stress factor of 1 mT EMF was not potent enough to induce alterations in rats behaviour. Similarly, mice that were prenatally exposed to EMF (50 Hz, 1 mT) did not exhibit changes in anxiety-like behaviour (Alsaeed et al., 2014). Moreover, we have not found differences in exploratory activity between all experimental groups. In our study rats exposed to EMF of 7 mT showed moderately increased anxiety-related behaviour in comparison to that noticed in rats, both control and exposed to 1 mT EMF. The results are opposite to those previously received in open-field test in this group (Klimek et al., 2023). The recommendation to perform open-field test before the plus maze aimed at increasing exploratory activity in the plus maze to receive more differentiated results in anxiety parameters (Walf and Frye, 2007). It was not such effect in our experiments.

An association between EMF exposure and emotional behaviour has been indicated in many but not all studies. Behavioural effects of the EMF depend on the length, frequency, and intensity of exposure (Mahdavi et al., 2014; Janać et al., 2012). Some reports noted reduced activity of animals after EMF exposure, and anxiety-like behaviour, others observed anxiolytic effect or did not observe any changes (Szemerszky et al., 2010; Alsaeed et al., 2014). The increased responsiveness of the LC–NA to presentation of an another (heterotypic) stressor have been noted (Finlay et al., 1995). A profound suppression of behavioral activity was also observed following beta receptor blockade, suggesting the involvement of these receptors in the regulation of behavioral state (Stone and Quartermain, 1999). Moreover, the alteration of behavioral patterns by a 3-wk-long 10 μT EMF treatment was correlated with the decrease of NA-activated β-adrenoceptor function (Laszlo et al., 2018). Thus the low level of β2-AR in 7 mT group might be one of the mechanisms explaining the higher anxiety behavior in the plus maze. Existent observations suggest that even in the presence of a significant effect of a noradrenergic lesion on behavioral or physiological processes, care should be taken in the interpretation of these results (Cirulli and Alleva, 2009). In the majority of cases, there is scant evidence establishing a direct, causal relationship between the noradrenergic neurotransmission dysfunction and a specific behavioural disorder (Ressler and Nemeroff, 2000). It appears that dysregulation of noradrenergic systems is not a primary etiological factor contributing to cognitive and/or affective dysfunction commonly associated with psychiatric/behavioural disorders. Rather it can be recognised as the element of the more sophisticated process including many brain structures and other neurotransmitter systems, which as a whole determines the behavioural phenotype. What is sure, in animals exposed to EMF of 7 mT both the LC-NA system activity and stress-induced behaviour are disturbed.

Conclusion

Our research proved that mechanisms underlying the effects of EMF on the stress response include LC-NA system. The exposure to EMF can establish a new "set-point" for stress-system activity, with the direction and dynamics of this process contingent upon the strength of the field and the number of exposures. The changes evoked by EMF of 1 mT in LC-NA system were weak. However, we can conclude that repeated exposure to EMF of 1 mT induces some shift in the set-point of the LC-NA system activity directed to adaptation to this particular type of stress. The same pattern of change in HPA axis activity, however much clear, was observed in our previous study (Klimek et al., 2023). Thus it suggest the generally positive impact of EMF of 1 mT intensity on stress systems directed into activation of compensatory mechanisms. Conversely, in animals exposed to EMF of 7 mT, we observed a shift in the set-point of stress system activity towards increased vulnerability. The effect of EMF of 7 mT on the LC-NA system is long-lasting and influence the responsiveness to other heterotypic stress factors. Consequently, dysregulation of LC-NA neurotransmission has the potential to influence various cognitive and affective processes. In line with this, several neurological or psychiatric disorders have been suggested to involve a dysregulation of noradrenergic neurotransmission (Moret and Briley, 2011; Holland et al., 2021; Dahl et al., 2023; Krohn et al. 2023). It suggest that EMF of high intensity can be recognized as the risk factor for development of diseases, particularly those associated with the nervous system. Summarizing, the effect of EMF on LC-NA is still under-researched. Our study provides new knowledge concerning this important issues, which should be continued concerning the proved in this research EMF impact on noradrenergic system.

Acknowledgement

We would like to thank Prof. Maria Stankiewicz (Nicolaus Copernicus University) for her valuable comments on the manuscript.

Statement of Ethics

This study protocol was reviewed and approved by the Local Committee for Ethics in Animal Research in Bydgoszcz, Poland; approval number 3/2018.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was supported by the National Science Centre, Poland (grant no. 2017/25/B/NZ7/00638) and project no. POWR.03.05.00-00-Z302/17 “Universitas Copernicana Thoruniensis In Futuro”, co-financed by the European Social Fund – the Operational Programme Knowledge Education Development. Module 5. Interdisciplinary PhD School “Academia Copernicana”.

Author Contributions

Justyna Rogalska conceived the project and got funding; Angelika Klimek, Hanna Kletkiewicz, and Joanna Wyszkowska prepared experimental protocols; Angelika Klimek, Hanna Kletkiewicz, Maciej Klimiuk, Agnieszka Siejka, Justyna Maliszewska, Milena Jankowska conducted the experiments; Angelika Klimek, Hanna Kletkiewicz analyzed the results; Angelika Klimek, Justyna Rogalska drafted the manuscript; Hanna Kletkiewicz, Justyna Maliszewska, Milena Jankowska, Joanna Wyszkowska, Anna Nowakowska reviewed the manuscript; Angelika Klimek, Agnieszka Siejka prepared data visualization;

Data Availability Statement

The data used to support the findings of this study are included in the article and supplementary material file.

Literature:

1) Touitou Y, Selmaoui B. The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system. Dialogues Clin Neurosci. 2012 Dec;14(4):381-99. doi: 10.31887/DCNS.2012.14.4/ytouitou.
2) Directive 2013/35/EU of the European Parliament and of the Council of 26 June 2013 on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (electromagnetic fields).
3) Duan Y, Wang Z, Zhang H, He Y, Lu R, Zhang R, Sun G, Sun X. The preventive effect of lotus seedpod procyanidins on cognitive impairment and oxidative damage induced by extremely low frequency electromagnetic field exposure. Food Funct. 2013 Aug;4(8):1252-62. doi: 10.1039/c3fo60116a.
4) Yin C, Luo X, Duan Y, Duan W, Zhang H, He Y, Sun G, Sun X. Neuroprotective effects of lotus seedpod procyanidins on extremely low frequency electromagnetic field-induced neurotoxicity in primary cultured hippocampal neurons. Biomed Pharmacother. 2016 Aug;82:628-39. doi: 10.1016/j.biopha.2016.05.032.

5) Klimek A, Nowakowska A, Kletkiewicz H, Wyszkowska J, Maliszewska J, Jankowska M, Peplowski L, Rogalska J. Bidirectional Effect of Repeated Exposure to Extremely Low-Frequency Electromagnetic Field (50 Hz) of 1 and 7 mT on Oxidative/Antioxidative Status in Rat's Brain: The Prediction for the Vulnerability to Diseases. Oxid Med Cell Longev. 2022 Jun 14;2022:1031211. doi: 10.1155/2022/1031211.
6) Sedghi H, Zare S, Hayatgeibi H, Alivandi S, Ebadi A. G. Effects of 50 HZ Magnetic Field on Some Factors of Immune System in the Male Guinea Pigs. Am. J. Immunol. 2005;1:37–41. doi: 10.3844/ajisp.2005.37.41.
7) Szemerszky R, Zelena D, Barna I, Bárdos G. Stress-related endocrinological and psychopathological effects of short- and long-term 50Hz electromagnetic field exposure in rats. Brain Res Bull. 2010 Jan 15;81(1):92-9. doi: 10.1016/j.brainresbull.2009.10.015.
8) Kitaoka K, Kitamura M, Aoi S, Shimizu N, Yoshizaki K. Chronic exposure to an extremely low-frequency magnetic field induces depression-like behavior and corticosterone secretion without enhancement of the hypothalamic-pituitary-adrenal axis in mice. Bioelectromagnetics. 2013 Jan;34(1):43-51. doi: 10.1002/bem.21743.
9) Mahdavi SM, Sahraei H, Yaghmaei P, Tavakoli H. Effects of electromagnetic radiation exposure on stress-related behaviors and stress hormones in male wistar rats. Biomol Ther (Seoul). 2014 Nov;22(6):570-6. doi: 10.4062/biomolther.2014.054.
10) Ebrahimzadeh Abarghoee S, Halvani GhH, Kargar-Shouroki F, Mihanpour H, Madadizadeh F. Job Stress among Workers Exposed to Extremely Low Frequency Electric and Magnetic Fields in a Combined Cycle Power Plant. J Environ Health Sustain Dev. 2022;7(2):1614-22. doi: 10.18502/jehsd.v7i2.9785.
11) Huang Z, Ito M, Zhang S, Toda T, Takeda JI, Ogi T, Ohno K. Extremely low-frequency electromagnetic field induces acetylation of heat shock proteins and enhances protein folding. Ecotoxicol Environ Saf. 2023 Oct 1;264:115482. doi: 10.1016/j.ecoenv.2023.115482.
12) Klimek A, Kletkiewicz H, Siejka A, Wyszkowska J, Maliszewska J, Klimiuk M, Jankowska M, Seckl J, Rogalska J. New View on the Impact of the Low-Frequency Electromagnetic Field (50 Hz) on Stress Responses: Hormesis Effect. Neuroendocrinology. 2023;113(4):423-441. doi: 10.1159/000527878.
13) Hosseinabadi M, Khanjani N, Ebrahimi MH, Haji B, Abdolahfard M. The effect of chronic exposure to extremely low-frequency electromagnetic fields on sleep quality, stress, depression and anxiety. Electromagn Biol Med. 2019;38(1):96-101. doi: 10.1080/15368378.2018.1545665.
14) Klimek A, Rogalska J. Extremely Low-Frequency Magnetic Field as a Stress Factor-Really Detrimental?-Insight into Literature from the Last Decade. Brain Sci. 2021 Jan 31;11(2):174. doi: 10.3390/brainsci11020174.
15) Krugers HJ, Karst H, Joels M. Interactions between noradrenaline and corticosteroids in the brain: from electrical activity to cognitive performance. Front Cell Neurosci. 2012 Apr 9;6:15. doi: 10.3389/fncel.2012.00015.
16) Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev. 2003 Apr;42(1):33-84. doi: 10.1016/s0165-0173(03)00143-7.
17) Tafet GE, Bernardini R. Psychoneuroendocrinological links between chronic stress and depression. Prog Neuropsychopharmacol Biol Psychiatry. 2003 Sep;27(6):893-903. doi: 10.1016/S0278-5846(03)00162-3.

