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Effects of electromagnetic fields on neuronal ion channels: a systematic review

Federico Bertagna,[1,2] Rebecca Lewis,[1,2] S. Ravi P. Silva,[1,3] Johnjoe McFadden,[1,4] and Kamalan Jeevaratnam[1,2]

[1]Leverhulme Quantum Biology Doctoral Training Centre, University of Surrey, Guildford, Surrey, UK. [2]School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK. [3]Advanced Technology Institute, University of Surrey, Guildford, Surrey, UK. [4]School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK

Address for correspondence: Kamalan Jeevaratnam, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Daphne Jackson Road, Guildford, Surrey GU2 7AL, UK. drkamalanjeeva@gmail.com

Many aspects of chemistry and biology are mediated by electromagnetic field (EMF) interactions. The central nervous system (CNS) is particularly sensitive to EMF stimuli. Studies have explored the direct effect of different EMFs on the electrical properties of neurons in the last two decades, particularly focusing on the role of voltage-gated ion channels (VGCs). This work aims to systematically review published evidence in the last two decades detailing the effects of EMFs on neuronal ion channels as per the PRISM guidelines. Following a predetermined exclusion and inclusion criteria, 22 papers were included after searches on three online databases. Changes in calcium homeostasis, attributable to the voltage-gated calcium channels, were found to be the most commonly reported result of EMF exposure. EMF effects on the neuronal landscape appear to be diverse and greatly dependent on parameters, such as the field's frequency, exposure time, and intrinsic properties of the irradiated tissue, such as the expression of VGCs. Here, we systematically clarify how neuronal ion channels are particularly affected and differentially modulated by EMFs at multiple levels, such as gating dynamics, ion conductance, concentration in the membrane, and gene and protein expression. Ion channels represent a major transducer for EMF-related effects on the CNS.

Keywords: brain; electromagnetic fields; electrophysiology; ion channels

Introduction

As the use of electromagnetic devices continues to grow, we have had to consider ways in which such devices may impact our health. There has been a dramatic increase in exposure to different types of electromagnetic fields (EMFs), ranging from extremely low-frequency electromagnetic fields (ELF-EMFs) up to 300 Hz and radiofrequency electromagnetic fields (RF-EMFs), ranging from 10 MHz to 300 GHz, and, more recently, devices that operate at terahertz (0.3–3 THz) frequencies, but also 0 frequency fields, such as static magnetic fields (SMFs). These frequencies are mainly derived from man-made sources, such as electricity power supplies, power amplifier circuits, voltage-controlled oscillators, cellular telephone antennas, and smartphones.[1,2] Exposure resulting from these sources is several orders of magnitude greater than those from natural sources, so not surprisingly, along with the increased usage of a wide range of electronic devices producing a manifold of EMF types, concern about their possible detrimental effect has been raised. Since the 1980s, various studies have investigated the link between different frequency EMFs and the risk of developing chronic diseases, such as cancer, along with cardiovascular and neurodegenerative diseases.[1,3,4] Epidemiological studies reported a significant association between exposure to ELF-EMF and

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the development of childhood leukemia,[5,6] and in 2011, WHO's International Agency for Research on Cancer highlighted the possible carcinogenic effect of RF-EMFs.[7] However, EMFs have also been extensively used in the treatment of many forms of neoplasia[8] and neurodegenerative diseases,[9] highlighting a possible beneficial use of these fields as therapeutic treatments for diverse chronic diseases. The effects on the central nervous system (CNS) appear to be particularly relevant, as the CNS is reliant on many voltage-dependent processes.[10]

EMFs are wave-like propagating fields exerting forces on every charged object within their vicinity. They consist of two different but inextricable field components (electric and magnetic) running perpendicularly to each other and continuously inducing each other by varying in time, as described by Maxwell's equations. The electric component is the stronger, but its interaction is attenuated by endogenous charges, so its magnitude is dependent on the dielectric constant of the tissue it crosses and falls of according to an inverse square law; whereas the weaker magnetic component, despite its reduced strength, is not attenuated by charge but falls off according to an inverse cube law. Moreover, despite their differences in force, the energies associated with the electric and magnetic components within an EM wave are equal. These characteristics lead to the need for considering them as both equally relevant in the study of their effects on living systems.

Typically, we are continuously exposed to natural EMF radiation, such as every form of light radiation, cosmic microwaves, or the magnetic field generated by the planet. However, two key points must be considered when comparing natural exposure to that of artificial exposure. First, the magnitude of the two exposures is very different, with the artificial one being higher by a few to several orders of magnitude in comparison with natural fields.[1] Nevertheless, in the delicately balanced system of the CNS of higher animals, even a small increase in intensity could generate significant effects.[10] Second, many types of common man-made EMFs, in contrast to natural ones (produced by a huge number of atomic or molecular transitions that are randomly oriented between them), are polarized, meaning that they oscillate in a specific and determined plane called the "polarization plane."[11] This peculiar characteristic gives artificial EMF an additional electrostatic force deriving from the synchronization of every type of charged/polar molecule with that polarization plane (so in phase with the field).

Furthermore, the well-organized orientation of these electromagnetic waves permits the generation of constructive interference effects by polarized fields that can amplify their intensity locally, increasing the subsequent biological activity.[11] It is for these reasons that this paper focuses on the effect of artificial EMFs.

Different types of EMFs have been shown to have a major impact on CNS physiology, but recent research has primarily been focused on the role of low-frequency EMFs. These forms of electromagnetic radiation are not ionizing because of the low energy contained in their quanta, in contrast to other higher frequency fields. Therefore, they do not have enough energy to remove electrons from atom and molecules,[12] but they can produce thermal effects and induce in the human body circulating currents that, if sufficiently large, could cause stimulation of nerves and muscles or affect other biological processes in many tissues.[13] ELF-EMFs were recently reported to have significant effects on synaptic plasticity of both adult[14] and newborn[15] rats, where they have been reported to modulate the development of long-term potentiation (LTP) in the hippocampus and neocortex. However, the specific impact of ELF-EMFs on LTP is controversial, as this phenomenon is both reported to be increased[14] or decreased[15,16] by this type of EMF. In addition, ELF-EMFs are known to modulate the cell cycle in several cell lines.[17] RF-EMF exposure has been linked to developmental abnormalities in specific areas of the brain[18] and to decreases in specific types of neurons,[19] and has been shown to activate the autophagic pathway.[20] Despite this, the potential beneficial impact of EMFs on CNS physiology has been extensively investigated. ELF-EMFs have been reported to increase neurogenesis both in isolated neuronal stem cells (NSCs)[21] and mouse hippocampus,[22] in addition to ameliorating the remyelination process through enhanced proliferation of NSCs.[23] On the other hand, RF-EMFs have been used in the treatment of chronic pain,[24] and studies have suggested a novel therapeutic use of low-intensity RF-EMFs in the treatment of severe brain cancer,[8] such as in recurrent glioblastoma patients, where this approach aims to replace the classical chemotherapy paradigm.[25] Both ELF-EMFs[26,27] and RF-EMFs[28,29] have been shown to

interfere with the physiology and functional activity of neurons. The effects exerted by EMF exposure appear to be dependent on the developmental stage of the exposed neurons. Indeed, one of the biggest EMF-related concerns that the scientific community is facing is the impact of the electromagnetic radiation on neural development, particularly as several reports have described severe effects on neural development,[18,30] although other studies have failed to observe significant effects.[31] One common explanation for this is the low thickness of a child's skull,[32] but the higher severity could be due to the higher density of stem cells present in the first stages of development.[33,34] Indeed, many studies have reported significant effects on embryonic stem cells.[30,35,36] This fact is particularly notable since a good correlation exists between the neonatal development of the CNS and late brain development in humans.[37]

Nonetheless, the electromagnetic force produced by EMFs is likely to influence neuronal activities through the interaction with charged cellular components that are particularly sensitive to changes in their charge, such as voltage-gated ion channels (VGCs).

Specifically, both acute and chronic ELF-EMF exposures have been reported to increase the ion transport rate of many types of VGCs, such as voltage-gated sodium channels (VGSCs),[38] high-threshold calcium channels, and calcium-activated potassium (KCa) channels,[39] maybe directly acting on their voltage-sensing domain (VSD) and pore-forming domain, and to trigger an increase in the expression of the VGCs themselves[40] that could be caused by the same change in ion channel conduction and permeability, leading to an altered ionic equilibrium within the cell. Chronic exposure to RF-EMFs, on the contrary, has been found to cause a decrease in pan calcium channel gene and protein expression in mouse hippocampus and hypothalamus[41,42] and alter the afterhyperpolarization amplitude and spike frequency of rat Purkinje cerebellar neurons.[43] However, acute exposure to this type of field apparently fails to elicit similar effects,[39,44] suggesting a different mechanism of action with respect to ELF-EMF exposure. ELF-EMFs and RF-EMFs are not the only types of fields reported to have effects on neurons. SMFs that do not change their intensity and direction in time have been shown to deeply impact the physiological properties of several types of neurons, through their interaction with VGCs.[45–47] Additionally, studies have also shown some frequencies to be more biologically active than others[48] and have reported diverse biological effects for different frequencies of EMFs.[39,48,49] This difference has been linked to microthermal effects elicited by RF-EMFs (and not by ELF-EMFs),[50] although numerous studies reported nonthermal effects related to RF-EMF exposure that seem to be strictly related to modifications of the calcium signaling pathway.[41,42,51] The mechanism underlying this diversity of effects, however, has not been completely unraveled and is still a matter of debate.

