Original Article

The Screening of Genes Sensitive to Long-Term, Low-Level Microwave Exposure and Bioinformatic Analysis of Potential Correlations to Learning and Memory*

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Abstract

Objective To gain a better understanding of gene expression changes in the brain following microwave exposure in mice. This study hopes to reveal mechanisms contributing to microwave-induced learning and memory dysfunction.

Methods Mice were exposed to whole body 2100 MHz microwaves with specific absorption rates (SARs) of 0.45 W/kg, 1.8 W/kg, and 3.6 W/kg for 1 hour daily for 8 weeks. Differentially expressing genes in the brains were screened using high-density oligonucleotide arrays, with genes showing more significant differences further confirmed by RT-PCR.

Results The gene chip results demonstrated that 41 genes (0.45 W/kg group), 29 genes (1.8 W/kg group), and 219 genes (3.6 W/kg group) were differentially expressed. GO analysis revealed that these differentially expressed genes were primarily involved in metabolic processes, cellular metabolic processes, regulation of biological processes, macromolecular metabolic processes, biosynthetic processes, cellular protein metabolic processes, transport, developmental processes, cellular component organization, etc. KEGG pathway analysis showed that these genes are mainly involved in pathways related to ribosome, Alzheimer's disease, Parkinson's disease, long-term potentiation, Huntington's disease, and Neurotrophin signaling. Construction of a protein interaction network identified several important regulatory genes including synbindin (sbdn), Crystallin (CryaB), PPP1CA, Ywhaq, Psap, Psmb1, Pcbp2, etc., which play important roles in the processes of learning and memory.

Conclusion Long-term, low-level microwave exposure may inhibit learning and memory by affecting protein and energy metabolic processes and signaling pathways relating to neurological functions or diseases.

Key words: Long-term; Low-level; Microwave; Gene chip; Learning and memory

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INTRODUCTION

Because microwaves have become widely applied in aerospace, both military and public, and particularly in communications, at 900-2100 MHz, the effects of microwaves on human health, especially on learning and memory, were the focus of this study. A large number of studies have shown that low level microwaves affect the Central Nervens System (CNS) functions. For

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instance, animal behavior, acetylcholine (Ach) intake and energy metabolism were affected even at a specific absorption rate (SAR) of 0.1-1.2 W/kg[1-3]. Rats exposed to 900 MHz microwave at a SAR of 0.6 and 60 mW/kg for 2 h/week for 4 weeks had impaired memory regarding object recall and impaired temporal order discrimination[4]. Rats exposed to pulsed microwaves at 2.45 GHz and an average power density of 1 mW/cm^2 for 3 h daily for up to 30 days showed inhibited spatial learning and memory performance[5]. Zhao et al. revealed that daily microwave exposure for 6 min over 1 month at average power densities of 2.5, 5, and 10 mW/cm^2 disrupted learning and memory abilities in rats[6]. Moreover, some evidence suggests that long-term, low-level exposure to electromagnetic fields can exert detrimental effects on learning and memory, with the causative mechanisms relating to oxidative stress, energy metabolism, inflammation, neurotransmitters, the mitochondria, long-term potentiation (LTP) and reduced NR2A protein and mRNA expression[7-11].

Collectively, previous studies have focused on individual biological processes or molecular functions, despite physiological responses being induced through complex signal networks. Therefore, microarray analysis, which allows for the simultaneous analysis of thousands of genes and is extensively used to describe biological responses, was utilized to gain further insight into the mechanisms of microwave induced biological effects. Following whole body exposure to low level microwaves (2100 MHz), alterations in brain gene expression were characterized using microarrays as a means to identify global mechanisms pertaining to microwave exposure, which is of high public concern.

MATERIALS AND METHODS

Animals

Male Kunming mice (16-20 g) were obtained from the Laboratory Animal Center (Beijing, China) and maintained at 22±2 °C with a 12 h light-dark cycle (lights on at 7 am). Food and water were freely available and all efforts were made to minimize suffering.

Microwave Exposure Experiment

Mice were divided randomly into exposure and sham groups (6 animals/group), with the exposure groups further divided into groups 1, 2, and 3, with average whole-body SARs of 0.45 W/kg, 1.8 W/kg, and 3.6 W/kg. Subjects were placed at a distance of 30 cm from the exposure source at power densities of 1, 4, and 8 mW/cm^2. The SAR calculation was based on the finite difference time domain (FDTD) method. Animals in the exposure groups were placed in polypropylene cages with apertures and partitions. Mice whole bodies were exposed to microwaves, with their body axes oriented in parallel to the electric field polarization for 1 h daily in a temperature controlled room at a continuous frequency of 2100 MHz for 8 weeks. To negate any other type of psychophysiological effects, sham group animals were processed in parallel to the exposed groups, but without microwave exposure. After irradiation, RNA was extracted from each brain independently, with RNA from each group pooled prior to gene microarray analysis. Three RNA samples from the 3.6 W/kg group were utilized for RT-PCR.