18) Morris LS, McCall JG, Charney DS, Murrough JW. The role of the locus coeruleus in the generation of pathological anxiety. Brain Neurosci Adv. 2020 Jul 21;4:2398212820930321. doi: 10.1177/2398212820930321.
19) Bacon TJ, Pickering AE, Mellor JR. Noradrenaline Release from Locus Coeruleus Terminals in the Hippocampus Enhances Excitation-Spike Coupling in CA1 Pyramidal Neurons Via β-Adrenoceptors. Cereb Cortex. 2020 Nov 3;30(12):6135-6151. doi: 10.1093/cercor/bhaa159.
20) Aloyz R, Fawcett JP, Kaplan DR, Murphy RA, Miller FD. Activity-dependent activation of TrkB neurotrophin receptors in the adult CNS. Learn Mem. 1999 May-Jun;6(3):216-31. PMID: 10492004; PMCID: PMC311290.
21) Roszkowski M, Manuella F, von Ziegler L, Durán-Pacheco G, Moreau JL, Mansuy IM, Bohacek J. Rapid stress-induced transcriptomic changes in the brain depend on beta-adrenergic signaling. Neuropharmacology. 2016 Aug;107:329-338. doi: 10.1016/j.neuropharm.2016.03.046.
22) Privitera M, von Ziegler LM, Floriou-Servou A, Duss SN, Zhang R, Leimbacher S. et al. Noradrenaline release from the locus coeruleus shapes stress-induced hippocampal gene expression. eLife. 2023;12:RP88559. doi: 10.7554/eLife.88559.1.
23) Marien MR, Colpaert FC, Rosenquist AC. Noradrenergic mechanisms in neurodegenerative diseases: a theory. Brain Res Brain Res Rev. 2004 Apr;45(1):38-78. doi: 10.1016/j.brainresrev.2004.02.002.
24) Maity S, Abbaspour R, Nahabedian D, Connor SA. Norepinephrine, beyond the Synapse: Coordinating Epigenetic Codes for Memory. Int J Mol Sci. 2022 Aug 31;23(17):9916. doi: 10.3390/ijms23179916.
25) Calabrese EJ. Overcompensation stimulation: a mechanism for hormetic effects. Crit Rev Toxicol. 2001 Jul;31(4-5):425-70. doi: 10.1080/20014091111749.
26) Mushak P. Temporal stability of chemical hormesis (CH): Is CH just a temporary stop on the road to thresholds and toxic responses? Sci Total Environ. 2016 Nov 1;569-570:1446-1456. doi: 10.1016/j.scitotenv.2016.06.233.
27) Calabrese EJ, Mattson MP. How does hormesis impact biology, toxicology, and medicine? NPJ Aging Mech Dis. 2017 Sep 15;3:13. doi: 10.1038/s41514-017-0013-z.
28) Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.
29) Bieńkowski P, Wyszkowska J. TECHNICZNE ASPEKTY EKSPOZYCJI NA POLE MAGNETYCZNE EKSTREMALNIE NISKICH CZĘSTOTLIWOŚCI (ELF) W BADANIACH BIOMEDYCZNYCH [TECHNICAL ASPECTS OF EXPOSURE TO MAGNETIC FIELDS OF EXTREMELY LOW FREQUENCIES (ELF) IN BIOMEDICAL RESEARCH]. Med Pr. 2015;66(2):185-97. Polish. doi: 10.13075/mp.5893.00164.
30) Trawiński T, Szczygieł M, Wyszkowska J, Kluszczyński K. Analysis of Magnetic Field Distribution and Mechanical Vibration of Magnetic Field Exciter under Different Voltage Supply. In: Piętka E, Kawa J. (eds) Information Technologies in Biomedicine. Advances in Intelligent and Soft Computing, 2010, vol 69. Springer, Berlin, Heidelberg. doi: 10.1007/978-3-642-13105-9_60.
31) Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 2002 May 1;30(9):e36. doi: 10.1093/nar/30.9.e36.
32) Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am J Physiol Endocrinol Metab. 2006 Nov;291(5):E965-73. doi: 10.1152/ajpendo.00070.2006.

33) McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP, Bruchas MR. CRH Engagement of the Locus Coeruleus Noradrenergic System Mediates Stress-Induced Anxiety. Neuron. 2015 Aug 5;87(3):605-20. doi: 10.1016/j.neuron.2015.07.002.
34) Ochedalski T, Rabadan-Diehl C, Aguilera G. Interaction between glucocorticoids and corticotropin releasing hormone (CRH) in the regulation of the pituitary CRH receptor in vivo in the rat. J Neuroendocrinol. 1998 May;10(5):363-9. doi: 10.1046/j.1365-2826.1998.00212.x.
35) O'Dell TJ, Connor SA, Guglietta R, Nguyen PV. β-Adrenergic receptor signaling and modulation of long-term potentiation in the mammalian hippocampus. Learn Mem. 2015 Aug 18;22(9):461-71. doi: 10.1101/lm.031088.113.
36) Giorgi FS, Galgani A, Puglisi-Allegra S, Busceti CL, Fornai F. The connections of Locus Coeruleus with hypothalamus: potential involvement in Alzheimer's disease. J Neural Transm (Vienna). 2021 May;128(5):589-613. doi: 10.1007/s00702-021-02338-8. Epub 2021 May 3. PMID: 33942174; PMCID: PMC8105225.
37) Rajendra P, Sujatha H, Devendranath D, Gunasekaran B, Sashidhar R, Subramanyam C, Channakeshava. Biological effects of power frequency magnetic fields: Neurochemical and toxicological changes in developing chick embryos. Biomagn Res Technol. 2004 Jan 31;2(1):1. doi: 10.1186/1477-044X-2-1.
38) Garvey M, Hollon S, DeRubeis R. Is there a relationship between MHPG and anxiety in depressed patients? Psychiatry Res. 1991 Aug;38(2):187-95. doi: 10.1016/0165-1781(91)90043-o.
39) Kurita M, Nishino S, Numata Y, Okubo Y, Sato T. The noradrenaline metabolite MHPG is a candidate biomarker between the depressive, remission, and manic states in bipolar disorder I: two long-term naturalistic case reports. Neuropsychiatr Dis Treat. 2015 Feb 11;11:353-8. doi: 10.2147/NDT.S74550.
40) van der Zee S, Vermeiren Y, Fransen E, Van Dam D, Aerts T, Gerritsen MJ, Spikman JM, van Laar T, De Deyn PP. Monoaminergic Markers Across the Cognitive Spectrum of Lewy Body Disease. J Parkinsons Dis. 2018;8(1):71-84. doi: 10.3233/JPD-171228.
41) Dekker AD, Coppus AM, Vermeiren Y, Aerts T, van Duijn CM, Kremer BP, Naudé PJ, Van Dam D, De Deyn PP. Serum MHPG strongly predicts conversion to Alzheimer's disease in behaviorally characterized subjects with Down syndrome. J Alzheimers Dis. 2015;43(3):871-91. doi: 10.3233/JAD-140783.
42) Abercrombie ED, Jacobs BL. Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. II. Adaptation to chronically presented stressful stimuli. J Neurosci. 1987 Sep;7(9):2844-8. doi: 10.1523/JNEUROSCI.07-09-02844.1987.
43) Nisenbaum LK, Zigmond MJ, Sved AF, Abercrombie ED. Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. J Neurosci. 1991 May;11(5):1478-84. doi: 10.1523/JNEUROSCI.11-05-01478.1991.
44) Pavcovich LA, Cancela LM, Volosin M, Molina VA, Ramirez OA. Chronic stress-induced changes in locus coeruleus neuronal activity. Brain Res Bull. 1990 Feb;24(2):293-6. doi: 10.1016/0361-9230(90)90219-p.
45) Hagena H, Hansen N, Manahan-Vaughan D. β-Adrenergic Control of Hippocampal Function: Subserving the Choreography of Synaptic Information Storage and Memory. Cereb Cortex. 2016 Apr;26(4):1349-64. doi: 10.1093/cercor/bhv330.
46) Stone EA, Platt JE. Brain adrenergic receptors and resistance to stress. Brain Res. 1982 Apr 15;237(2):405-14. doi: 10.1016/0006-8993(82)90452-8.