The molecular mechanisms underlying the effects of EMF exposure are less well known. A role for calcium is, however, well established. Exposure to both acute and chronic ELF-EMFs promotes calcium influx, resulting in an increased intracellular calcium concentration in various types of neurons[39,40,52] that is conversely decreased following chronic exposure to RF-EMFs.[41,42,53] The involvement of calcium is remarkable because of the many related calcium signaling pathways involved in various essential neurophysiological processes, such as neural differentiation, survival, and apoptosis[54,55] that could ultimately explain the many effects of EMF exposure on learning and memory.[56] In areas of the brain involved in the modulation of these cognitive tasks, such as the hippocampus and prefrontal cortex, exposure to ELF-EMFs resulted in abnormal calcium signaling that led to a decrease in the binding between the *N*-methyl-D-aspartate receptor (NMDAR) and its ligand glutamate, which could directly result in important effects on synaptic plasticity.[57,58]

However, the effects of EMFs reported in the literature are often conflicting. Recent studies report no increase in intracellular calcium or production of reactive oxygen species (ROS)[59] subsequent to both acute and subchronic exposure to ELF-EMFs,[31,44,59] and these disagreements also remain when considering studies focused on the same model, such as in the case of PC12 cells.[60] Also, the impact of EMFs on the CNS has been questioned, with studies reporting (in different models) no effects on the expression of synaptic receptors as NMDAR,[61] no relation between EMF exposure and brain electrical activity,[62,63] and no neurotoxic effect subsequent to EMF exposure in pre- and postnatal

development.[64] However, the experimental setups are often different in many key parameters, such as the time, intensity, and type of exposure, making results difficult to compare so that there remains no consensus on the effects of EMF exposure on ion channels within the CNS. The principal aim of this review is, therefore, to provide a systematic analysis of EMF effects on neuronal ion channels.

Review methodology

Research question

Using the PICOT structure (Population, Intervention, Comparator, Outcomes, and Time), the following research question was formulated: "Are EMFs capable of influencing ion channel conductance and expression in the central nervous system?" We then followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines to perform this systematic review.[65]

Search strategy

A systematic review of the literature was carried out in April 2020 by submitting the chosen combination of keywords into three different databases: PubMed, Scopus, and Web of Science. The agreed combination with which the search was performed was "ion channel*" AND "electromagnetic field*" OR "EMF" AND "neuron." A time filter was applied to the search to isolate solely the works published in the last 15 years (2005–2020).

Search eligibility criteria

The papers resulting from the above searches were screened for the presence of duplicates and narrowed down further using the predefined inclusion and exclusion criteria based on the journal title and abstract. The chosen exclusion criteria were (1) the paper is not in English, (2) the paper is not an original research paper (i.e., it is a review article, editorial, or book chapter), or (3) the paper is not freely available (using the institutional credentials of the University of Surrey).

This resulting list was then subject to an inclusion round in which we considered only original laboratory research studies, conducted on neurons, neuron-like cells, or neural tissue as a model, that met our original research questions. Thus, all the papers that did not discuss in the title or abstract the effects of EMFs on ion channel conductance or expression were excluded from the study. In summary, the inclusion criteria were (1) the paper covers original laboratory research; (2) the model of the study is neurons, neuron-like cells, or neural tissue; and (3) the paper is relevant based on its title and abstract.

Article selection and processing

The final list obtained by this iterative selection process was independently reviewed by three different investigators. The full text was obtained for each of the included articles. Where an article was not readily available, the author was personally contacted, and the manuscript of the relevant study was obtained. No papers were excluded in this step.

Quality appraisal

The Checklist of Review Criteria published by the Task Force of Academic Medicine and GEA-RIME committee[66] was used as a further inclusion instrument to highlight the quality and completeness of the papers resulting from research. This screened publications for their quality by using a set of 13 predetermined criteria. We considered papers that satisfied at least 12 out of the 13 criteria.

Data extraction

In order to facilitate the analysis and to better compare key features of the studies, a custom data extraction table was designed and used to extract relevant information. The features evaluated were: material used (cell type and eventual modifications, brain regions, and animal provenance); EMF type and exposure time; EMF generation system (type of exposure system and specifics); delivery mode; techniques used to study ion channels; type of ion channel studied; key results and discussion; and proposed mechanism of EMF effect on the ion channel.

Results

Search results

The initial search yielded 157 titles from PubMed, 38 titles from Scopus, and 19 titles from Web of Science, for a total of 214 (Fig. 1). Removal of duplicates reduced this number to 175, and the subsequent exclusion and inclusion rounds led to the final number of 24 articles.

Following quality appraisal (Table 1), there were 21 publications deemed of suitable quality for inclusion in this review.

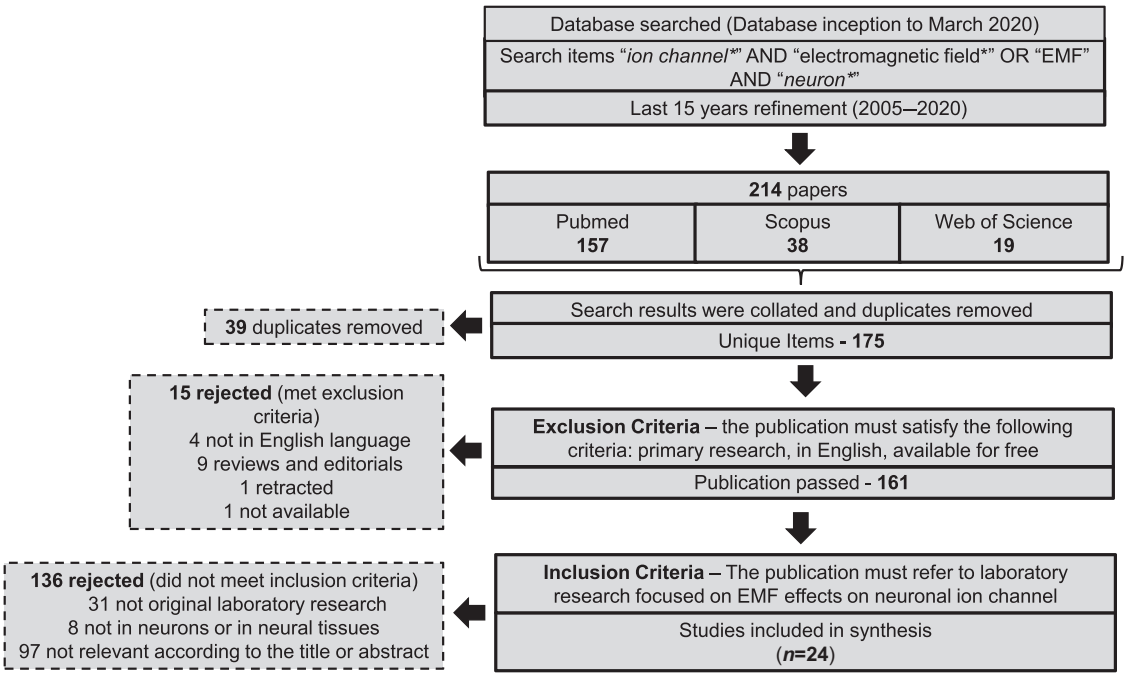

Figure 1. Publication attrition process depicted in a step-by-step flowchart that details the method by which the final set of reviewed publications was produced. PubMed, Web of Science, and Scopus publications were searched using a selected combination of keywords to gather publications relating to EMF effects on neuronal ion channels. After removing duplicate items, exclusion and inclusion criteria were applied to select primary research publications specifically relating to the review question.

Types of EMFs studied

Central to any discussion related to EMFs is the definition of particular parameters, such as the frequency and intensity of these fields, as they are directly correlated with the different effects that such fields have on cells. Accurately defining the type of field delivered and its exposure time is, therefore, necessary to allow any reasonable comparison. To mimic the effects of the principal human-generated sources of EMFs, relative EMF exposures have been used. The most commonly studied fields (57% of total papers) were ELF-EMFs at 50 Hz, delivered with various magnetic intensities, among which the most used did not exceed 1 mT (33% of total cases). ELF-EMFs were preferentially delivered following an acute or subchronic ($\leq$48 h) delivery (75% of cases). This trend was inverted in the case of RF-EMF exposure (33% of total), where 57% of studies used chronic exposure to simulate the daily exposure to fields derived from mobile phone antennas.

Experimental platforms

Many different systems have been used to generate the EMFs, ranging from conventional EMF generators to custom-made devices arranged to fit particular experimental queries. Helmholtz coils, consisting of two circular electromagnets placed along a common axis, were the preferred generator for ELF-EMFs (67% of papers), while the Wave Exposer V20 custom device (extensively described in Ref. 53) was the most common generator for RF-EMFs (43%), maybe by virtue of the multiple studies published by the same team. Other highly represented RF-EMF generators were the Coplanar Waveguide (CPW) (28.5%), consisting of a single conducting track printed onto a dielectric substrate and arranged to be in the middle of a pair of return conductors, and mobile phones (28.5%). All EMF types and exposure systems used are summarized in Figure 2 and more extensively reported in Table 2.