RNA Preparation

Whole brains were dissected, snap frozen on dry ice and pooled and stored at -80 °C. Total RNA was isolated using Trizol. The supernatant was transferred to a fresh sterile tube and sheared by aspiration through a 23-gauge needle. The homogenate was then layered on a cushion of cesium trifluoroacetate and centrifuged at 12,000 rpm for 15 min at 4 °C. After centrifugation, the supernatant was discarded and the RNA pellet was resuspended, ethanol precipitated and stored at -80 °C.

High-Density Oligonucleotide Microarray Analysis

The U133Av2 Gene Chips (Affymetrix, Santa Clara, CA, USA) hybridization procedures were conducted in biostar gene chip corporation. Probe intensities were summarized and normalized using log scale robust multi-array analysis (RMA). The genes with fluorescence density ratio, Cy3/Cy5, of less than 0.5 or higher than 2.0, were identified as differential expression genes. The raw data of microarray were further analyzed through GO, KEGG pathway, Cluster analyses and protein network.

RT-PCR

Primer Design and Synthesis Some highly differentially expressed genes relating to learning and memory were validated by reverse transcription PCR (RT-PCR). Primers were designed for these genes and β-actin (Table 1), which served as an

internal reference gene was provided by the Saibaisheng Corporation (Beijing, China).

Semi-quantitative RT-PCR RT-PCR was performed using the Reverse Transcription System (Promega) according to the manufacture's protocols with the above primers (Table 1). Amplification was performed in a thermal cycler at 94 °C for 30 s, 55.2 °C for 1 min and 68 °C for 2 min for over 35 cycles. Two μL of the single-stranded cDNA amplicons were resolved by electrophoresis on a 5% agarose gel. Each amplicon was confirmed in triplicate and normalized to β-actin levels.

Statistical Analysis

For microarray analyses, an ANOVA was performed using the GeneSpring GX software (Agilent, Santa Clara, CA, USA) with a 1.5-fold minimum expression cut-off and a false discovery rate (FDR) of 5%. Determination of differential gene expression was performed using varying doses (sham irradiation, 0.45 W/kg, 1.8 W/kg, or 3.6 W/kg). A KEGG pathway was generated using the integrated Expression Analysis System Explorer (EASE) at a threshold of 0.1 (a modified Fisher's exact *P*-value), with a count threshold of 2 (minimum number of genes for a particular term). A Student's *t*-test was used for statistical comparison of RT-PCR results, with the results presented as a mean±SD and $P<0.05$ considered statistically significant.

RESULTS

Global Gene Expression Changes in Response to Microwave Exposure

At SARs of 0.45 W/kg, 1.8 W/kg, and 3.6 W/kg, 41, 29, and 219 genes were shown to be differentially expressed; 94 of differential expression genes displayed a ratio higher than 2.5 or less than 0.4 and were provided in Tables S1-S3 (www.besjournal.com for details).

Validation of RT-PCR and Reproducibility

The expressional trends of the 30 differentially expressed genes in the three radiation groups were completely consistent. Furthermore, 9 differentially expressed genes with higher fold changes were verified in the 3.6 W/kg exposure group by semi-quantitative RT-PCR and showed consistent results (Figure 1).

Table 1. Primers Used for RT-PCR Reactions

Name of Gene	Sequence of Primer (5'-3')
β-actin	Forward: GCT CTT TTC CAG CCT TCC TT
	Reverse: GTG CTA GGA GCCAGA GCA GT
Synbindin (Sbdn)	Forward: ACT TTC AGT TAC CCG CTG GA
	Reverse: GCT CGC ATC TGA TAG GCA TT
Fas death domain-associated protein (Daxx)	Forward: TCT GTG GTG TCC GTC ATC TC
	Reverse: CAG TTC ATG GCT GGG AGA GT
mitogen-activated protein kinase 8 interacting protein 2 (MAPK8ip2)	Forward: GTG TTC CCT GCC TTC TAT GC
	Reverse: CCT CTG TAG GGC AGG CAA A
melanoma antigen, family D, 1 (Maged1)	Forward: GAC TAC GTC CTG GGG TGA GA
	Reverse: CAA TGA TGG GGC CAG TAA AG
mitogen-activated protein kinase 10 (MAPK10)	Forward: TGA CTC CGT ATG TGG TGA CG
	Reverse: ATG GTG TGC TCC CTT TCA TC
N-myc downstream regulated gene 2 (Ndr2）	Forward: TGG CCT TAC GTC TTC CAT TC
	Reverse: ACT CGC TACT CT GCG ACA GG
RAB6A, member RAS oncogene family (Rab6)	Forward: GTG TTC CTG GGA GAG CA GAG
	Reverse: GTG TGC TTT CCA TTC CAG GT
BCL2-interacting killer (Biklk)	Forward: TAC AGC CTC CGC TCT GAA TA
	Reverse: TCA CTG AAG CTG CAA ATA CCA
Fas apoptotic inhibitory molecule (Faim)	Forward: GCT GTT TGG GAC GTA GCA TT
	Reverse: TGG GAT TTC CCT GTT ATC CG