47) Laszlo AM, Ladanyi M, Boda K, Csicsman J, Bari F, Serester A, Molnar Z, Sepp K, Galfi M, Radacs M. Effects of extremely low frequency electromagnetic fields on turkeys. Poult Sci. 2018 Feb 1;97(2):634-642. doi: 10.3382/ps/pex304.
48) Roozendaal B, Okuda S, de Quervain DJ, McGaugh JL. Glucocorticoids interact with emotion-induced noradrenergic activation in influencing different memory functions. Neuroscience. 2006;138(3):901-10. doi: 10.1016/j.neuroscience.2005.07.049. Epub 2005 Nov 28. PMID: 16310958.
49) O'Dell TJ, Connor SA, Gelinas JN, Nguyen PV. Viagra for your synapses: Enhancement of hippocampal long-term potentiation by activation of beta-adrenergic receptors. Cell Signal. 2010 May;22(5):728-36. doi: 10.1016/j.cellsig.2009.12.004.
50) Chai GS, Feng Q, Wang ZH, Hu Y, Sun DS, Li XG, Ke D, Li HL, Liu GP, Wang JZ. Downregulating ANP32A rescues synapse and memory loss via chromatin remodeling in Alzheimer model. Mol Neurodegener. 2017 May 4;12(1):34. doi: 10.1186/s13024-017-0178-8.
51) Bing G, Stone EA, Zhang Y, Filer D. Immunohistochemical studies of noradrenergic-induced expression of c-fos in the rat CNS. Brain Res. 1992 Oct 2;592(1-2):57-62. doi: 10.1016/0006-8993(92)91658-2.
52) Stone EA, Zhang Y, John SM, Bing G. c-Fos response to administration of catecholamines into brain by microdialysis. Neurosci Lett. 1991 Nov 25;133(1):33-5. doi: 10.1016/0304-3940(91)90050-4.
53) Calabrese EJ, Mattson MP. Hormesis provides a generalized quantitative estimate of biological plasticity. J Cell Commun Signal. 2011 Mar;5(1):25-38. doi: 10.1007/s12079-011-0119-1.
54) Chauhan E, Bali A, Singh N, Jaggi AS. Cross stress adaptation: Phenomenon of interactions between homotypic and heterotypic stressors. Life Sci. 2015 Sep 15;137:98-104. doi: 10.1016/j.lfs.2015.07.018.
55) Alsaeed I, Al-Somali F, Sakhnini L, Aljarallah OS, Hamdan RM, Bubishate SA, Sarfaraz ZK, Kamal A. Autism-relevant social abnormalities in mice exposed perinatally to extremely low frequency electromagnetic fields. Int J Dev Neurosci. 2014 Oct;37:58-64. doi: 10.1016/j.ijdevneu.2014.06.010.
56) Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc. 2007;2(2):322-8. doi: 10.1038/nprot.2007.44.
57) Janać B, Selaković V, Rauš S, Radenović L, Zrnić M, Prolić Z. Temporal patterns of extremely low frequency magnetic field-induced motor behavior changes in Mongolian gerbils of different age. Int J Radiat Biol. 2012 Apr;88(4):359-66. doi: 10.3109/09553002.2012.652725.
58) Finlay JM, Zigmond MJ, Abercrombie ED. Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: effects of diazepam. Neuroscience. 1995 Feb;64(3):619-28. doi: 10.1016/0306-4522(94)00331-x.
59) Stone EA, Quartermain D. Alpha-1-noradrenergic neurotransmission, corticosterone, and behavioral depression. Biol Psychiatry. 1999 Nov 1;46(9):1287-300. doi: 10.1016/s0006-3223(99)00234-6.
60) Cirulli F, Alleva E. The NGF saga: from animal models of psychosocial stress to stress-related psychopathology. Front Neuroendocrinol. 2009 Aug;30(3):379-95. doi: 10.1016/j.yfrne.2009.05.002.
61) Ressler KJ, Nemeroff CB. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress Anxiety. 2000;12 Suppl 1:2-19. doi: 10.1002/1520-6394(2000)12:1+<2::AID-DA2>3.0.CO;2-4.

62) Moret C, Briley M. The importance of norepinephrine in depression. Neuropsychiatr Dis Treat. 2011;7(Suppl 1):9-13. doi: 10.2147/NDT.S19619.
63) Holland N, Robbins TW, Rowe JB. The role of noradrenaline in cognition and cognitive disorders. Brain. 2021 Sep 4;144(8):2243-2256. doi: 10.1093/brain/awab111.
64) Dahl MJ, Kulesza A, Werkle-Bergner M, Mather M. Declining locus coeruleus-dopaminergic and noradrenergic modulation of long-term memory in aging and Alzheimer's disease. Neurosci Biobehav Rev. 2023 Oct;153:105358. doi: 10.1016/j.neubiorev.2023.105358.
65) Krohn F, Lancini E, Ludwig M, Leiman M, Guruprasath G, Haag L, Panczyszyn J, Düzel E, Hämmerer D, Betts M. Noradrenergic neuromodulation in ageing and disease. Neurosci Biobehav Rev. 2023 Sep;152:105311. doi: 10.1016/j.neubiorev.2023.105311.

Supplementary Materials

The electromagnetic field (50 Hz) can establish a new "set-point" for the activity of the locus coeruleus–noradrenergic (LC-NA) system in rat

Angelika Klimek[1#], Hanna Kletkiewicz[1], Agnieszka Siejka[1], Joanna Wyszkowska[1], Justyna Maliszewska[1], Maciej Klimiuk[1], Milena Jankowska[1], Justyna Rogalska[1#]

[1] Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences Nicolaus Copernicus University in Torun, 87-100, Poland.

[#]***Angelika Klimek, Justyna Rogalska***

Email: klimek@doktorant.umk.pl, rogal@umk.pl

Tel: +48 785 150 453

Supplement 1

Distribution of the EMF in coil

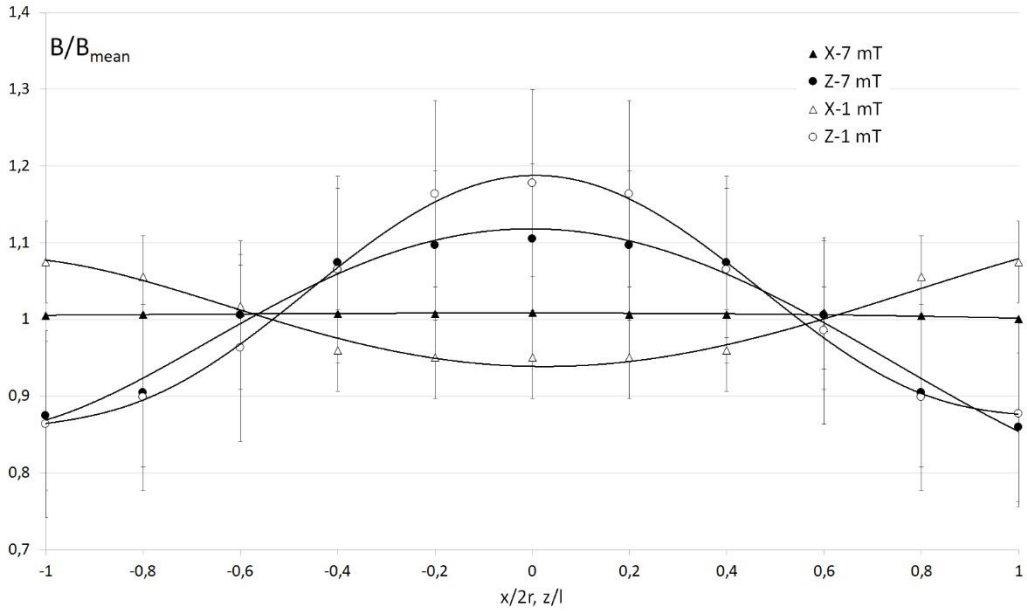


Fig. S1

The distribution of the EMF along the main axis of the coil within the area of the animal's cage. Abbreviations: **B** – magnetic flux density vector, **B/B$_{mean}$**: normalized magnetic flux density relative to the mean value; x/2r: normalized distance from the solenoid centre along the x-axis; z/l: normalized distance from the coil centre along the z-axis; r: coil radius; l: coil length.