Choice of biological system

The majority of the studies (95%) chose to focus on rodent-derived cell lines or tissues, among which

Table 1. Summary of the quality appraisal of the 24 papers

Publication	Problem statement, conceptual framework, and research question	Reference to the literature and documentation	Relevance	Research design	Instru-mentation, data collection, and quality control	Population and sample	Data analysis and statistics	Reporting of statistical analyses	Present-ation of results	Discussion and conclusion; interpretation	Title, authors, and abstract	Presentation and docu-mentation	Scientific conduct	Total criteria met
Akiyama *et al.*[95]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Calabrò and Magazù[71]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
de Groot *et al.*[59]	√	√	√	√	√	√	√	√	√	—	√	√	√	12
de Groot *et al.*[31]	√	√	√	√	√	√	√	√	√	—	√	√	√	12
Duan *et al.*[58]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Foletti *et al.*[105]	√	√	—	√	√	√	√	√	√	—	√	√	√	11
Haghani *et al.*[43]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
He *et al.*[38]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Kim *et al.*[41]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Kim *et al.*[42]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Ledda *et al.*[106]	√	√	—	√	√	√	√	√	√	—	√	√	√	11
Lisi *et al.*[88]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Liu *et al.*[89]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Luo *et al.*[90]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Marchionni *et al.*[39]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Maskey *et al.*[53]	√	√	√	√	√	√	√	√	√	—	√	√	√	12
Morabito *et al.*[60]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Platano *et al.*[44]	√	√	√	√	√	√	√	√	√	—	√	√	√	12
Prina-Mello *et al.*[47]	√	√	√	√	—	√	√	√	√	√	√	√	√	12
Prucha *et al.*[109]	√	√	√	√	—	√	√	√	√	√	√	√	√	12
Shen *et al.*[46]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Sun *et al.*[40]	√	√	√	√	√	√	√	√	√	√	√	√	√	13
Vernier *et al.*[110]	√	√	—	√	√	√	√	√	√	—	√	√	√	11
Yin *et al.*[52]	√	√	√	√	√	√	√	√	√	√	√	√	√	13

Note: A total of 13 criteria were assessed, based on the quality checklist formulated by the *Checklist of Review Criteria* established by the Task Force of Academic Medicine and the GEA-RIME Committee.[66] Twenty-two out of the 24 papers met at least 12 out of the 13 criteria and were included in the study.

the rat (*R. norvegicus*) represented the favored source. However, the present study revealed great variability in the specific type of rodent cell lines. Sixty-seven percent of the studies used cell culture as a model, 71% of which had been isolated from neural tissue (among which 30% were from the cortex, 20% were from the cerebellum and dorsal ganglion, and 10% were from the hippocampus, trigeminal nerve, and entorhinal cortex), while 29% used cancer cell lines. The lines used in this latter case were PC12 cells (derived from rat pheochromocytoma), NG108-15 (a hybrid between mouse neuroblastoma and rat glioma), SH-SY5Y (derived from human neuroblastoma), and F11 (a hybrid between mouse neuroblastoma and rat dorsal root ganglion neurons). A single study used the AtT20 D16V cell line, derived from the pituitary gland. Notably, brain-derived slices have been used only in 19% of the papers. Among the different regions of the CNS studied, the hippocampus was used the most (24% of the articles), followed by the cortex and cerebellum (14% each). A small proportion of studies derived their cells and tissues from the hypothalamus, trapezoid body, entorhinal cortex, pituitary gland, trigeminal nerve, and spinal cord. These results are summarized in Table 2.

Ion channels studied

Seventy-six percent of articles focused on different types of calcium channels (P/Q, N, R, but mainly L subtypes), with 24% reporting effects on VGSCs and only 19% studying the role of potassium channels (A-type K^+, delayed rectifier K^+, M-type K^+, KCa, fast-inactivating transient (IK, A), and dominant-sustained (IK,V) channels). Some studies also investigated the role of GABAA, HCN, TRPA1, and TRPV1 channels in the modulation of EMF effects on the cell. (Note that the studies reviewed here do not always explore only one ion channel, thus the percentages reported cannot always be summed to 100%.)

Experimental techniques

Forty-eight percent of total papers used the patch clamp technique to assess the effects of EMF exposure on ion channels. In particular, the whole-cell recording configuration appeared to be the most frequently used (90%), with only one study using the single channel recording configuration (10%), maybe due to the technical limitation of this configuration despite its great relevance for the study of single ion channel conductance. In 60% of cases, researchers added various channel inhibitors and agonists to better discriminate between different

Table 2. The data extraction table for the 22 studies analyzed in this review

Publication	Tissue/ cell type	EMF type and exposure time	Exposure system	Delivery mode	Techniques used to study ion channel	Ion channel studied	Results and discussion	Proposed mechanism of action
Akiyama *et al.*[95]	Rat hippocampal slices and rat CA1 pyramidal neurons	High-intensity DC fields (±40 mV mm^{-1})	A square-wave pulse generated by an electrical stimulator was converted to constant current through a custom-made stimulus isolation unit	The current was applied to hippocampal slices via two parallel Ag–AgCl electrodes placed on a submerged-type recording chamber	Voltage-sensitive dye imaging and whole-cell patch clamp recordings, using various channel blockers. The drugs used were 1 μM TTX, 5 mM 4-aminopyridine (4-AP), 20 mM tetraethylammonium (TEA), 5 mM CsCl, 2 mM $BaCl_2$, 20 μM ZD7288, 20 μM XE991, and 10 mM bicuculline-methiodide	Voltage-dependent Na^+, GABAA, A-type K^+, delayed rectifier K^+, M-type K^+, HCN channels	DC electrical fields induce membrane polarization in CA1 pyramidal neurons that is not spatially and temporally uniform along the cell. These nonuniformities in the membrane potential response to DC electric fields are possibly implicated in the unique information processing of CA1 pyramidal neurons, such as frequency preferences and slow temporal integration in the soma. Voltage-activated conductance, including HCN channel–mediated conductance, had only minor contributions to both the monophasic and biphasic characteristic current profiles induced by the exposure to DC fields	The presence of the leaky distal dendrites increases the sensitivity of the somatic membrane potential in response to the extracellular electric fields. Such amplification of somatic polarization could physiologically enable weak extracellular electric fields to control the probability of firing and thus to orchestrate the ensemble firing of neuronal population *in vivo*
Calabrò and Magazù[71]	Human-derived neuroblastoma cell line SH-SY5Y	ELF-EMFs and RF-EMFs at different frequencies (0, 50 Hz, and 900 MHz) and same intensity (2 mT); subchronic (6 h) exposure	Two Helmholtz coils driven by a power amplifier in current mode and an arbitrary function generator. Microwave sources at frequencies ranging up to 900 MHz were obtained using mobile phone devices	Cells grown in 25-cm^2 culture flasks were placed in the center of the field generated between the coils. The coils and cell samples were placed into an incubator	Fourier transform infrared (FTIR) spectroscopy	Different ion channels (effect studied on α-helices)	A significant increase in the intensity of the amide I band was observed after exposures to 2 mT electromagnetic fields at 0, 50 Hz, and 900 MHz, with $P < 0.05$. Interestingly, the integrated area of the amide I band increased significantly ($P < 0.05$) with the increase in the frequency of the applied electromagnetic field, demonstrating that the displacement of protein α-helices is closely dependent on the frequency of the applied electromagnetic field	α-Helices had aligned with the applied electromagnetic fields, showing that the exposure of proteins in bidistilled water solution to an applied electromagnetic field causes a torque which induces the alignment of protein α-helices toward the direction of the applied field. As a result, an enlargement in the diameter of the cellular membrane channel should occur, inducing a decrease in the resistance of the channel and a consequent increase in ion flux, changing the delicate equilibrium of cellular functions
de Groot *et al.*[59]	Naive and chemically stressed rat pheochromocytoma (PC12) cells	50-Hz ELF-EMF up to 1000 μT rms; acute (30 min) and subchronic (48 h) exposure	ELF-EMFs were generated using two custom-made devices: a copper coil fitted to the stage of the fluorescence microscope for acute exposure during Ca^{2+} imaging and a copper coil fitted into an incubator for subchronic exposure. Both exposure devices consist of double copper-wired solenoid coils connected to a signal generator with preprogrammed exposure protocols	For acute exposure experiments, a subset of cells was placed in the switched-off microscope-fitted coil for sham exposure (approximately 0.2 μT, i.e., background ELF-EMFs from the fluorescence microscope set-up). For subchronic exposure experiments, a subset of cells was placed in a second incubator and received sham exposure (approximately 0.4 μT, i.e., incubator-generated background ELF-EMFs), while cells in the incubator-fitted coils in the exposure incubator received subchronic exposure	Intracellular Ca^{2+} imaging using the Ca^{2+}-sensitive fluorescent ratio dye Fura-2 AM	Ca^{2+} homeostasis (Ca^{2+} channels, but maybe other mechanisms)	Acute exposure of naive PC12 cells to 50-Hz ELF-EMF up to 1000 mT fails to affect basal or depolarization-evoked $[Ca^{2+}]_i$. Subchronic ELF-EMF exposure up to 1000 mT has no consistent effects on Ca^{2+} homeostasis in naive PC12 cells and does not affect ROS production and membrane integrity. In chemically stressed PC12 cells, both acute and subchronic ELF-EMF exposure also failed to exert consistent effects on Ca^{2+} homeostasis, ROS production, and membrane integrity. Exposure to 50-Hz ELF-EMF up to 1000 mT does not induce neurotoxic effects *in vitro*, either in naive or in chemically stressed PC12 cells	N/A