Identification of Cell Processes Modified by Exposure

Gene ontology (GO) analysis revealed that the differentially expressed genes within the 3 irradiated groups were involved in primary metabolic process, cellular metabolic process, regulation of biological process, macromolecular metabolic process, biosynthetic process, cellular protein metabolic process, transport, developmental process, cellular component organization, cell communication, response to stress and cellular homeostasis (Figure 2).

Identification of Pathways Modulated by Microwaves

KEGG pathway analysis revealed that the signaling pathways affected the most by microwaves were fatty acid elongation in the mitochondria (25%), vitamin B6 metabolism (14.29%), citrate cycle (TCA cycle) (9.38%), ribosome (9.09%), Alzheimer's disease (6.84%), Parkinson's disease (5.96%), Long-term potentiation (5.80%), Huntington's disease (5.50%), neurotrophin signaling (4.55%), glioma (4.55%), and long-term depression (4.17%) pathways. At the lower density, microwaves only affected a fewer number of genes in the ribosome, Alzheimer's disease, Parkinson's disease, long-term potential, Huntington's disease and neurotrophin signaling pathways. However, in the higher density group, fatty acid elongation in the mitochondria, vitamin B6 metabolism and the TCA cycle pathways were also affected, with nerve disease, learning and memory affected the most. Overall, radiation at a lower density induced a lower number of altered genes relative to a 3.6 W/kg exposure (Table 2).

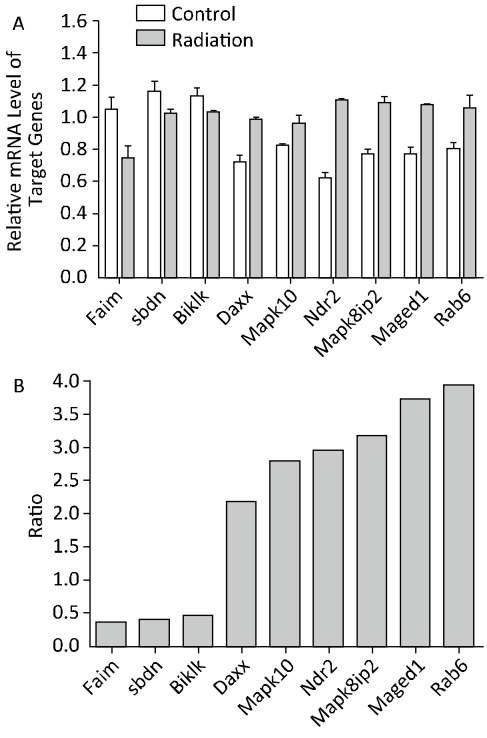

Figure 1. Examined gene expression alterations following a 3.6 W/kg exposure via RT-PCR (A) and gene microarray (B). Genes that were unaffected had a ratio lower than 0.5, while others exhibited over a two-fold increase relative to the control, which was consistent with the other approaches.

Identification of Clusters

Of the differentially expressed genes in the 3 irradiation groups, 91 with a ratio lower than 0.4 or higher than 2.5 were analyzed by cluster analysis (Figure 3). These differentially expressed genes were divided into two classes, upregulated and downregulated, and further subdivided into 9 subclasses, with the cluster information provided in Table S4 (www.besjournal.com for details).

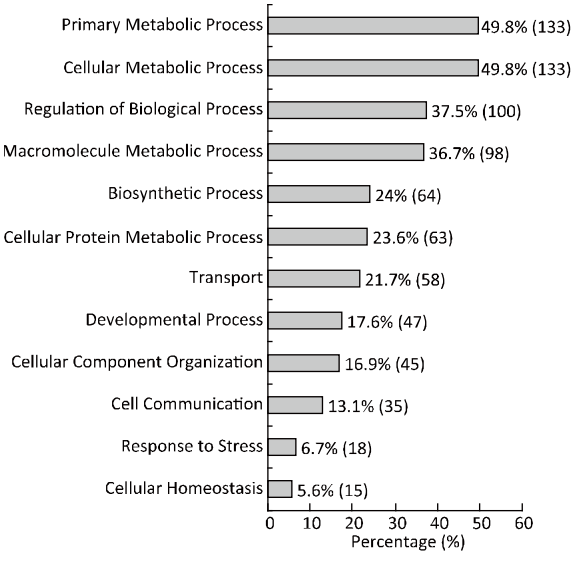

Figure 2. Functional annotations of the differentially expressed genes from 3 irradiation groups in GO Terms. The vertical-axis represents gene function by GO classification, the horizontal-axis represents the percentage of differentially expressed genes in that classification and the numbers in parentheses are the number of differentially expressed genes.