Supplement 2

The scheme of the plus maze

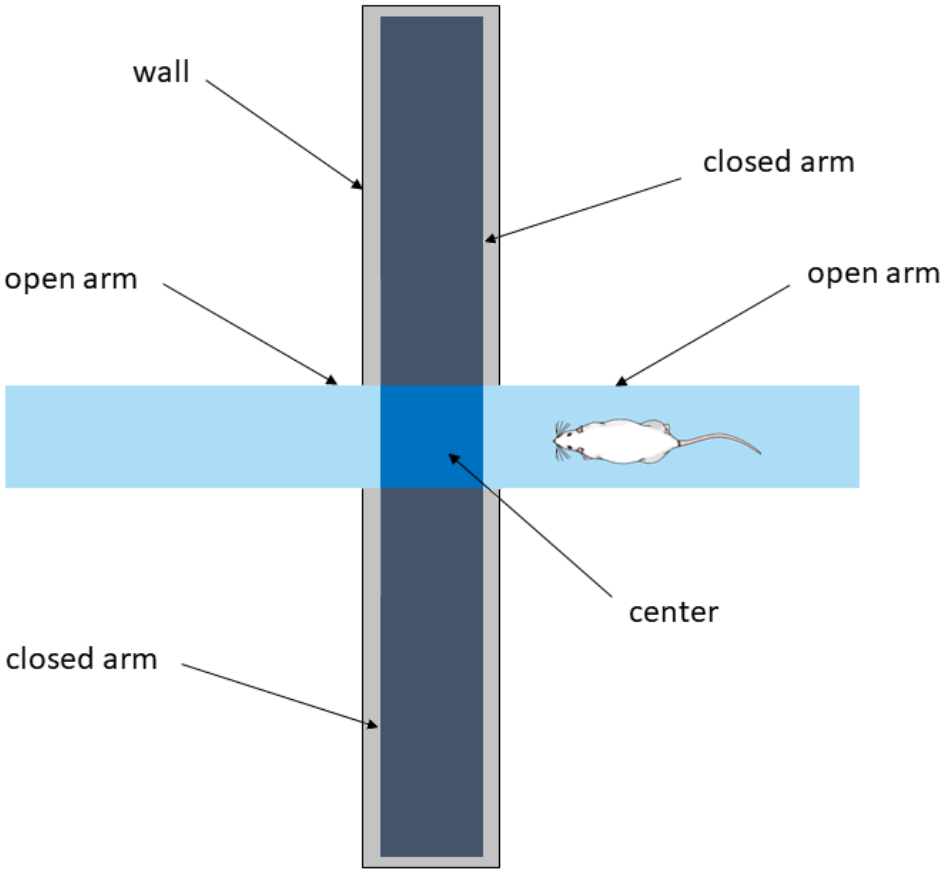


Fig. S2

The figure shows a schematic representation of a plus maze (PM). The scheme includes open and closed arms (40 cm in length) and the centre of the maze (10 cm x 10 cm). Arms are limited by 40 cm high dark walls (closed arms) or plexiglass (open arms).

Supplement 3

Percentage changes in the values of hormones

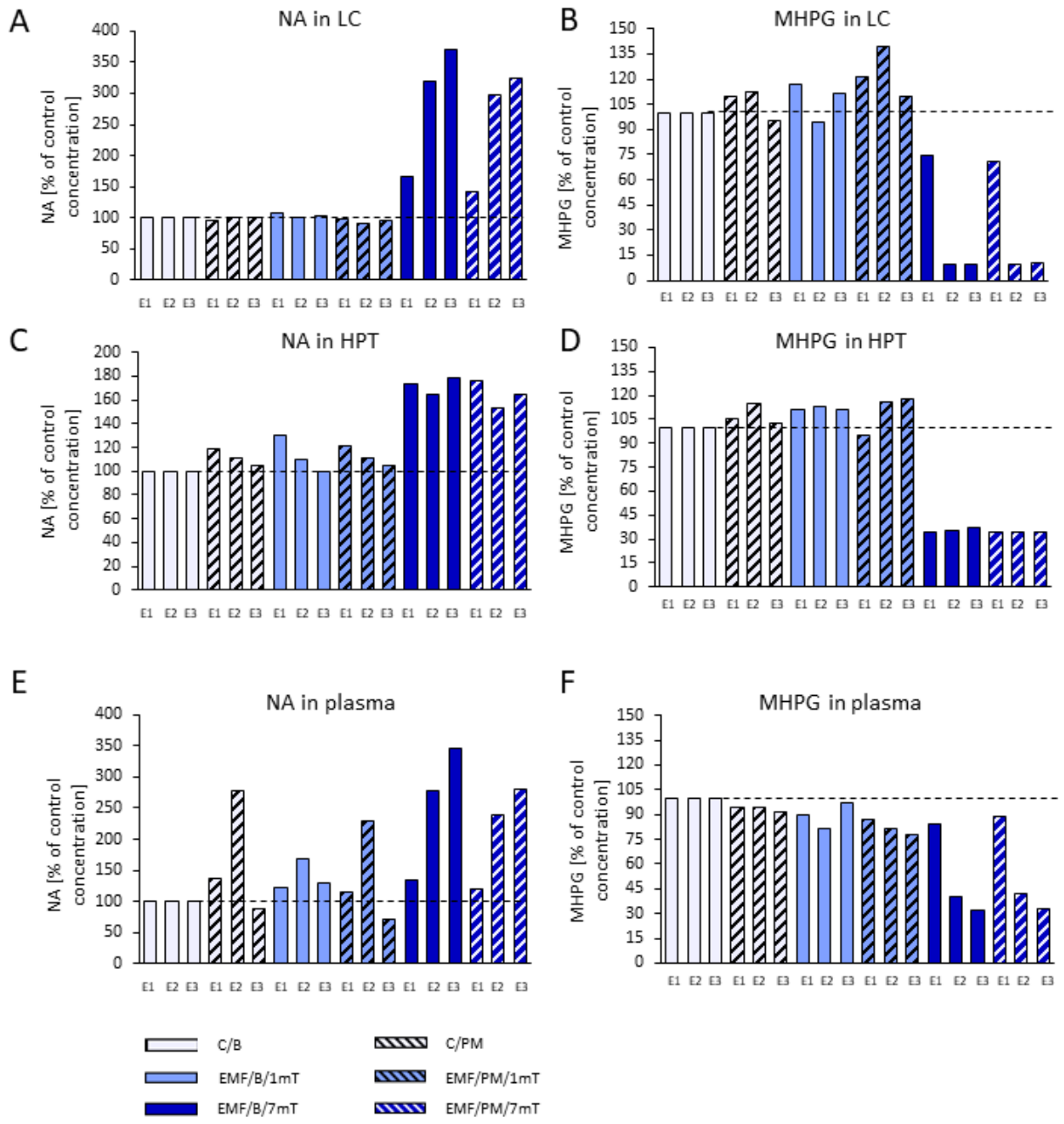


Fig. S3

Percentage changes in the values of hormones in each group compared to their concentrations in the control group (C/B; set at 100 % - reference value) separately after each exposure.

Supplement 4

Percentage changes in the values of MHPG/NA ratio

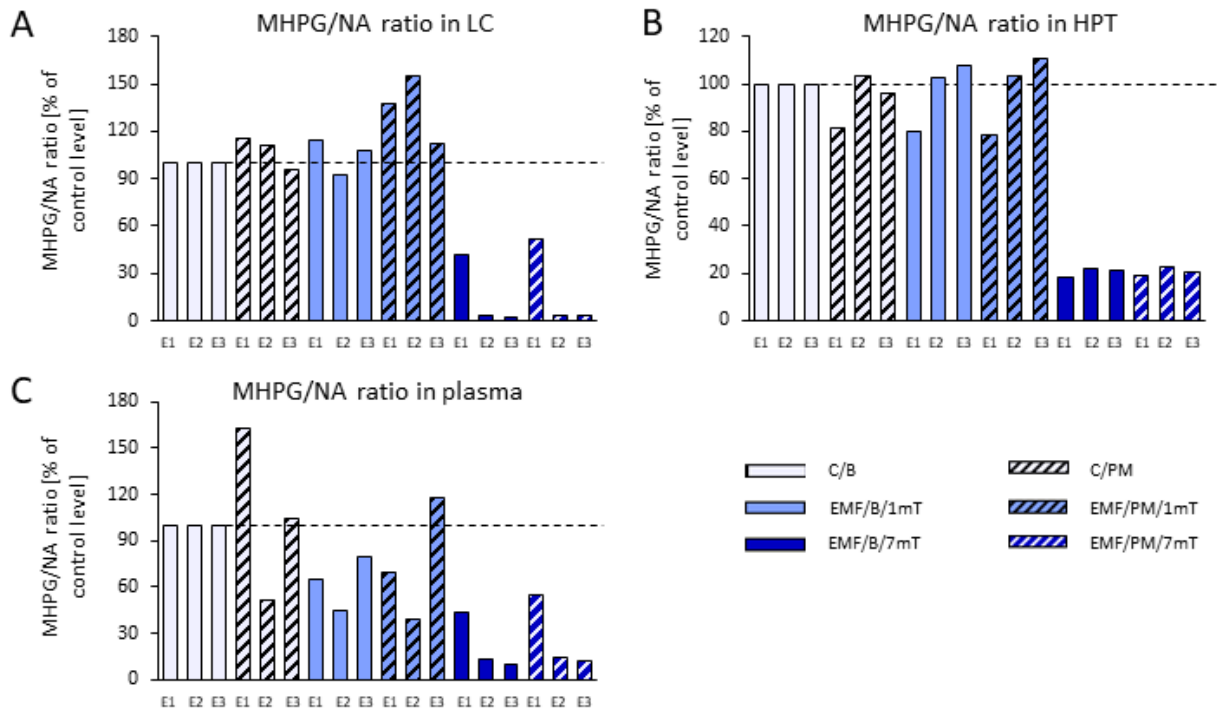


Fig. S4

Percentage changes in the values of MHPG/NA ratio in each group compared to their values in the control group (C/B; set at 100 % - reference value) separately after each exposure.