Continued

Table 2. ***(Continued)***

Publication	Tissue/ cell type	EMF type and exposure time	Exposure system	Delivery mode	Techniques used to study ion channel	Ion channel studied	Results and discussion	Proposed mechanism of action
de Groot *et al.*[31]	Primary rat cortical cultures	50-Hz ELF-EMF up to 1000 μT; 7-day developmental exposure	EMFs were generated using a custom-made device consisting of double copper-wired solenoid coils fitted into an incubator and connected to a signal generator with preprogramed exposure protocols	A subset of cells was placed in a second incubator fitted with the same double copper-wired solenoid coils connected to a signal generator and received 7-day sham exposure (0.4 μT, i.e., incubator-generated background ELF-EMFs)	Intracellular Ca^{2+} imaging using the Ca^{2+}-sensitive fluorescent ratio dye Fura- 2AM; MEA measurements	Ca^{2+} homeostasis (Ca^{2+} channels, but maybe other mechanisms)	Basal $[Ca^{2+}]_i$ was not affected by chronic block-pulsed ELF-EMFs up to 1000 μT. Only at 1000 μT, basal $[Ca^{2+}]_i$ is slightly decreased. Chronic ELF-EMF exposure has only limited (developmental) neurotoxic potential *in vitro*. The heterogeneity of such primary cultures also results in large biological variation, in particular when studying highly integrated endpoints, such as calcium homeostasis and neuronal activity. It is, therefore, possible that small ELF-EMF–induced effects remained undetected in this study	N/A
Duan *et al.*[58]	Mouse hippocampus	50-Hz ELF-EMF, 8 mT; chronic exposure (28 days, 4 h per day)	Helmholtz coils were connected to a 250 V adjustable transformer and were fixed on a ventilated Perspex box. Two Helmholtz coils, vertical to the ground, were connected in parallel to a 220 V AC power supply via the adjustable transformer. The AC current (50 Hz) was passed through a pair of coils in the same direction, producing an EMF of 0−15 mT at the center of the coils	An EMF-generating device was placed in a room at a controlled temperature. Mice were placed in a well-ventilated Perspex box and were placed in the middle of a pair of coils, which was not in contact with the coils. A nonmagnetic support was used to place the ventilated Perspex box	Intracellular calcium imaging using the Ca^{2+}-sensitive fluorescent ratio dye Fluo-3 AM	Ca^{2+} homeostasis (Ca^{2+} channels, but maybe other mechanisms)	ELF-EMF exposure increased glutamate and GABA release and excessively activated NMDA receptors, increasing the number of NMDA receptor 2B (NR2B) molecules and $[Ca^{2+}]_i$ in the hippocampus. LSPC treatment did significantly prevent the rise of $[Ca^{2+}]_i$ in mice hippocampus in ELF-EMF + LSPC60 and ELF-EMF + LSPC90 groups ($P < 0.05$ and $P < 0.01$, respectively)	ELF-EMF exposure can cause massive release of glutamate and cause the excessive activation of NMDA receptors. Overactivated NMDA receptors can increase the expression of NR2B and $[Ca^{2+}]_i$. The high $[Ca^{2+}]_i$ signal can be transferred to the nucleus by MAPK, causing oxidative damage of the hippocampus and changing the expression of downstream CREB signaling, further damaging the learning and memory of mice
Haghani *et al.*[43]	Rat Purkinje cerebellar neurons	900-MHz pulse EMF; 6 h per day for the entire gestation period	To generate the EMF, a cell phone (K750 Sony Ericsson) was used as the source of EMF radiation. The cell phone was operated in test mode and was powered through a stabilizer power supply so that the antenna power supply as well as the field intensity was constant	A cell phone and Plexiglas cage (containing animals) was placed inside a Faraday cage. For maximum radiation, the Plexiglas cage was placed 40 cm away from the EMF source	Whole-cell patch clamp recordings in current clamp mode. Purkinje neurons were visualized with a 60× water immersion objective using Nomarski-type differential interference contrast (DIC) imaging with infrared illumination	General investigation on the electrical properties of the cell (action potentials evoked, repetitive firing properties, and first spike latency)	Prenatal EMF exposure results in altered electrophysiological properties of Purkinje neurons. The most prominent changes included afterhyperpolarization amplitude, spike frequency, half width, and first spike latency. However, these changes may not be severe enough to alter the cerebellum-dependent functional tasks	Increases in action potential half-width in EMF-exposed pups may be a result of high Ca^{2+} entry into Purkinje neurons. It is possible that prenatal EMF exposure raised intracellular Ca^{2+} concentrations, which caused an increase in K^+ conductance by Ca^{2+}-dependent K^+ channels, which, in turn, leads to the increase in size of AHP and decreased neuronal excitability observed in EMF-exposed pups. An increase in K^+ current could be implicated as a cause for prolongation of the refractory period

Continued

Table 2. ***(Continued)***

Publication	Tissue/ cell type	EMF type and exposure time	Exposure system	Delivery mode	Techniques used to study ion channel	Ion channel studied	Results and discussion	Proposed mechanism of action
He *et al.*[38]	Rat cerebellar granular cells	50-Hz ELF-EMF up to 1 mT; acute exposure (10—60 min)	The EMF was generated by a pair of Helmholtz coils placed in opposition to each other. The coils were powered by a generator system that produced the input voltage of the pulse, and the magnetic flux densities could be regulated within the range of 0–1.0 mT. The device was powered by an AC power generator, and the EMF frequency and density were monitored by an EMF sensor that was connected to a digital multimeter	The incubator was kept closed all throughout the EMF or non-EMF experiments to make sure that the conditions were stable. The non-EMF–exposed groups were incubated in the same incubator in which the conditions were the same as for the exposed groups but without EMF	Whole-cell patch clamp recordings and siRNAs	Voltage-gated Na^+ channels	The activity of neuronal Na^+ channels is significantly increased by ELF-EMF stimulation, although the steady-state inactivation curve of the Na_V current (I_{Na}) in cerebellar GCs did not significantly shift upon exposure to ELF-EMFs. Notably, the effect of ELF-EMFs is mediated by an increase in cPLA2 activity, and subsequent changes in intracellular concentration of arachidonic acid (AA) and EP receptor–mediated activation of the cAMP/PKA signaling pathway are involved. Exposure to ELF-EMFs induced similar effects on I_{Na} in rat cerebellar GCs whether the condition is 1-mT stimulation for a short time or 0.4-mT stimulation for a longer time	Exposure to ELF-EMFs activates PKA, which then modulates I_{Na}, in part, by insertion of new Na_V channels into the membrane. Exposure of cerebellar GCs to ELF-EMFs influences the activity of PLA2, thus stimulating the production of intracellular AA, which is then converted to PGE2. PGE2 then enters the extracellular space and binds to EP receptors, activating the cAMP/PKA pathway and accounting for the induction of I_{Na}
Kim *et al.*[41]	Mouse hippocampus	835-MHz RF-EMF, with a specific absorption rate (SAR) of 4.0 W/kg for 4 weeks	Wave Exposer V20 RF generator	The cage for RF-EMF exposure was placed in the RF-EMF generator; 835-MHz RF-EMF was delivered to the mice from a horn antenna, installed above the mouse cage. The bottom and walls of the cage were covered by ceramic wave absorption material	Western blot	Voltage-gated Ca^{2+} channels	Pan Ca^{2+} channel expression in hippocampal neurons was significantly decreased after exposure to RF-EMF. However, downregulation of the apoptotic pathway may contribute to the decrease in Ca^{2+} channel expression, and thus lower levels of Ca^{2+} in hippocampal neurons	Exposure to RF-EMFs could alter intracellular Ca^{2+} homeostasis by decreasing Ca^{2+} channel expression in the hippocampus, presumably by activating the autophagy pathway, while inhibiting apoptosis as an adaptation process for exposure to 835-MHz RF-EMF
Kim *et al.*[42]	Mouse hypothalamus	RF-EMF of 835-MHz (4.0 W/kg specific absorption rate (SAR) 5 h/day for 12 weeks)	Wave Exposer V20 RF generator	The cage for RF-EMF exposure was placed in the RF-EMF generator; 835-MHz RF-EMF was delivered to the mice from a horn antenna, installed above the mouse cage. The bottom and walls of the cage were covered by ceramic wave absorption material	Quantitative real-time PCR and western blot	Voltage-gated Ca^{2+} channels	The number and size of synaptic vesicles (SVs) were significantly decreased by exposure to 835-MHz RF-EMF (SAR of 4.0 W/kg for 5 h/day for 12 weeks). Moreover, the number of SVs in the active zone was decreased, suggesting that trafficking of SVs in hypothalamic neurons was affected by RF-EMF exposure. In parallel, synapsin I/II and SYT1, two regulatory factors of SV trafficking, were significantly decreased in hypothalamic presynaptic neurons. The expression of VGCCs was also significantly reduced in the hypothalamus	The decreased expression of synapsin I/II, SYT1, and VGCCs by RF-EMF exposure may contribute to a decrease in the number and size of the SVs in hypothalamic neurons
Lisi *et al.*[88]	Mouse pituitary corticotrope–derived AtT20 D16VCells	Sinusoidal 50-Hz ELF-EMF with flux density of 2 mT (rms)	AMF coil, consisting of two turns of 1.2-mm diameter copper wire formed into a circle (radius 1/4 10 mm)	The apparatus was lowered into the incubation chamber by a stereotactic controller and carefully adjusted to be concentric with the center of the visual field	Cell labeling with fluorescent probes (Indo/SNARF)	L-type Ca^{2+} channel	Pretreatment with nifedipine, to block AtT20 D16 V cell L-type Ca^{2+} channels, with ELF-EMF exposure, resulted in an impairment of NF-200 expression compared with field-exposed, nifedipine-untreated cells	The main target of field exposure in the system is at the level of voltage-gated L-type Ca^{2+} channels