Cluster analysis found that the unknown genes, m0124c07, m2404h07, m0054d01, m0013b11, m2343d08, m2388g02, and m2337c05 had the same expression pattern as glutathione peroxidase 4 (Gpx4), succinate-CoA ligase, GDP-forming, beta subunit (Suclg2), tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein, theta polypeptide (Ywhaq), cholinergic receptor, nicotinic, beta polypeptide 3 (Chrnb3), N-myc downstream regulated gene 2 (Ndrg2), prosaposin (Psap) and protein phosphatase 2, regulatory subunit A, alpha (PPP2R1A). Notably, Sbdn, crystallin, alpha B(Cryab), ATPase, Ca^{2+} transporting, plasma membrane 2 (Atp2b2) and RAB GTPase activating protein 1 (Rabgap1) were clustered in the same class and showed a similar expressional pattern with an increased SAR.

Identification of Protein Interaction Networks

A protein interaction network was constructed for the differentially expressed genes (Figure 4). Proteins of nondifferentially expressed genes, tumor protein p53 (TP53), SMAD family member 2 (SMAD52), estrogen receptor 1 (alpha) (ESR1), epidermal growth factor receptor (EGFR), heat shock protein 90, alpha (cytosolic), class A member 1 (HSP90AA1) and mitogen-activated protein kinase 1 (MAPK1) and that of differentially expressing genes, sbdn, Cryab and protein phosphatase 1, catalytic subunit, alpha isoform (PPP1CA), YWHAQ, Ras homolog enriched in brain like 1 (RHEBL1), PSAP, ATP synthase, H^+ transporting, mitochondrial F1 complex, gamma polypeptide 1 (ATP5c1), proteasome (prosome, macropain) subunit, beta type 1 (PSMB1), poly(rC) binding protein 2 (PCBP2), protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform (PPP2CA), PPP2R1A, calmodulin 1 (Calm1), guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1 (Gnb2l1) and ATPase, Ca^{2+} transporting, plasma membrane 2 (Atp2b2) had a higher degree of linkage, which indicates that these proteins are important network regulators.

DISCUSSION

Due to its wide use, the effects of microwaves on learning and memory have been gaining attention. It has been reported that long term microwave exposure induces learning and memory deficits via mechanism including oxidative stress, mitochondrial damage, impairment of glutamate receptors and LTP induction, but more remained to be elucidated[7,10-11]. The effects of long-term, non-thermal microwave exposure on learning and memory abilities were dependent on the exposure parameters and research subjects. In small animal models, the long-term effects of protracted microwave exposure at a SAR level of 0.06-0.18 W/kg resulted in learning and memory reduction[4,12]. In the present study, mice were continuously irradiated with 2100 MHz microwaves at a SAR of 0.45 W/kg to 3.6 W/kg for 1 h/d for 8 weeks. These findings suggest that expressional changes within the brain may lead to learning and memory inhibition, with most of the gene alternations occurring at the highest SAR (3.6 W/kg). However, the number of gene alterations did

Table 2. KEGG Signal Pathways Modulated by Microwaves

Pathway Names	% of Genes to Total Number of Genes	Number of Altered Genes	Number of Altered Genes in Each Group		
			0.45 W/kg	1.8 W /kg	3.6 W/kg
Fatty acid elongation in mitochondria	25%	2	0	0	2
Vitamin B6 metabolism	14.29%	1	0	0	1
Citrate cycle (TCA cycle)	9.38%	3	0	0	3
Ribosome	9.09%	11	3	1	7
Alzheimer's disease	6.84%	13	3	2	10
Parkinson's disease	5.96%	9	2	2	7
Long-term potentiation	5.80%	4	1	0	4
Huntington's disease	5.50%	11	3	2	10
Neurotrophin signaling	4.55%	6	2	0	5
Glioma	4.55%	3	0	0	3
Long-term depression	4.17%	3	0	1	2