Supplement 5

Percentage changes in the values of β2-AR mRNA relative expression

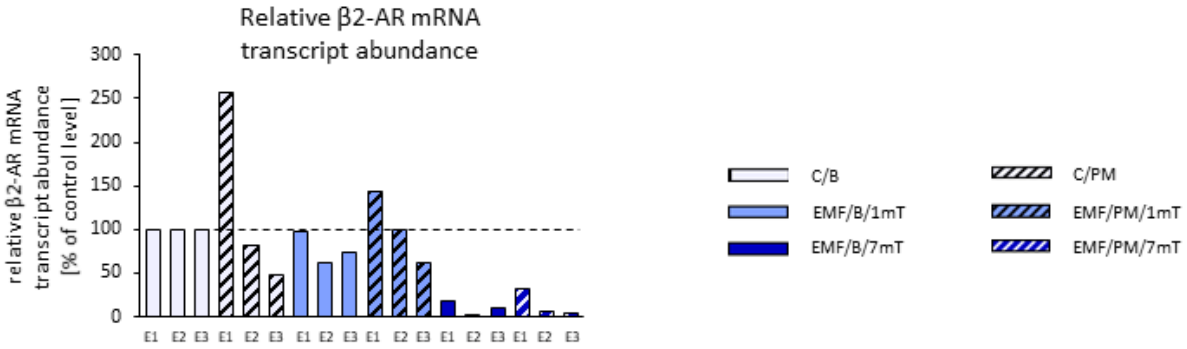


Fig. S5

Percentage changes in the values of β2-AR mRNA relative expression in each group compared to their expression in the control group (C/B; set at 100 % - reference value) separately after each exposure.

Supplement 6

Table S1 Primers for reverse transcription-quantitative polymerase chain reaction

Gene	Forward primer	Reverse primer	Annealing
β2-AR	CTCCTTAACTGGTTGGGCTATG	CCTGGAAGGCAATCCTGAAA	56˚C, 20 sec
β-actin	CGTTGACATCCGTAAAGACCTC	TAGGAGCCAGGGCAGTAATCT	61˚C, 20 sec
GAPDH	TAAAGAACAGGCTCTTAGCACA	AGTCTTGGAAATGGATTGTCTC	61˚C, 20 sec

β2-AR, β2-adrenergic receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

Table S2 Results of statistical analysis of the 'basal' concentrations of NA, MHPG and MHPG/NA ratio in examined brain areas and plasma

	Dependent variable	Effect	*df*	*F/H*	*P*
A	NA in LC	intensity of the electromagnetic field (mT)	2	261.053 (F)	**<0.001**
		number of exposures (E1-E3)	2	1.076 (F)	0.347
		(mT) x (E1-E3)	4	16.474 (F)	**<0.001**
		Error	63		
B	MHPG in LC	intensity of the electromagnetic field (mT)	2	581.570 (F)	**<0.001**
		number of exposures (E1-E3)	2	45.377 (F)	**<0.001**
		(mT) x (E1-E3)	4	74.472 (F)	**<0.001**
		Error	81		
C	MHPG/NA ratio in LC	intensity of the electromagnetic field (mT)	2	218.096 (F)	**<0.001**
		number of exposures (E1-E3)	2	3.028 (F)	0.055
		(mT) x (E1-E3)	4	10.108 (F)	**<0.001**
		Error	63		
D	NA in HPT	intensity of the electromagnetic field (mT)	2	151.950 (F)	**<0.001**
		number of exposures (E1-E3)	2	7.213 (F)	**0.001**
		(mT) x (E1-E3)	4	3.463 (F)	**0.013**
		Error	65		
E	MHPG in HPT	intensity of the electromagnetic field (mT)	2	61.724 (H)	**<0.001**
		number of exposures (E1-E3)	2	1.967 (H)	0.374
F	MHPG/NA ratio in HPT	intensity of the electromagnetic field (mT)	2	137.464 (F)	**<0.001**
		number of exposures (E1-E3)	2	2.898 (F)	0.062
		(mT) x (E1-E3)	4	1.248 (F)	0.299
		Error	65		
G	NA in plasma	intensity of the electromagnetic field (mT)	2	39.418 (F)	**<0.001**
		number of exposures (E1-E3)	2	8.207 (F)	**<0.001**
		(mT) x (E1-E3)	4	5.081 (F)	**0.001**
		Error	69		
H	MHPG in plasma	intensity of the electromagnetic field (mT)	2	40.056 (F)	**<0.001**
		number of exposures (E1-E3)	2	5.386 (F)	**0.006**
		(mT) x (E1-E3)	4	6.766 (F)	**<0.001**
		Error	77		
I	MHPG/NA ratio in plasma	intensity of the electromagnetic field (mT)	2	28.957 (F)	**<0.001**
		number of exposures (E1-E3)	2	1.979 (F)	0.146
		(mT) x (E1-E3)	4	2.998 (F)	**0.024**
		Error	67		

F - ANOVA test, H- Kruskal-Wallis test, statistically significant P value - indicated in bold, NA - noradrenaline, MHPG - 3-Methoxy-4-hydroxyphenylglycol

Table S3 Results of statistical analysis of the 'plus maze-induced' concentrations of NA, MHPG and MHPG/NA ratio in examined brain areas and plasma

	Dependent variable	Effect	*df*	*F/H*	*P*
A	NA in LC	intensity of the electromagnetic field (mT)	2	51.748 (H)	**<0.001**
		number of exposures (E1-E3)	2	3.711 (H)	0.156
B	MHPG in LC	intensity of the electromagnetic field (mT)	2	59.529 (H)	**<0.001**
		number of exposures (E1-E3)	2	0.994 (H)	0.608
C	MHPG/NA ratio in LC	intensity of the electromagnetic field (mT)	2	147.663 (F)	**<0.001**
		number of exposures (E1-E3)	2	2.941 (F)	0.060
		(mT) x (E1-E3)	4	7.170 (F)	**<0.001**
		Error	66		
D	NA in HPT	intensity of the electromagnetic field (mT)	2	43.437 (H)	**<0.001**
		number of exposures (E1-E3)	2	6.078 (H)	**0.048**
E	MHPG in HPT	intensity of the electromagnetic field (mT)	2	59.455 (H)	**<0.001**
		number of exposures (E1-E3)	2	4.773 (H)	0.092
F	MHPG/NA ratio in HPT	intensity of the electromagnetic field (mT)	2	126.480 (F)	**<0.001**
		number of exposures (E1-E3)	2	7.244 (F)	**0.001**
		(mT) x (E1-E3)	4	1.672 (F)	0.167
		Error	66		
G	NA in plasma	intensity of the electromagnetic field (mT)	2	14.662 (H)	**<0.001**
		number of exposures (E1-E3)	2	6.450 (H)	**0.040**
H	MHPG in plasma	intensity of the electromagnetic field (mT)	2	60.363 (F)	**<0.001**
		number of exposures (E1-E3)	2	20.613 (F)	**<0.001**
		(mT) x (E1-E3)	4	14.042 (F)	**<0.001**
		Error	79		
I	MHPG/NA ratio in plasma	intensity of the electromagnetic field (mT)	2	28.932 (F)	**<0.001**
		number of exposures (E1-E3)	2	2.147 (F)	0.125
		(mT) x (E1-E3)	4	7.582 (F)	**<0.001**
		Error	69		

F - ANOVA test, H- Kruskal-Wallis test, statistically significant P value - indicated in bold, NA - noradrenaline, MHPG - 3-Methoxy-4-hydroxyphenylglycol

Table S4 Results of statistical analysis of effects of plusmaze on concentrations of NA, MHPG and MHPG/NA ratio in relation to their 'basal' levels in each experimental group in locus coeruleus