Continued

Table 2. ***(Continued)***

Publication	Tissue/ cell type	EMF type and exposure time	Exposure system	Delivery mode	Techniques used to study ion channel	Ion channel studied	Results and discussion	Proposed mechanism of action
Liu *et al.*[89]	Rat cerebellar granule cells	50-Hz ELF-EMF up to 1 mT; acute exposure (60 min)	A 50-Hz magnetic field was generated by a pair of Helmholtz coils placed in opposition to each other. The coils were powered by a generator system that produced sinusoidal input voltage, and the magnetic flux densities could be regulated within the range of 0−1.0 mT. The device was powered by an AC power generator, and the EMF frequency and density were monitored by an EMF sensor that was connected to a digital multimeter	The incubator was kept closed all throughout the EMF or non-EMF experiments to make sure that the conditions stable. The non-EMF–exposed groups were incubated in the same incubator in which the conditions were the same as for the exposed groups but without EMF	Whole-cell patch clamp recordings. Measurement of intracellular Ca^{2+} level by single-cell Fura-2 AM fluorescence	Voltage-gated Na^+ and Ca^{2+} channels	ELF-EMF exposure significantly increased the Na_V current (I_{Na}) densities by 62.5%. Melatonin (MT; 5 μM) inhibited the ELF-EMF–induced I_{Na} increase. The Na_V channel steady-state activation curve was significantly shifted toward hyperpolarization by ELF-EMF stimulation but remained unchanged by MT in cerebellar GCs that were either exposed or not exposed to ELF-EMF. The inhibitory effects of MT on ELF-EMF–induced Na_V activity was greatly reduced by the calmodulin inhibitor KN93. Ca^{2+} imaging showed that MT did not increase the basal intracellular Ca^{2+} level, but it significantly elevated the intracellular Ca^{2+} level evoked by the high Na^+ stimulation in cerebellar GCs that were either exposed or not exposed to ELF-EMF. In the presence of ruthenium red, a ryanodine-sensitive receptor blocker, the MT-induced increase in intracellular Ca^{2+} levels was reduced	MT itself was unable to modify I_{Na} but might inhibit I_{Na} enhancement resulting from ELF-EMF exposure in cerebellar GCs by increasing the concentration of intracellular Ca^{2+}
Luo *et al.*[90]	Cultured rat entorhinal cortex neurons	ELF-EMF exposure (sinusoidal waveform, 50 Hz, 1 or 3 mT); 24 h exposure procedure, in which sham or ELF-EMF exposure was applied alternately, 5 min on and 10 min off	The exposure system consisted of two four-coil setups (two coils with 56 and two coils with 50 windings), each of which was placed inside a Mu-metal box. The currents in the bifilar coils could be switched between parallel for field exposure and nonparallel for sham control	Both setups were placed inside a commercial incubator to ensure constant environmental conditions (37 °C, 5% CO_2, 95% humidity)	Whole-cell patch clamp recordings. While recording the activity of Ca^{2+} channels, the culture medium was replaced by modified ACSF to change the charge carrier from Ca^{2+} to Ba^{2+}. Then, TTX (1 μM) was added to the modified ACSF to block Na^+ channels. The pipette was filled with Cs-based internal solution to block K^+ channels; Ca^{2+} imaging by single cell Fluo-4 AM fluorescence	Low and high voltage–activated Ca^{2+} channels. Mixture of inward currents (mainly reflecting the fast Na_V currents) and transient and sustained outward currents (mainly reflecting the K^+ currents)	ELF-EMF exposure does not influence Ca^{2+} currents or the activation dynamics of both low and high voltage–activated Ca^{2+} channels, but influences the intracellular Ca^{2+} dynamics of cultured EC neurons via a Ca^{2+} channel–independent mechanism	ELF-EMFs affect the Ca^{2+} dynamics via an intracellular Ca^{2+} store–dependent process. Indeed, the Ca^{2+} dynamics can be tightly controlled by intracellular Ca^{2+} stores primarily via the two secondary processes of Ca^{2+} release from intracellular stores and/or subsequent uptake

Continued

Table 2. ***(Continued)***

Publication	Tissue/ cell type	EMF type and exposure time	Exposure system	Delivery mode	Techniques used to study ion channel	Ion channel studied	Results and discussion	Proposed mechanism of action
Marchionni *et al.*[39]	Rat dorsal root ganglion isolated neurons	125 μT (rms) 50-Hz ELF-EMF, 900-MHz RF-EMF; experimental exposure (acute)	The experimental chamber was encircled with a Helmholtz device using coils made of isolated copper wire, each field intensity being calibrated. The exposure system used for RF-EMF was based on a coplanar waveguide (CPW), an open propagating structure with a dielectric substrate on which three metallic strips are deposited. Choosing glass as the dielectric substrate, it was possible to design a system suitable to replace the microscope head stage	The different EMFs were continuously exposed to the cell under observation on the inverted microscope head stage	Patch clamp recordings (current-clamp and single-channel recording techniques) using TEA and 4-AP to isolate single ion currents	L-type Ca^{2+}, delayed rectifier (DR) K^+, and Ca^{2+}-activated K^+ (KCa) channels	The firing rate of rat sensory neurons can be modified by 50/60-Hz magnetic fields but not by low level 900-MHz fields. The action of the 50/60-Hz magnetic fields is biphasic. The fields do not affect action potentials but influence the gating dynamics and the meantime open probability of ion channels. RF-EMF, inducing an SAR of 1 W/kg in the biological specimen, does not interact, either directly or indirectly, with ionic membrane permeability. Conversely, ELF-EMF modulates the currents flowing through at least two ionic channels: high-threshold Ca^{2+} channel and one of the Ca^{2+}-activated K^+ channels	The behavior of action potential repetitive firing under the influence of the ELF-EMF could be explained by the potentiation of Ca^{2+} and alteration of the ionic permeability of the KCa channel. The effect of the field on Ca^{2+} current is probably mediated by the action on the membrane surface charges
Maskey *et al.*[53]	Mouse hippocampus	835-MHz RF-EMF, with a specific absorption rate (SAR) of 4.0 W/kg for 4 weeks (5 h per day)	Wave Exposer V20 emitting 835 MHz equivalent to the Korean Code Division Multiple Access (CDMA) mobile phone frequency. SAR was controlled from 1.6 to 4.0 W/kg. Waves were generated and amplified in an electronic unit and eventually were radiated by a pyramidal rectangular horn antenna connected by a waveguide to coaxial transition	The entire body of mice was exposed to 835 MHz radiation for 1 month with average SAR of 1.6 W/kg by using the Wave Exposer V20 instrument	Immunoreactivity (IR) analysis	Ca^{2+} homeostasis study on CaBP	Loss of dendritic arborization was noted with the CaBP in the cornu ammonis areas as well as a decrease in staining intensity of the granule cells in the dentate gyrus after exposure, while no loss was observed in the ginseng-treated group. A significant difference in the relative mean density was noted between the control and exposed groups but was nonsignificant in the ginseng-treated group	A decrease in CaBP IR with changes in the neuronal staining as observed in the exposed group would affect the hippocampal presynaptic circuit by alteration of the Ca^{2+} concentration, which could be prevented by ginseng. Hence, ginseng could act as a radioprotective agent against EMF exposure, contributing to the maintenance of Ca^{2+} homeostasis by preventing impairment of intracellular Ca^{2+} levels in the hippocampus
Morabito *et al.*[60]	Rat pheochromocytoma (PC12) cells	50-Hz ELF-EMF up to 1 mT; acute (30 min) and chronic (7 days) exposure	The electromagnetic fields were generated by two different devices: a solenoid for chronic exposure and a pair of Helmholtz coils for acute exposure	Cells were cultured in plastic dishes transparent to the ELF-EMF	Spectrofluorimetric determination of basal $[Ca^{2+}]_i$	Ca^{2+} homeostasis (not better specified)	The chronic ELF-EMF exposure did not appear to significantly affect the biological response (proliferation and neurogenesis). However, during the acute ELF-EMF exposure (30 min) of undifferentiated PC12 cells, there were increased ROS levels and decreased catalase activity, that, conversely, were increased after chronic exposure. Acute exposure affected the spontaneous intracellular Ca^{2+} variations in undifferentiated cells, in which basal intracellular Ca^{2+} increased after chronic exposure. Acute exposure affected cell response to a depolarizing agent, while basal membrane potential was not changed	ROS and Ca^{2+} could be the primary cellular causes of ELF-EMF–induced effects on biological systems