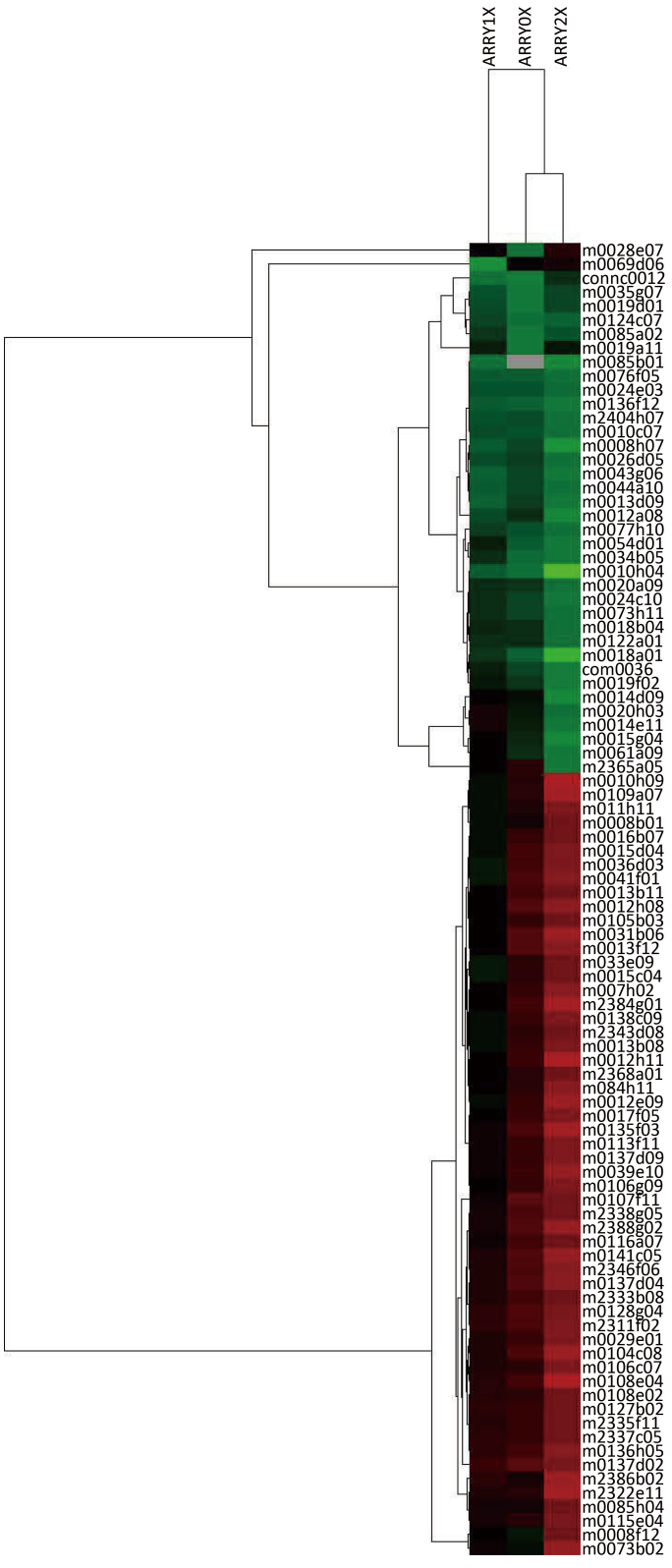

Figure 3. Hierarchical clustering of mouse brain gene expression profiles. Arrays 1, 0, and 2 represent exposures at 0.45 W/kg, 1.8 W/kg, and 3.6 W/kg respectively. This heatmap depicts hierarchical clustering, where genes are depicted as upregulated (red) or downregulated (green) using the GeneSpring GX software.

not increase with an increasing SAR since more genes were affected at 0.45 W/kg than at 1.8 W/kg, which may indicate an amplitude window effect. However, these surmises will require further investigation. Furthermore, the differentially expressed genes that were identified *via* gene microarray and confirmed by RT-PCR (Figure 1) were bioinformatically analyzed to globally characterize their mediating effects on learning and memory following long-term microwave exposure.

The Major Cellular Processes, Signal Pathways, and Clusters Pertaining to Microwaves Affected Learning and Memory

It has been proven that long-term memory is relative to metabolism, especially for protein synthesis in the brain, with continuous gene transduction and translation at synapses necessary for persistent alterations of synaptic contents[13]. In the present study, microwaves inducing differential gene expression were majorly involved in these cellular processes, such as cellular protein synthesis (23.6%), transport (21.7%) and development (17.6%), which indicates that microwaves affect learning and memory (Figure 2). Among the pathways affected by microwave exposure, ribosome, Alzheimer's disease, Parkinson's disease, LTP and Huntington's disease were the most sensitive due to their alterations at lower SAR levels, while fatty acid elongation in the mitochondria, vitamin B6 metabolism, TCA cycle and