	Dependent variable/group	Effect	*df*	*F/H*	*P*
A	NA in LC	PM effect	1	0.368 (F)	0.547
	control group	number of exposures (E1-E3)	2	17.743 (F)	**<0.001**
		number of exposures x PM effect	2	0.623 (F)	0.541
		Error	45		
B	NA in LC	PM effect	1	7.275 (F)	**0.010**
	EMF/1 mT	number of exposures (E1-E3)	2	14.232 (F)	**<0.001**
		number of exposures x PM effect	2	0.470 (F)	0.628
		Error	43		
C	NA in LC	PM effect	1	2.020 (F)	0.163
	EMF/7 mT	number of exposures (E1-E3)	2	20.611 (F)	**<0.001**
		number of exposures x PM effect	2	0.112 (F)	0.894
		Error	41		
D	MHPG in LC	PM effect	1	0.839 (F)	0.364
	control group	number of exposures (E1-E3)	2	2.732 (F)	0.074
		number of exposures x PM effect	2	0.718 (F)	0.493
		Error	54		
E	MHPG in LC	PM effect	1	9.907 (F)	**0.003**
	EMF/1 mT	number of exposures (E1-E3)	2	3.272 (F)	**0.046**
		number of exposures x PM effect	2	8.413 (F)	**<0.001**
		Error	54		
F	MHPG in LC	PM effect	1	0.108 (F)	0.744
	EMF/7 mT	number of exposures (E1-E3)	2	338.428 (F)	**<0.001**
		number of exposures x PM effect	2	0.364 (F)	0.697
		Error	54		
G	MHPG/NA ratio in	PM effect	1	0.706 (F)	0.405
	LC	number of exposures (E1-E3)	2	3.984 (F)	**0.026**
	control group	number of exposures x PM effect	2	0.685 (F)	0.509
		Error	45		
H	MHPG/NA ratio in	PM effect	1	15.884 (F)	**<0.001**
	LC	number of exposures (E1-E3)	2	4.538 (F)	**0.016**
	EMF/1 mT	number of exposures x PM effect	2	7.594 (F)	**0.001**
		Error	43		
I	MHPG/NA ratio in	PM effect	1	1.798 (H)	0.180
	LC	number of exposures (E1-E3)	2	31.501 (H)	**<0.001**
	EMF/7 mT				

F - ANOVA test, H- Kruskal-Wallis test, statistically significant P value - indicated in bold, NA - noradrenaline, MHPG - 3-methoxy-4-hydroxyphenylglycol

Table S5 Results of statistical analysis of effects of plus maze on concentrations of NA, MHPG and MHPG/NA ratio in relation to their 'basal' levels in each experimental group in the hypothalamus

	Dependent variable/group	Effect	*df*	*F/H*	*P*
A	NA in HPT	PM effect	1	15.197 (F)	**<0.001**
	control group	number of exposures (E1-E3)	2	3.319 (F)	**0.045**
		number of exposures x PM effect	2	1.760 (F)	0.183
		Error	46		
B	NA in HPT	PM effect	1	0.068 (F)	0.795
	EMF/1 mT	number of exposures (E1-E3)	2	13.776 (F)	**<0.001**
		number of exposures x PM effect	2	1.114 (F)	0.337
		Error	43		
C	NA in HPT	PM effect	1	1.219 (F)	0.276
	EMF/7 mT	number of exposures (E1-E3)	2	4.675 (F)	**0.015**
		number of exposures x PM effect	2	0.400 (F)	0.673
		Error	42		
D	MHPG in HPT	PM effect	1	1.674 (H)	0.195
	control group	number of exposures (E1-E3)	2	4.337 (H)	0.114
E	MHPG in HPT	PM effect	1	0.167 (F)	0.685
	EMF/1 mT	number of exposures (E1-E3)	2	4.539 (F)	**0.015**
		number of exposures x PM effect	2	1.773 (F)	0.180
		Error	54		
F	MHPG in HPT	PM effect	1	0.210 (H)	0.647
	EMF/7 mT	number of exposures (E1-E3)	2	11.424 (H)	**0.003**
G	MHPG/NA ratio in	PM effect	1	1.153 (F)	0.288
	HPT	number of exposures (E1-E3)	2	2.742 (F)	0.075
	control group	number of exposures x PM effect	2	0.942 (F)	0.397
		Error	46		
H	MHPG/NA ratio in	PM effect	1	0.010 (F)	0.920
	HPT	number of exposures (E1-E3)	2	7.896 (F)	**0.001**
	EMF/1 mT	number of exposures x PM effect	2	0.049 (F)	0.952
		Error	43		
I	MHPG/NA ratio in	PM effect	1	0.100 (F)	0.753
	HPT	number of exposures (E1-E3)	2	10.376 (F)	**<0.001**
	EMF/7 mT	number of exposures x PM effect	2	0.157 (F)	0.855
		Error	42		

F - ANOVA test, H- Kruskal-Wallis test, statistically significant P value - indicated in bold, NA - noradrenaline, MHPG - 3-methoxy-4-hydroxyphenylglycol

Table S6 Results of statistical analysis of effects of plus maze on concentrations of NA, MHPG and MHPG/NA ratio in relation to their 'basal' levels in each experimental group in plasma

	Dependent variable/group	Effect	*df*	*F*	*P*
A	NA in plasma	PM effect	1	3.249	0.077
	control group	number of exposures (E1-E3)	2	3.830	**0.028**
		number of exposures x PM effect	2	3.158	0.051
		Error	50		
B	NA in plasma	PM effect	1	1.447	0.235
	EMF/1 mT	number of exposures (E1-E3)	2	8.263	**<0.001**
		number of exposures x PM effect	2	3.443	**0.040**
		Error	47		
C	NA in plasma	PM effect	1	7.128	**0.011**
	EMF/7 mT	number of exposures (E1-E3)	2	23.589	**<0.001**
		number of exposures x PM effect	2	0.726	0.490
		Error	42		
D	MHPG in plasma	PM effect	1	0.836	0.365
	control group	number of exposures (E1-E3)	2	0.412	0.665
		number of exposures x PM effect	2	0.021	0.979
		Error	51		
E	MHPG in plasma	PM effect	1	2.618	0.112
	EMF/1 mT	number of exposures (E1-E3)	2	2.565	0.087
		number of exposures x PM effect	2	1.717	0.190
		Error	51		
F	MHPG in plasma	PM effect	1	0.496	0.484
	EMF/7 mT	number of exposures (E1-E3)	2	107.625	**<0.001**
		number of exposures x PM effect	2	0.065	0.937
		Error	54		
G	MHPG/NA ratio in	PM effect	1	1.743	0.193
	plasma	number of exposures (E1-E3)	2	1.765	0.182
	control group	number of exposures x PM effect	2	2.503	0.093
		Error	47		
H	MHPG/NA ratio in	PM effect	1	1.715	0.197
	plasma	number of exposures (E1-E3)	2	12.200	**<0.001**
	EMF/1 mT	number of exposures x PM effect	2	2.958	0.062
		Error	47		
I	MHPG/NA ratio in	PM effect	1	6.779	**0.013**
	plasma	number of exposures (E1-E3)	2	64.205	**<0.001**
	EMF/7 mT	number of exposures x PM effect	2	0.177	0.839
		Error	42		

F - ANOVA test, statistically significant P value - indicated in bold, NA - noradrenaline, MHPG - 3-methoxy-4-hydroxyphenylglycol

Table S7 Results of statistical analysis of the 'basal' and 'plus maze-induced' hippocampal β2-AR mRNA relative expression

	Dependent variable	Effect	*df*	*F*	*P*
A	'Basal' β2-AR	intensity of the electromagnetic field (mT)	2	41.722 (F)	**<0.001**
		number of exposures (E1-E3)	2	3.862 (F)	**0.030**
		(mT) x (E1-E3)	4	2.047 (F)	0.108
		Error	36		
B	'PM-induced' β2-AR	intensity of the electromagnetic field (mT)	2	32.857 (F)	**<0.001**
		number of exposures (E1-E3)	2	12.978 (F)	**<0.001**
		(mT) x (E1-E3)	4	2.837 (F)	**0.038**
		Error	36		

F - ANOVA test, statistically significant P value - indicated in bold, β2-AR –β2-adrenergic receptor

Table S8 Results of statistical analysis of effects of plus maze on hippocampal β2-AR mRNA relative expression in relation to its 'basal' level in each experimental group

	Dependent variable/group	Effect	*df*	*F*	*P*
A	β2-AR	PM effect	1	0.548	0.466
	control group	number of exposures (E1-E3)	2	4.042	**0.031**
		number of exposures x PM effect	2	1.890	0.173
		Error	24		
B	β2-AR	PM effect	1	0.852	0.365
	EMF/1 mT	number of exposures (E1-E3)	2	8.210	**0.002**
		number of exposures x PM effect	2	0.715	0.499
		Error	24		
C	β2-AR	PM effect	1	0.088	0.770
	EMF/7 mT	number of exposures (E1-E3)	2	0.957	0.398
		number of exposures x PM effect	2	3.951	**0.033**
		Error	24		

F - ANOVA test, statistically significant P value - indicated in bold, β2-AR –β2-adrenergic receptor

Table S9 Results of statistical analysis of behavioural variables in a plus maze test

	Dependent variable	Effect	*df*	*F*	*P*
A	Total distance moved (cm)	intensity of the electromagnetic field (mT)	2	0.982	0.379
		number of exposures (E1-E3)	2	4.121	**0.020**
		(mT) x (E1-E3)	4	2.646	**0.039**
		Error	81		
B	Movement velocity (cm/s)	intensity of the electromagnetic field (mT)	2	1.367	0.261
		number of exposures (E1-E3)	2	4.489	**0.014**
		(mT) x (E1-E3)	4	2.629	**0.040**
		Error	81		
C	Movement duration (s)	intensity of the electromagnetic field (mT)	2	0.532	0.589
		number of exposures (E1-E3)	2	3.176	**0.047**
		(mT) x (E1-E3)	4	2.610	**0.041**
		Error	81		
D	Time spent in closed arms (s)	intensity of the electromagnetic field (mT)	2	4.453	**0.015**
		number of exposures (E1-E3)	2	2.240	0.113
		(mT) x (E1-E3)	4	0.843	0.502
		Error	81		
E	Open arms frequency	intensity of the electromagnetic field (mT)	2	3.602	**0.032**
		number of exposures (E1-E3)	2	4.875	**0.010**
		(mT) x (E1-E3)	4	2.061	0.094
		Error	81		