Continued

Table 2. ***(Continued)***

Publication	Tissue/ cell type	EMF type and exposure time	Exposure system	Delivery mode	Techniques used to study ion channel	Ion channel studied	Results and discussion	Proposed mechanism of action
Platano *et al.*[44]	Primary cultured rat cortical neurons	900-Hz RF-EMF; acute exposure (1−3 periods of 90 s); specific absorption rates (SARs) were 2 W/kg for continuous wave (CW) and 2 W/kg for GSM-modulated signals	The exposure system is an open propagating structure consisting of a CPW. The upper side of the dielectric substrate (glass) was plated by three thin conductive gold layers in a way to leave two windows for the visualization of the cell culture under the microscope	The Petri dish was maintained in the center of the exposure system by using a 3-mm polystyrene mask	Whole-cell patch clamp recordings using Ba^{2+} as ion carrier to avoid Ca^{2+} -dependent inactivation of the currents, and with TEA and TTX in the external solution. $CdCl_2$ (200 mM; a specific blocker of VGCCs) was added to the external solution at the end of the experiments	Voltage-gated Ca^{2+} channels	Ba^{2+} currents through VGCCs in rat cortical neurons are not affected by single or multiple (two or three) acute exposures to either CW-(2 W/kg) or GSM-modulated (2 W/kg time average value) 900-MHz RF-EMFs	N/A
Prina-Mello *et al.*[47]	Primary cultured rat cortical neurons	Strong SMFs up to 5 T and different static magnetic field strengths (0.1, 0.5, 0.75, and 1 T); acute (2 min) exposure	Exposures to static magnetic fields were conducted using a superconducting solenoid with a wide, open, circular bore and equipped with dedicated software for real-time measurement of the magnetic field	The primary cultured cortical neurons (PCNs) were exposed to one of six different static magnetic field strengths, (0.1, 0.5, 0.75, 1, 2, and 5 T), for an exposure time of 1 h	Ca^{2+} imaging by single-cell Fura-2 AM fluorescence	Voltage-gated Ca^{2+} channels	When cells were exposed to a static magnetic field strength of 0.75 T, the resting Ca^{2+} concentration was significantly increased and this may account for the increase in ERK activity induced by 0.75 T since mitogen-activated protein kinase (MAPK) activation has a Ca^{2+}-dependent component. By contrast, magnetic stimulation (0.75 T) resulted in reduced Ca^{2+} influx following KCl depolarization	In neurons, there are additional ion currents associated with signal transmission. Typically, these are in the range of 1−20 pA. If these relate to a single ion channel, local current densities as great as 10 A/cm^2 are present. Magnetic field–induced shifts of rest potential are, therefore, to be expected and it is likely that they will influence the signaling pathways. A static magnetic field alters the activation kinetics of voltage-dependent Ca^{2+} channels
Prucha *et al.*[109]	Rat F11 cells derived from dorsal root ganglia neurons	LF-EMF; short-term (<180 s) exposure	LF-EMF generated by three different medical devices: VAS-07 STRONG, SALUTER MOTI, and DIPOL SETA-D, I-100.	Not specified	Whole-cell patch clamp recordings and Ca^{2+} imaging by single cell Fura-2 AM fluorescence	Voltage-gated Ca^{2+} channels and inward currents, typical for voltage-gated Na^+ channels	Repetitive electromagnetic stimulation has acute effects on Ca^{2+} responses in model peripheral sensory neurons. Short-term exposure of naive F11 cells to LF-EMF reduces Ca^{2+} transients in response to bradykinin and demonstrates a potentiating effect of LF-EMF on the spontaneous activity of F11 cells under two different conditions	Bradykinin stimulates the hydrolysis of phosphatidylinositol 4,5-bisphosphate. This membrane phospholipid is a necessary cofactor whose requirement is clearly established for many ion channels, receptors, and transporters
Shen *et al.*[46]	Rat trigeminal root ganglion neurons	125-mT SMF; acute (15 min) exposure	The SMF exposure device was designed by an electromagnetic design software. The U-shaped device has two 60 mm × 60 mm square NdFeB magnets covered by polar caps and connected by a steel yoke	In the course of SMF exposure, a 35-mm cell culture dish was placed on a plastic base to reach the position at the vertical and horizontal centers of the 50-mm–wide air gaps between the two caps	Whole-cell patch clamp recordings using 4-AP and TEA to pharmacologically select the different channels	Voltage-gated K^+ channels: fast inactivating transient (IK, A) and dominant-sustained (IK, V) channels	125-mT SMF showed no effect on the peak current density and I–V relationship of IK, A, and IK, V activation, but changes were found in the inactivation kinetics of both types of VGPCs between the SMF exposure and control groups	A biological membrane would be deformed in an SMF and the ion channels on the membrane would be affected. The alterations of ion channel activity caused by SMF exposure are indirect. The primary effect of magnetic fields is to induce rotation and reorientation of the membrane lipid molecules and such reorientation could affect conformational changes of ion channels

Continued

Table 2. ***(Continued)***

Publication	Tissue/ cell type	EMF type and exposure time	Exposure system	Delivery mode	Techniques used to study ion channel	Ion channel studied	Results and discussion	Proposed mechanism of action
Sun *et al.*[40]	Mouse medial nucleus of the trapezoid body (MNTB)	50-Hz ELF-EMF up to 1 mT; chronic exposure (8−10 days)	A 50-Hz magnetic field was generated by a pair of Helmholtz coils powered by a generator system producing the input pulse. The magnetic flux densities were adjusted to 1 mT and monitored by an electromagnetic field sensor with a digital multimeter	The ELF-EMF exposure group was raised in the electromagnetic field from the day of birth (p0)	Whole-cell patch clamp recordings where kynurenic acid (KYN) was added to the bath solution to relieve AMPA receptor saturation and desensitization; western blots and real-time PCR	Ca^{2+} channels (P/Q, N, and R subtypes)	ELF-EMF facilitates all forms of endocytosis and potentiates PTP. Furthermore, the enhanced expression of Ca^{2+} channels at the presynaptic nerve terminal, especially the P/Q type, increases Ca^{2+} influx upon stimulation and facilitates vesicle endocytosis and synaptic plasticity	Enhanced expression of Ca^{2+} channels at the presynaptic nerve terminal, mostly the P/Q subtype, accounts for the increased Ca^{2+} influx upon stimulation, facilitating vesicle endocytosis and synaptic plasticity
Yin *et al.*[52]	Primary cultured rat hippocampal neurons	50-Hz ELF-EMF up to 8 mT; acute exposure (90 min)	The electromagnetic field was generated with a pair of Helmholtz coils of 400 turns. A pair of parallel Helmholtz coils, parallel to the ground, was connected to a 220 V AC power supply via an adjustable transformer	Cell culture plates were exposed to ELF-EMF (50 Hz, 8 mT) for 90 min in the experiments	Intracellular Ca^{2+} imaging by Fluo-3 AM	Ca^{2+} homeostasis (not better specified)	The ELF-EMF group showed a significant increase in the level of Ca^{2+} compared with the control group ($P < 0.01$). The LSPC group (10 mg/mL) significantly decreased the Ca^{2+} level compared with the ELF-EMF group ($P < 0.01$)	Elevation of Ca^{2+} levels in the ELF-EMF group led to destabilization of the neuronal cells' structure and increased excitability, further leading to the activation of nNOS, which caused cell damage and eventually cell death

Note: For each paper, tissue/cell type, EMF type, type of exposure, delivery mode, studied ion channel, results, and proposed mechanism for EMF action are summarized.

channel currents, among which the most commonly used were the sodium channel blocker tetrodotoxin (TTX) and the potassium channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA). The first is a potent neurotoxin derived from pufferfish that binds the pore domain (PD) of fast VGSCs, blocking sodium conductance.[67] The second is a relatively selective blocker of members of the K_v1 (Shaker, KCNA) family of voltage-gated potassium channels (VGPCs),[68] which have interestingly been reported to directly potentiate ion conduction through voltage-gated calcium channels (VGCCs).[69] The third is thought to physically enter the pore of VGPCs and KCa channels, blocking potassium conductance.[70]

Fifty-two percent of papers also measured changes in intracellular calcium levels using various methodologies, including single-cell Fura-2 AM (54%), Fluo-3 AM (18%), and Fluo-4 AM (18%), and fluorescence cell labeling with fluorescent probes (Indo/SNARF) (9%). In addition to this, 14% and 9% of studies evaluated the impact of EMFs on ion channel expression using western blots and quantitative RT-PCR, respectively. Finally, an isolated study used short interfering RNA (siRNA) to target ion channels, while the other one used Fourier transform infrared spectroscopy to assess the effect of EMF exposure on the α-helices of ion channels (Fig. 3).