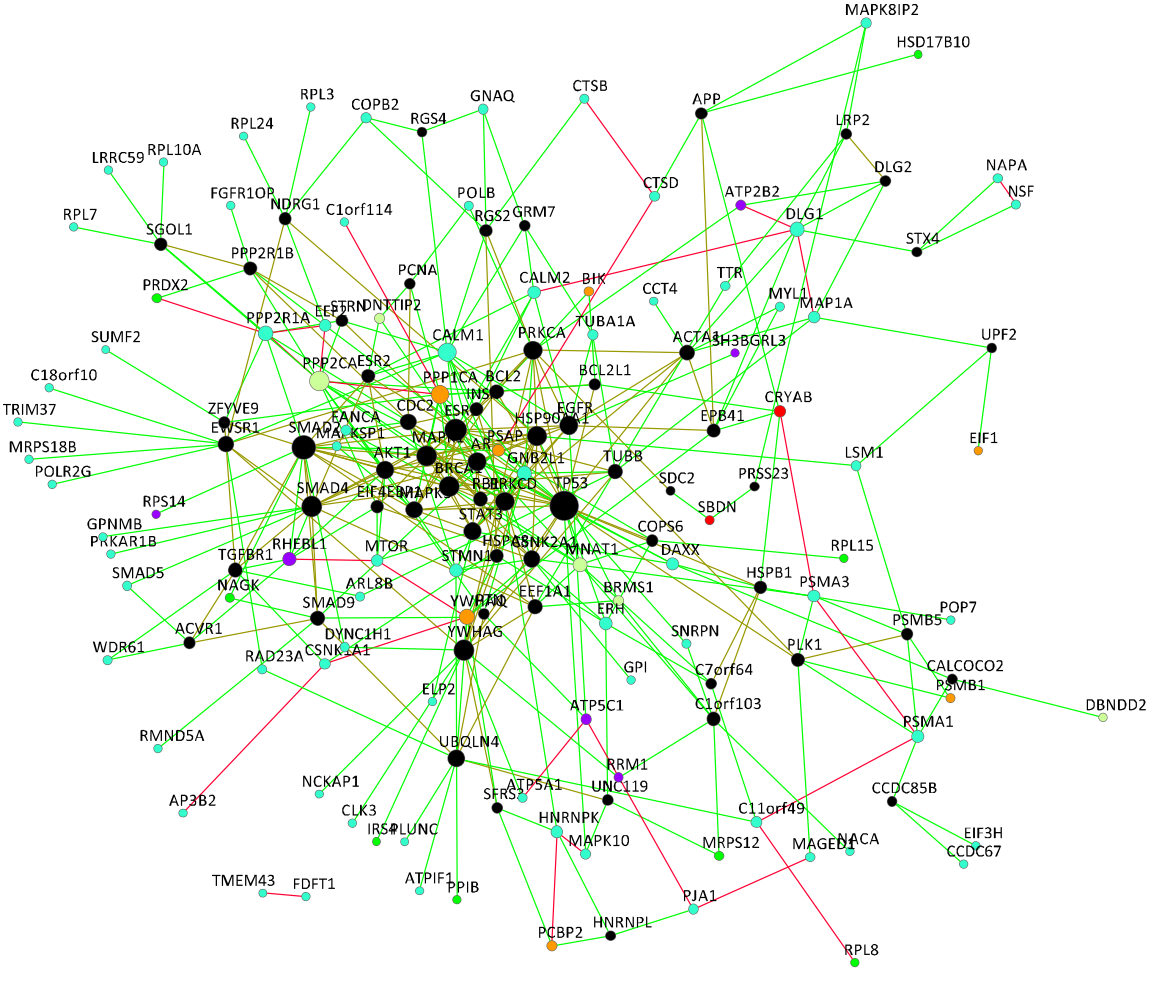

Figure 4. Protein interactions network for microwave exposure. Node colors represent experimental groups, to include, Group 1 (0.45 W/kg; green), Group 2 (1.8 W/kg; light green), Group 3 (3.6 W/kg; blue), Groups 1 and 3 (orange), Groups 2 and 3 (purple) and Groups 1-3 (red). Node size represents the degree of importance in the network. Edge colors represent interaction types, to include, interactions between differentially expressed genes (red), interactions between differentially expressed genes and others (brown) and interactions between other proteins (blue). Gene names highlighted in yellow are the most important in the network.

LTD pathways showed changes only at higher SAR levels, which also indicates that microwaves affect learning and memory differently depending on the SAR. Additionally, the highest density microwaves (3.6 W/kg) induced many metabolic genes, with this finding potentially correlated to the observed local heating or hotspots in the brain possibly due to high levels of energy absorption at 2100 MHz.