F - ANOVA test, statistically significant P value - indicated in bold

Streszczenie rozprawy doktorskiej w języku polskim

Wpływ pola elektromagnetycznego o niskiej częstotliwości (50 Hz) na status oksydacyjny i reakcje stresowe u szczura – efekt hormezy

Angelika Klimek

Pole elektromagnetyczne o skrajnie niskiej częstotliwości (EMF) jest generowane przez urządzenia elektryczne oraz napowietrzne linie elektroenergetyczne. Wzrastająca liczba antropogenicznych źródeł EMF skłania naukowców do rozważenia jego wpływu na organizmy żywe, w tym na funkcjonowanie układu nerwowego. Dla społeczeństw zurbanizowanych szczególnie ważna jest identyfikacja potencjalnych zagrożeń związanych z EMF i ustalenie bezpiecznych limitów narażenia. Efekty działania EMF są niejednoznaczne (pozytywne lub negatywne), natomiast na pewno nie pozostają obojętne, a analiza dostępnej literatury nie daje odpowiedzi na pytania dotyczące wszystkich potencjalnych skutków zdrowotnych narażenia na ten czynnik środowiskowy. Aktualny stan wiedzy pozwala na postawienie hipotezy, że EMF wykazuje hormezę, tzn. dwukierunkowe działanie uzależnione od wartości indukcji magnetycznej. EMF indukuje wiele modyfikacji procesów wewnątrzkomórkowych, których konsekwencje mogą być w znaczący sposób zaburzać funkcje organizmu. Szczególną uwagę zwraca się na indukowane przez EMF zmiany w równowadze oksydacyjno-antyoksydacyjnej. Ponadto, wiele badań wskazuje, że EMF jest czynnikiem stresowym, który wywołuje reakcje komórek/organizmów charakterystyczne dla ogólnych reakcji na stres.

Założono, że proponowany efekt hormetyczny (dwukierunkowy) działania EMF jest związany z różnym poziomem stresu oksydacyjnego oraz zmianami w aktywacji układów stresu, takich jak oś podwzgórzowo-przysadkowo-nadnerczowa (HPA) i układ nadnerczowy (LC-NA). Hormony stresu, głównie kortykosteron i noradrenalina, mogą modulować funkcjonowanie hipokampu i wpływać na procesy plastyczności w tym obszarze mózgu. Przyjęto, że powtarzana ekspozycja na pole elektromagnetyczne (EMF) prowadzi do ustalenia nowego punktu nastawczego „set-point" dla aktywności układów oksydacyjno-antyoksydacyjnych i układów stresu, przy czym kierunek i dynamika tych zmian zależą od wartości indukcji pola magnetycznego. W rezultacie EMF może modyfikować reakcję organizmu na kolejne czynniki stresogenne i zmieniać podatność na choroby, zwłaszcza te dotyczące układu nerwowego.

Celem badań było 1) określenie czy EMF wykazuje hormezę, tzn. dwukierunkowe działanie uzależnione od wartości indukcji magnetycznej i 2) wyjaśnienie mechanizmu tego zjawiska na modelu zwierzęcym.

W badaniach zweryfikowano cztery szczegółowe hipotezy robocze:

1. U podłoża dwukierunkowego działania EMF leżą zmiany statusu oksydacyjno-antyoksydacyjnego mózgowia (hipoteza I)
2. EMF inicjuje zmiany w odpowiedzi stresowej, których kierunek i nasilenie zależą od siły pola elektromagnetycznego (hipoteza II)
3. EMF trwale modyfikuje status oksydacyjno-antyoksydacyjny i poziom aktywności układów stresu i tym samym zmienia odpowiedź na kolejne czynniki stresogenne (hipoteza III)
4. Zmiany w odpowiedzi stresowej indukowanej przez EMF modulują plastyczność mózgową (hipoteza IV)

W celu weryfikacji hipotezy o hormetycznym działaniu EMF dorosłe szczury rasy Wistar były poddane powtarzanej ekspozycji na EMF (50 Hz) o dwóch wartościach indukcji magnetycznej (1 lub 7 mT). Ekspozycje (1h ekspozycji dziennie przez 7 kolejnych dni) były powtarzane 3-krotnie w 3-tygodniowych odstępach czasu. Oznaczono poziom markerów stresu oksydacyjnego i antyoksydantów w korze przedczołowej, zmiany poziomu hormonów stresu i ekspresji ich receptorów w strukturach i tkankach należących do osi HPA i układu LC-NA i z nimi powiązanych: oś HPA: podwzgórze, przysadka, nadnercza; układ LC-NA: miejsce sinawe, podwzgórze, i nadnercza; a także w osoczu. W hipokampie oznaczono poziomy receprorów glikokortykoidowych (GR) i mineralokortykoidowych (MR), a także receptorów β2-adrenergicznych (β2-AR), które odgrywają ważną rolę w modulacji plastyczności mózgowej. Ponadto zweryfikowano hormonalne i behawioralne zmiany u zwierząt eksponowanych na EMF w odpowiedzi na kolejny czynnik stresowy – test otwartego pola i test podwyższonego labiryntu krzyżowego. Wszystkie parametry były analizowane po każdej z 3 powtarzanych ekspozycji na EMF w celu oszacowania kierunku i dynamiki zmian ich poziomu.

Przeprowadzone eksperymenty wykazały, że powtarzana ekspozycja na EMF zmienia status oksydacyjno-antyoksydacyjny w korze przedczołowej szczurów w sposób zależny od wartości indukcji magnetycznej EMF oraz liczby ekspozycji. Poziom markerów stresu oksydacyjnego i całkowitego potencjału antyoksydacyjnego (ang. *total antioxidant capacity,* TAC) u szczurów eksponowanych na EMF o indukcji 1 mT nie różnił się znacząco od wartości kontrolnej. Natomiast ekspozycja na EMF o indukcji 7 mT spowodowała wzrost poziomu stresu oksydacyjnego i ograniczenie obrony antyoksydacyjnej.

Zmiany poziomu hormonów osi HPA i układu LC-NA u szczurów eksponowanych na EMF o indukcji 1 mT były maksymalne po pierwszej ekspozycji na EMF i następnie po kolejnych ekspozycjach obserwowano powrót ich poziomu do wartości kontrolnych. Natomiast, stwierdzono kumulację efektów poszczególnych ekspozycji na EMF o wartości indukcji 7 mT, z każdą kolejną ekspozycją zmiana w poziomie badanych parametrów była coraz bardziej widoczna.

Powtarzana ekspozycja na EMF o indukcji 1 mT wywołała umiarkowaną reakcję stresową, która uruchamia mechanizmy kompensacyjne prowadząc do adaptacji do tego

rodzaju stresu. W przeciwieństwie do tego, u zwierząt eksponowanych na EMF o indukcji magnetycznej 7 mT zauważono przesunięcie punktu set-point aktywności układów stresu w kierunku zwiększonej wrażliwości na ten czynnik stresowy. W rezultacie, przesunięcie punktu nastawczego regulacji endokrynologicznej zmieniło odpowiedź na kolejne bodźce stresowe - test otwartego pola i test podwyższonego labiryntu.

Po raz pierwszy wykazano również wzrost ekspresji MR mRNA w neuronach hipokampu w odpowiedzi na EMF o indukcji 1 mT. Zjawisko to może stanowić endogenną reakcję mającą na celu ochronę mózgu przed potencjalnymi uszkodzeniami. W tej grupie zwierząt stwierdzono również nieistotne statystycznie obniżenie mRNA receptora β2-AR w hipokampie. Jednakże w grupie eksponowanej na EMF 7 mT zaobserwowano zdecydowane obniżenie poziomu β2-AR po każdej kolejnej ekspozycji. Kluczowa rola receptora β2-AR w indukowaniu plastyczności mózgowej wskazuje, że ekspozycja na pole elektromagnetyczne o indukcji 7 mT hamuje procesy neuroplastyczne w hipokampie.

Przeprowadzone badania wykazały dwukierunkowy wpływ EMF zależny od wartości indukcji magnetycznej w odniesieniu do statusu oksydacyjnego-antyoksydacyjnego, odpowiedzi na stres, jak również modulowania plastyczności mózgowej. Ponadto stwierdzono, że skutki odziaływania EMF mogą być trwałe i wpływać na reakcję organizmu na kolejne wydarzenia stresowe. Z jednej strony, słabe EMF (1 mT) może pozytywnie wpływać na wzmacnianie plastyczności mózgu, co sprzyja neuroadaptacji do kolejnych czynników stresowych. Z drugiej strony, silne EMF (7 mT) może prowadzić do zakłócenia odpowiedzi na stres, zwiększając wrażliwość na kolejne stresujące bodźce i potencjalnie zwiększać ryzyko zaburzeń związanych ze stresem. Wyniki eksperymentów po raz pierwszy dokumentują istnienie "hormetycznego mechanizmu działania" EMF (50 Hz) u kręgowców, co zostało zweryfikowane na modelu szczura. Wyniki te mogą przyczynić się do lepszego zrozumienia fundamentalnych mechanizmów dwukierunkowej reakcji na EMF, dostarczają nowych danych dotyczących potencjalnych właściwości terapeutycznych pola elektromagnetycznego, jak również otwierają nowe perspektywy w ocenie ryzyka związanego z ekspozycją na EMF.