Experimental results

The most commonly recorded effect evoked by ELF-EMFs in neurons was an increase in the basal calcium concentration (reported in 42% of papers focusing on these types of fields). Nonetheless, the results of acute ELF-EMF exposure appear to be diverse. In addition to a rise in intracellular calcium concentration, altered gating dynamics of high-threshold calcium channels and calcium-activated potassium channels as well as increased activity and insertion in the membrane of Na^+ channels were described. On the other hand, two papers reported a lack of effects on both the calcium homeostasis and the electrophysiological properties of the cell under this condition. However, under chronic exposure, all the papers reported an increase in the intracellular calcium levels at intensities greater than 1000 μT, along with increases in the gene and protein expression of transmembrane calcium channels.

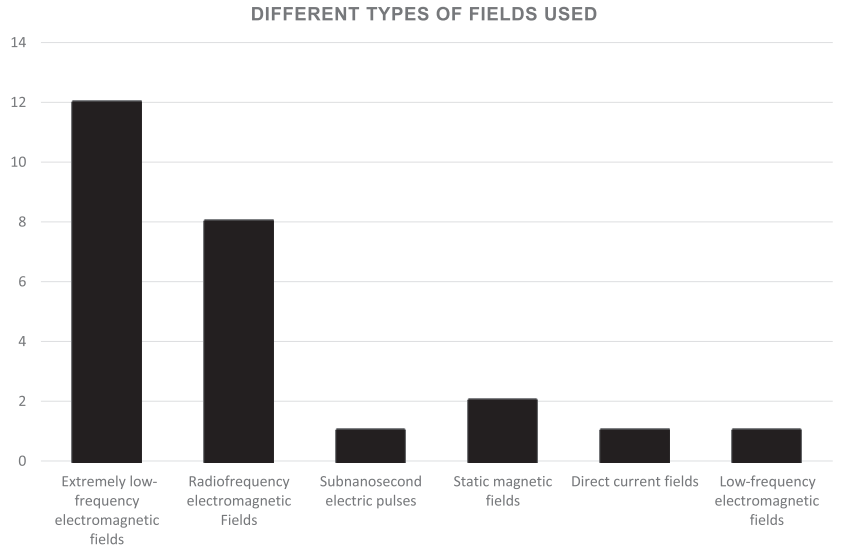

Figure 2. **Different types of EMFs employed in the 22 studies analyzed in this review. Fields of similar frequencies have been grouped in the same category.**

Acute exposure to RF-EMFs does not appear to exert a significant effect according to the two papers focusing on this type of exposure. However, under chronic exposure, 60% of studies reported a decrease in calcium channel expression and one study reported effects on the electrophysiological properties of neurons, such as altered afterhyperpolarization amplitude, spike frequency, half width, and first spike frequency.

A single study on direct current fields (DCFs) showed membrane polarization in hippocampal CA1 pyramidal neurons subsequent to DCF exposure that was, however, independent of any alteration in the dynamics of VGCs. This paper, as the ones investigating the role of SMFs, has been included since the electric and magnetic components are never totally separable (although in these cases the magnetic component of the field is likely to be negligible due to the constant magnetic flux generated by the DC). Acute exposure to SMFs is reported to have effects on both the inactivation kinetics of VGPCs and the intracellular basal calcium level. Finally, one other study[71] established a direct proportionality between the frequency of EMFs used (ELF-EMFs and RF-EMFs) and the displacement of protein α-helices of different ion channels.

Discussion

Biological effects of EMFs are widely reported in the literature, and extreme low frequencies in particular have been shown to have many effects, including changes in VGC conductance and neurotransmitter expression.

The effects of EMFs on VGCs are important due to the key role of these transmembrane proteins in physiological processes in the cell and particularly in the CNS, where they are at the center of the regulation of a myriad of neurophysiological processes, ranging from the generation of action potentials (APs) to synaptic transmission.[72] These transmembrane proteins are highly conserved throughout diverse biological kingdoms[73] and all share a similar structure, consisting of a variable number of subunits arranged to form a pore through which ions can travel in the direction of different electrochemical gradients.[74] The functionality of these VGCs is mediated by the VSD, a specialized region of the protein that is rich in charged residues and can trigger a conformational change in the channel, modulating its entire configuration.[75,76] Properties central to the functionality of VGCs are ion selectivity and ion conductance.[77] Ion selectivity is key to the specificity of each VGC family and is regulated

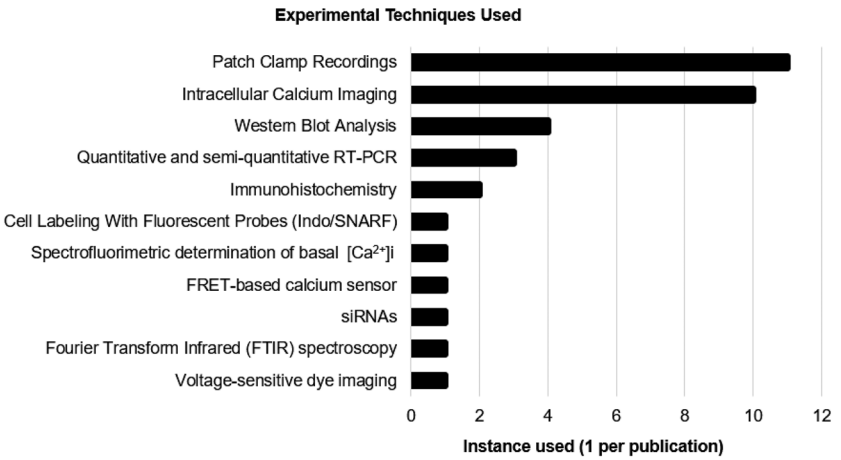

Figure 3. **Instances of experimental techniques used in the reviewed studies that were employed to either directly or indirectly assess the effects of EMF exposure on ion channels. Techniques using similar principles and materials were put into the same group.**

by a portion of the protein known as the selectivity filter, embedded in the upper extracellular side of the PD. This selectivity filter discriminates between different ions based on their diameter (of a few angstroms) and charge.[78] Ion conductance reflects, on the other hand, the inverse of channel resistance to ion flow, or in other words the number of ions that the VGCs translocate in a specific period of time. Different VGCs belonging to the same family can have the same selectivity but greatly differ in their conductance.[79] A great diversity of VGCs exist but three main families can be identified on the basis of their distinct selectivity for a particular permeant ion. First, VGSCs are responsible for the initiation of APs in excitable cells, such as neurons,[70] and represent a common target for the therapy of epilepsy and many neurodegenerative diseases.[80,81] Second, VGPCs are extremely important in shaping APs, where they are responsible for membrane's repolarization, and in the general modulation of neuronal excitability.[77] Their essential role is testified to by the existence of numerous channelopathies[79] in which a mutation in the genes encoding for structural subunits of these channel complexes is enough to cause severe conditions, ranging from epilepsy and related disorders to numerous forms of ataxia and dyskinesia.[82] Finally, VGCCs are involved in the modulation of an extremely broad variety of neurological processes, including (but not limited to) modulation of neurotransmitter release and intersynaptic short- and long-term communication, neuronal plasticity, neurite outgrowth, and gene expression.[83] Due to the major role of calcium as a second messenger, VGCC dysfunctions have been implicated in a wide variety of CNS pathologies, including epilepsy, neurodegenerative diseases, neuropathic pain, and neuropsychiatric disorders.[84] The particular electrical sensitivity of VGCs themselves makes them a perfect target for EMF effects. First, the charges located on the S4 helix voltage sensor in the VSD[85] are particularly exposed to the electrostatic forces resulting from an applied EMF, and this type of stimulation could easily trigger a displacement in these charges, similar to the one generated by the depolarizing wave of an AP, irregularly gating the channel.[11] Having the same direction at a particular time, the magnitude of this shift becomes greatly amplified. Furthermore, the location of the VSD, embedded in surrounding lipid environment, needs to be considered. Indeed, the forces exerted by an EMF tend to align the permanent phospholipid dipoles, which are normally randomly oriented because of thermal excitation, producing new fields.[86] Finally, transmembrane proteins allow a higher permeation of the EMF with respect to the surrounding membrane since the resistance of the membrane is extremely high and displays a dielectric constant up to 120 times lower than the aqueous phase of the cytoplasm where most of the charges are located.[87]

The effect of EMFs on VGCs is exerted at multiple levels

VGCs represent a perfect candidate for the transduction of the EMF effects on neural tissue. Here, we summarize the evidence for EMF-related effects on these ion channels in the studies analyzed in this review. Both ELF-EMFs and RF-EMFs have been found to modulate VGCs in many ways, including their expression,[40–42,58] gating dynamics,[38,39,43,88,89] and insertion into the membrane.[38] An increase[40] (or a decrease, as reported for RF-EMF exposure)[41,42] in VGC density could explain the altered ion flux and account for the many secondary effects reported in many papers, including, but not limited to, activation of the autophagic pathway,[41] altered spike frequencies and AP firing,[43] and facilitated vesicle endocytosis and synaptic plasticity.[40] However, this mechanism cannot account for the rapid effects elicited by acute exposure to ELF-EMFs, such as increased level of ROS,[52,60] altered firing rate,[39] and spontaneous intracellular calcium variations,[60] due to the longer time (ranging from tens of seconds to days) required by the cell to modify its gene expression patterns. Thus, an effect on the voltage-sensing and gating dynamics of VGCs is likely to be involved. Indeed, a shift in the steady state of VGSCs (Na_v) was observed in two different studies,[38,89] and single-ion channel studies revealed altered gating dynamics for both high-threshold VGCCs and KCa channels subsequent to acute ELF-EMF exposure.[39] Similar alterations were further observed in the inactivation kinetics of different types of VGPCs after acute exposure to SMFs.[46] Interestingly, one of the studies analyzed in this review reported a direct correlation between the frequency of EMFs (ELF-EMFs and RF-EMFs) and the displacement of the α-helices in ion channels,[71] which could be related to the changes in gating dynamics reported elsewhere.[38,39,43,88,89] In summary, it appears that the effect of EMFs on VGCs is exerted at multiple levels, one being a rapid modulation in the transport dynamics of VGC proteins, and the other being changes in both their gene and protein expression and density in the membrane. The latter seem to require a prolonged exposure to EMFs and could result from the same intracellular ionic concentration shift extensively reported as one of the major effects of EMF exposure.