Based on the premise that genes with similar expression patterns most likely have the same functions, the functions of unknown genes could be speculated based on the functions of the known genes with similar expression patterns. Therefore, it is likely that the functions of unknown genes, m0124c07, m2404h07, m0054d01, m0013b11, m2343d08, m2388g02, and m2337c05 are similar to those of Gpx4, Suclg2, Ywhaq, Chrnb3, Ndrg2, Psap, and pPP2R1a. Notably, a subclass of Sbdn that included 22 genes was significantly inhibited by microwaves, as denoted by ratios less than 1. Furthermore, the subclass containing sbdn, Cryab, Atp2b2 and Rabgap1 was down-regulated in over two groups, which suggests a critical role in microwaves inhibiting learning and memory (Table S4, www.besjournal.com for details).

Important Regulatory Proteins Following Microwave Exposure

The constructed protein interaction network revealed that differentially expressed genes sbdn, Cryab, PPP1CA, Ywhaq, RHEBL1, PSAP, ATP5c1, PSMB1, PCBP2, PPP2CA, PPP2R1A, Calm1, Gnb2l1, and Atp2b2 had a higher degree of linkage and were critical protein regulators executing the effects of microwaves. Learning and memory are affected by a variety of factors, such as the generation and maintenance of LTP and LTD and their balance from the perspective of neurophysiology and metabolism, with protein metabolism in brain affecting neurobiochemistry and the establishment of new synaptic contacts for neuroanatomy. Additionally, some pathological factors, such as AD, aging and nerve inflammation also result in learning and memory deficiencies[14]. According to microwave sensitivity, degree of difference, pathway effects and protein linkage degree, 15 differentially expressed genes were selected to explain the plausible mechanisms for global microwave inhibition of learning and memory (Table 3).

Table 3. Important Regulatory Proteins that Mediate Effects of Long-term Microwave Exposure and Possibly Effect Learning and Memory

Gene Name	Differentially Expressing Genes	Relationship between Gene Expression and Learning and Memory	Effects on Learning and Memory
LTP associated molecules			
Calm1 and Calm2	Underexpression at the highest SAR	These two genes are central signal integrators for synaptic plasticity and have unique regulatory properties allowing the integration of several forms of signal transduction that are required for LTP and LTD[15-16]. So they play positive roles in learning and memory.	Inhibition
Guanine nucleotide binding protein, alpha q polypeptide (Gnaq)	Underexpression at the highest SAR	A guanine nucleotide binding protein member that is expressed almost ubiquitously in the central nervous system. A large number of neurotransmitters, hormones and sense stimulators mediate their physiological responses by activating the heterotrimeric Gαq couple receptor to activate phospholipase C (PLC)-β isoforms, phosphatidyl inositol(PI) hydrolysis and downstream secondary messenger signaling systems. Use of the Y-maze revealed spatial memory deficits in Gαq knockout mice[17-18]. So Gαq are positive related to learning and memory.	Inhibition
PPP1CA	Overexpression at 2 level of SAR	A protein phosphatase 1 member, able to make CREB dephosphorylate, inactivate it and suppress gene expression and LTP induction. A gain-of-function study clearly confirmed that contextual memory formation involves CREB and PP1 as positive and negative regulators[19]. So, Ppp1ca can negatively relate long-term and short-term memory.	Inhibition

Continued

Gene Name	Differentially Expressing Genes	Relationship between Gene Expression and Learning and Memory	Effects on Learning and Memory
Sbdn	Underexpression at 3 levels of SAR	1) Sbdn, also known as TRAPPC4, is the fourth subunit of a multiprotein complex, plays a role in ER-to-Golgi trafficking to transport synthetic proteins to the Golgi. Meanwhile, Sbdn also interacts with and activates ERK in the Golgi to facilitate efficient vesicle trafficking[20-21]. 2) Sbdn also interacts with ERK in the cytoplasm and promotes ERK1/2 nuclear translocation, activating transcription[22-23]. Subsequently, ERK activation modulates gene expression and stimulates protein synthesis to further induce LTP in the hippocampus and cortex[24-25]. 3) Sbdn, a physiological syndecan-2 ligand on dendritic spines, induces spine formation and early maturation by recruiting intracellular vesicles toward postsynaptic sites through interactions with synbindin[26]. So, sbdn is positively related to learning and memory.	Inhibition
Ywhaq	Underexpression at 2 levels of SAR	Overexpression of YWHAQ in the optic tectum promotes the synthesis or delivery of instructive information, synaptic remodeling and ultimately the expression of learned behavior[27]. The interaction of GABABRs with the adaptor protein 14-3-3 and the transcription factor activating transcription factor 4 (ATF4) activates G-protein signaling, cytoskeletal reorganization and nuclear gene expression which contributes to synaptic plasticity[28]. So, Ywhaq is positively related to learning and memory.	Inhibition
Atp2b2	Underexpression at the highest SAR	The encoded protein, a family of P-type primary ion transport ATPases, removes bivalent calcium ions from eukaryotic cells against very large concentration gradients and plays a critical role in intracellular calcium homeostasis. It was also reported to play critical roles in calcium dependent synaptic transmission, for EPSCs amplitude and a LTP induction decrease if Ca^{2+}-ATPase activity decreased[29]. So, Atp2b2 is positively related to learning and memory.	Inhibition
Rabgap1l	Underexpression at 2 levels of SAR	Rabgap1l, a GTPase activator, participates in vesicle trafficking and therefore is helpful for LTP induction and maintenance. Reduced KIAA0471 (according Rabgap1L in human) mRNA expression was found in Alzheimer's patients[30]. So, Rabgap1l is positively related to learning and memory.	Inhibition
LTD associated molecules			
Gnaq	Underexpression at the highest SAR	Learning and memory requires sustaining LTP and LTD and depends on constant gene transcription and translation at synapses, so as to keep synaptic contents changing[13,31]. While Gnaq is upstream of gene expression, its activation is commonly the basis of LTP and LTD induction[32]. So, Gnaq is positively related to learning and memory.	Remains obscure
PPP2CA and PPP2R1A	Overexpression at the highest SAR	ppp2ca and ppp2r1a are involved in protein dephosphorylation[33]. So, they are positively related to LTD induction.	
Molecules involved in inflammation and AD pathways			
MAPK8ip2	Overexpression at the highest SAR	This protein is expressed in the brain and is shown to interact with and regulate MAPK8/JNK1 activity and MAP2K7/MKK7 kinases, which relates to inflammation[34]. So, MAPK8ip2 is negatively related to learning and memory.	Inhibition