Streszczenie rozprawy doktorskiej w języku angielskim

The influence of low-frequency electromagnetic field (50 hz) on oxidative status and stress reactions in rats – hormetic effect

Angelika Klimek

The extremely low-frequency electromagnetic field (EMF) is generated by electrical devices and overhead power lines. The increasing number of anthropogenic sources of EMF prompts scientists to consider its impact on living organisms, including the functioning of the nervous system. For urbanized societies, the identification of potential risks associated with EMF and establishing safe exposure limits is particularly important. The effects of EMF are ambiguous (positive or negative), but certainly not neutral, and the analysis of available literature does not provide answers to questions regarding all potential health effects of exposure to this environmental factor. The current state of knowledge allows for the hypothesis that EMF exhibits hormesis, i.e., a two-way action dependent on the value of magnetic induction. EMF induces many modifications in intracellular processes, the consequences of which may significantly disrupt the functions of the organism. Special attention is paid to the EMF-induced changes in the oxidative-antioxidative balance. Moreover, many studies indicate that EMF is a stress factor that triggers cellular/organismal reactions characteristic of general stress responses.

It is assumed that the proposed hormetic effect (bidirectional) of EMF action is associated with different levels of oxidative stress and changes in the activation of stress systems, such as the hypothalamic-pituitary-adrenal (HPA) axis and the noradrenergic system (LC-NA). Stress hormones, mainly corticosterone and noradrenaline, can modulate hippocampal function and influence plasticity processes in this brain region. It is hypothesized that repeated exposure to electromagnetic fields (EMF) leads to the establishment of a new set-point for the activity of oxidative-antioxidative processes and stress systems, with the direction and dynamics of these changes depending on the value of magnetic field induction. As a result, EMF may modify the organism's response to subsequent stressors and alter susceptibility to diseases, especially those related to the nervous system.

The aim of the research was 1) to determine whether EMF exhibits hormesis, i.e., a bidirectional effect dependent on the value of magnetic induction, and 2) to elucidate the mechanism of this phenomenon using an animal model.

The research verified four detailed working hypotheses:

1. The bidirectional action of the EMF is based on changes in oxidative-antioxidative status in the brain (Hypothesis I).

2. EMF initiates changes in the stress response, the direction and intensity of which depend on the magnetic induction of the field (Hypothesis II).
3. EMF permanently modifies the activity level of stress systems, thereby altering the stress response to subsequent stressors (Hypothesis III).
4. Changes in the stress response induced by EMF modulate brain plasticity (Hypothesis IV).

To verify the hypothesis of the hormetic action of EMF, adult Wistar rats were subjected to repeated exposure to EMF (50 Hz) of two values of magnetic induction (1 or 7 mT). Exposures (1 hour per day for 7 consecutive days) were repeated three times at 3-week intervals. Levels of oxidative stress markers and antioxidants in the prefrontal cortex were determined, as well as changes in the levels of stress hormones and the expression of their receptors in structures and tissues belonging or related to the HPA axis and the LC-NA system: HPA axis: hypothalamus, pituitary gland, adrenal glands; LC-NA system: locus coeruleus, hypothalamus, and adrenal glands; as well as in serum. In the hippocampus, levels of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) were measured, along with β2-adrenergic receptors (β2-AR), which play an important role in modulating brain plasticity. Additionally, hormonal and behavioural changes in animals exposed to EMF in response to a subsequent stressor were verified using the open field test and elevated plus maze test. All parameters were analyzed after each of the 3 repeated exposures to EMF to assess the direction and dynamics of changes in their levels.

The conducted experiments revealed that repeated exposure to EMF alters the oxidative-antioxidant status in the prefrontal cortex of rats in a manner dependent on the magnetic induction value of EMF and the number of exposures. The level of oxidative stress markers and total antioxidant capacity (TAC) in rats exposed to EMF of 1 mT did not significantly differ from the control values. However, exposure to EMF of 7 mT resulted in an increase in oxidative stress levels and a limitation of antioxidant defence.

Changes in the levels of hormones of the HPA axis and LC-NA system in rats exposed to EMF of 1 mT were maximal after the first exposure, with subsequent exposures leading to a return to baseline levels. Conversely, a cumulative effect of individual exposures to EMF of 7 mT was observed, with each successive exposure resulting in increasingly visible changes in the levels of the verified parameters.

Repeated exposure to EMF of 1 mT induced a moderate stress response, activating compensatory mechanisms leading to adaptation to this type of stress. In contrast, in animals exposed to EMF of 7 mT, a shift in the set-point of stress system activity towards increased sensitivity to this stressor was found. Consequently, the shift in the set-point of endocrinological regulation altered the response to subsequent stressors - the open field test and elevated plus maze test.

For the first time, an increase in MR mRNA expression in hippocampal neurons in response to EMF of 1 mT has been demonstrated. This phenomenon may represent an endogenous response aimed at protecting the brain from potential damage. Conversely, a statistically significant decrease in β2-AR receptor mRNA levels in the hippocampus was observed in the group exposed to EMF of 7 mT after each successive exposure. The crucial role of β2-AR receptors in inducing brain plasticity suggests that exposure to EMF at 7 mT strongly inhibits neuroplastic processes in the hippocampus.

The performed studies demonstrated a bidirectional influence of EMF dependent on the magnetic induction value regarding the oxidative-antioxidant status, stress response, as well as modulation of brain plasticity. Additionally, it was found that the effects of EMF exposure may be long-lasting and influence the organism's response to subsequent stressful events. On one hand, weak EMF (1 mT) may positively impact brain plasticity reinforcement, facilitating neuroadaptation to subsequent stressors. On the other hand, strong EMF (7 mT) may disrupt stress response and in this way increase the sensitivity to subsequent stressful stimuli and potentially increase the risk of stress-related disorders. The results of the experiments for the first time document the existence of the "hormetic mechanism of action" of EMF (50 Hz) in vertebrates, which has been verified in the rat model. These findings may contribute to a better understanding of the fundamental mechanisms of bidirectional response to EMF, provide new data on the potential therapeutic properties of electromagnetic fields, and open new perspectives in the assessment of the risk associated with EMF exposure.

Wkład w autorstwo publikacji

Oświadczam, że mój udział w realizacji pracy 1:

Klimek A, Rogalska J. Extremely Low-Frequency Magnetic Field as a Stress Factor—Really Detrimental?—Insight into Literature from the Last Decade. Brain Sci. 2021, Vol. 11, No 2, p. 174. doi: 10.3390/brainsci11020174 polegał na analizie literatury, przygotowaniu manuskryptu, przygotowaniu wizualizacji danych oraz abstraktu graficznego.

Oświadczam, że mój udział w realizacji pracy 2:

Klimek A, Nowakowska A, Kletkiewicz H, Wyszkowska J, Maliszewska J, Jankowska M, Peplowski L, Rogalska J. Bidirectional Effect of Repeated Exposure to Extremely Low-Frequency Electromagnetic Field (50 Hz) of 1 and 7 mT on Oxidative/Antioxidative Status in Rat's Brain: The Prediction for the Vulnerability to Diseases. Oxid Med Cell Longev. 2022, Vol. 14, No 2022, p. 1031211. doi: 10.1155/2022/1031211 polegał na udziale w przygotowaniu protokołów eksperymentalnych, wykonaniu części eksperymentów, analizie rezultatów, przygotowaniu wizualizacji danych oraz przygotowaniu manuskryptu.

Oświadczam, że mój udział w realizacji pracy 3:

Klimek A, Kletkiewicz H, Siejka A, Wyszkowska J, Maliszewska J, Klimiuk M, Jankowska M, Seckl J, Rogalska J. New View on the Impact of the Low-Frequency Electromagnetic Field (50 Hz) on Stress Responses: Hormesis Effect. Neuroendocrinology. 2023, Vol. 113, No 4, p. 423-441. doi: 10.1159/000527878 polegał na udziale w przygotowaniu protokołów eksperymentalnych, przeprowadzeniu doświadczeń, analizie otrzymanych wyników, przygotowaniu manuskryptu oraz przygotowaniu wizualizacji danych.

Oświadczam, że mój udział w realizacji pracy 4:

Klimek A, Kletkiewicz H, Siejka A , Wyszkowska J, Maliszewska J, Klimiuk M, Jankowska M, Rogalska J. The electromagnetic field (50 Hz) can establish a new “set-point” for the activity of the locus coeruleus–noradrenergic (LC-NA) system in rat polegał na polegał na udziale w przygotowaniu protokołów eksperymentalnych, wykonaniu części eksperymentów, analizie rezultatów, przygotowaniu wizualizacji danych oraz przygotowaniu manuskryptu.