On the other hand, three studies reported no (or at least no significant) changes to VGC transport dynamics related to EMF exposure. However, it must be noted that these studies used very low field intensities ($\leq$1000 μT)[31,59] or a particular combination of radio frequencies and acute exposure[44] that other studies have shown to be less effective,[39] thus explaining the lack of identifiable effects reported. Finally, the different ion channels in different brain regions must be taken into account. The only paper proposing a VGC-independent mechanism (based on the release of intracellular Ca^{2+} stores) for the effect triggered by EMF exposure on calcium homeostasis also points out how the model used is characterized by a poor expression of VGCCs.[90]

The role of calcium

The important role of calcium in the induction of EMF exposure–related effects was already established[91] and is further confirmed in this review by the many papers reporting an increase in the basal level of calcium following EMF exposure.[39,40,52,88] This is not surprising, since calcium is universally known as the most important and widespread regulator of neurological processes, such as neural differentiation, survival, apoptosis,[54,55] neurotransmitter release, excitability, and synaptic plasticity.[92] The complex and different calcium-related signaling pathways have variable importance in different areas of the brain. This very reason could explain the complex and contrary effects reported in the literature. Such an increase in intracellular calcium influx could be due to different mechanisms, and the papers reviewed here propose many mechanisms: a direct effect on the mean opening time of VGCCs,[39,88] both an increase[40] or a decrease[41,42] in their expression (depending on the frequency of the field and exposure time), and the augmented release of glutamate that through the activation of NMDAR would stimulate such an increase in the hippocampus.[58] This last hypothesis is particularly intriguing because it could easily explain the many reported effects that the EMFs exert on learning, memory, and synaptic plasticity.[57,93] The particularly relevant role of VGCCs in the transduction of EMF effects on neurons seems to be attributable to the great number of effects produced by an increase in intracellular calcium levels, as previously suggested.[94] Indeed, the few studies focusing on other VGCs, such as VGPCs and VGSCs, reviewed here[38,39,43,46,90,95] reported significant effects specific to these ion

channels, suggesting how the effect of EMFs could target the VSD of every VGC.

Different effects for different fields

An extensive literature exists reporting a myriad of EMF exposure–related effects on many biological processes, ranging from cell differentiation, survival, and changes in gene expression[96,97] to effects on cell membranes and signal transduction pathways.[30] However, many other studies indicated the absence of significant effects elicited by these fields.[59,61] A possible explanation for the different effects reported could be related to the fact that the way in which EMFs interact with the body depends on what combination of frequencies are used and the related wavelengths.

It is well known that the effects of exposure to EMFs differ significantly based on the exposure intensities and the exposure time,[1,98] and because of this any reasonable comparison must be made between groups having the same experimental conditions. In this study, we found different types of EMFs employed, although the two most commonly represented categories were ELF-EMFs and RF-EMFs, in line with the well-documented biological relevance of these fields.[94,99,100] It is important to point out that the effects exerted by these two types of exposures are not equal due to the intrinsic electrical properties of the neuronal membrane. For instance, electrical phenomena involving a redistribution of charges in the membrane subsequent to EMF exposure, such as counterion polarization and phospholipid reorientation, are not likely to occur in RF-EMF exposure, due to the high inertia of charged particles at this high frequency.[101]

Moreover, pulsed EMFs are often reported to be more active relative to static EMFs, which are characterized by a continuous electromagnetic wave to which the cell could be more adapted,[102] and they could affect the gating properties of VGCs since these proteins are intrinsically sensitive to minimal electrical variations.[100] Likewise, the effect of SMFs could similarly influence VGCs through a deformation of the membrane involving a reorientation of the phospholipid bilayer, as suggested by Rosen's study.[103] Indeed, both of the studies reviewed here that focused on SMF effects reported effects on VGCs, specifically on the gating dynamics of VGCCs[47] and the inactivation dynamics of VGPCs.[46]

Lastly, it is worth mentioning that the frequency-related impact of the various type of EMFs has not been totally clarified, and theories exist suggesting that only specific frequencies would relevantly interact with the cell.[104–106] However, although many different types of fields have been used, the frequencies used were similar (specifically 50 Hz for ELF-EMFs and 835 and 900 Hz for RF-EMFs).

Relevance of EMF exposure time

It is worth noting that, in the studies analyzed here, many different exposure times have been used. This variability might account for the many different and sometimes opposite effects reported. The time dependency of EMF exposure–related effects is well known. A 1973 study by Tolgskaya and Gordon reported how, in the first months of exposure to radio waves, the morphological and physiological effects on animals brain are poor and modest, becoming evident and irreversible after longer exposure.[107] Most papers reviewed here investigated acute (up to 2 h) or subchronic exposure (from 2 to 48 h) and could, therefore, have overlooked the effects elicited by longer exposures. Interestingly, all the papers except one[31] reported significant effects after chronic (>48 h) exposure, whatever the type of field used. On the other hand, the rapid increase in intracellular calcium reported in many papers after ELF-EMF exposure seems to go against this line of thinking, pointing toward a direct effect on VGCs. These reasons, in line with the different and various effects of EMF exposure reported, seem to suggest that EMFs could act through more than one mechanism, to differentially influence particular brain areas or neuronal populations according to the exposure time.

Effects of EMFs on neural development

As stated above, there is great interest regarding EMF-related effects on neural development since less differentiated cells have been proposed to be particularly susceptible to these fields. Supporting this idea, one of the studies reviewed here reported significant effects on the electrical properties of Purkinje cerebellar neurons subsequent to exposure during development (6 h per day for the gestation period) to RF-EMFs.[43] On the other hand, de Groot and colleagues failed to observe important anomalies in an ELF-EMF development exposure model, although the short exposure time (7 days) that did not cover the entire length of mouse pregnancy

could underestimate long-term effects, as discussed in the previous section.[31] Interestingly, Morabito *et al.* reported, using the same model, a more severe impact on undifferentiated PC12 cells.[60]

Finally, VGCC expression dynamics are far from stable throughout development, ranging from a prevalent expression of T-type calcium channels in the initial stages to the higher presence of N- and L-type channels in the mature neuron.[108] This differential expression and the preferential effect of EMF on specific types of VGCCs should, therefore, be considered in the interpretation of every study involving EMF exposure.

Limitations of this study

This study investigates a complex field, with sometimes conflicting results. The many variables that influence the impact of EMF exposure on neural tissue, such as the physiological state of the cell, its developmental stage, and the various physical characteristics of the many fields involved, complicate the reproducibility and often impede a consistent comparison between different studies. In spite of having highlighted some recurring patterns in the reported results, this review is, therefore, limited by the intrinsic differences of the studies reviewed.

Conclusion

The studies reviewed here show VGCs as an important transducer of the effect of EMFs in neurons, and the central role played by these proteins in the regulation of important biological processes, central in the regulation of brain physiology, sheds a light on the influence that modern exposure to EMFs could have on human health. While a diverse range of biological systems were used, cell lines were the preferred option, and VGCCs were the most studied ion channels, in line with their central role in the regulation of many physiological processes in neurons. However, many other VGCs have been shown to be affected by EMFs and the results are often conflicting. In spite of the controversy, this systematic review reports significant correlation between EMFs and multiple changes in the electrophysiological properties of diverse neuronal tissues, and these results, if interpreted well, could pave the way to a new understanding of the relationship between electromagnetic stimulation and brain functions. In conclusion, we systematically demonstrate how the complex effects of EMFs in neuronal ion channels are exerted at multiple levels and how their significance in the alteration of neuronal functions is strictly dependent on different parameters relative to the type of field used and the studied cell or tissue. Improved experimental reproducibility will be key to any advances in this field, and the development of new experimental procedures capable of measuring the small but profound way in which certain types of EMF exposure seem to affect our brain might help us to establish whether it is harmful and its therapeutic potential. We hope this work will help in improving our knowledge about the molecular dynamics of neuronal VGCs, which will be key both for any progress in the treatment of neurodegenerative diseases and for an advancement in the general understanding of the relationship between technological progress and cellular dynamics.

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Authors contributions

F.B., K.J., R.L., J.M., and S.R.P.S. designed the research. F.B., K.J., and R.L. analyzed the data. F.B. performed research. F.B., K.J., and R.L. wrote the paper. All authors approved the final manuscript.

Competing interests

The authors declare no competing interests.

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