Continued

Gene Name	Differentially Expressing Genes	Relationship between Gene Expression and Learning and Memory	Effects on Learning and Memory
MAPK10	Overexpression at the highest SAR	Is positively related to LTP and positively related to LTP inhibition and inflammation. MAPK10, also known as JNK3, is majorly expressed in the brain, especially the cortex and hippocampus[35]. JNK activation was reported in AD patient and transgenic mice brains with Aβ overexpression, indicating that JNK mediates LTP impairment[36]. So, MAPK10 is negatively related to learning and memory.	Inhibition
Cryab	Underexpression at 3 SAR levels	Encodes a member of the small heat-shock protein (HSP20) family. The encoded protein is a molecular chaperone that protects proteins against thermal denaturation and other stresses[37-38]. Accumulated evidences reveal that CRYAB plays a critical role in suppressing neuroinflammation. Cryab also has the capability to protect cells against Aβ toxicity in AD mice models[39]. So, Cyrab is positively related to learning and memory.	Inhibition
RHEBL1	Underexpression at 2 SAR levels	It plays critical roles in cellular proliferation and transformation. The activation of mTOR is regulated by a small G-protein, Rheb1, essential for mTORC1 signaling and myelination in the brain and normal learning and memory[40]. So RHEBL1 is positively related to learning and memory.	Inhibition
ATP5c1	Underexpression at 2 SAR levels	ATP5c1, which encodes ATP synthetic kinases, is positively related to learning and memory.	Inhibition Coincided with previous studies[41-42].
PSMB1	Underexpression at 2 SAR levels	The encoded protein, a 20S proteasome, promotes cell growth and migration, as well as colony formation. PSMB1 is a transcriptional activator of Rbp4 and therefore promotes LTP induction[43-44]. So, PSMB1 is positively related to learning and memory.	Inhibition
PCBP2	Underexpression at 2 SAR levels	The encoded protein, α-CP2, functions as a transcriptional activator by binding to a single-stranded poly(C) sequence[45]. PCBP2 also provides for the selective expression of cell survival factors through posttranslational events[46]. So, PCBP2 is positively related to learning and memory.	Inhibition
Rab6	Overexpression at the highest SAR	Rab6, a subfamily of small GTPases, is predominantly expressed in brain. It regulates retrograde transport from the late endosomes via the Golgi to the ER and in the transition from anaphase to metaphase during mitosis[47]. AD patients have increased brain levels, suggesting that Rab6 activation is increased in response to early pathogenic changes in AD[48-49]. So, Rab6 is negatively related to learning and memory.	Inhibition

Molecules Involved in the LTP Pathway

Previous studies have shown that LTP induction and maintenance is the neurophysiological basis of learning and memory[14]. In the KEGG pathway database, the LTP pathway included presynaptic neurotransmitter release, modulation of postsynaptic transmitter receptors (AMPAR, NMDAR, mGlu, VDCC), secondary messengers (calcium/calmodulin, G protein-q, IP3), kinase cascade (PKA, PKC, Ras-Raf-MEK1/2-ERK1/2, CaMKII, PP1), and effector kinases (CREB, CaMKIV), which induce and sustain LTP by gene expression regulation and synaptic protein synthesis. This study showed that 5.8 percent of the total genes within the pathway were differentially expressed, including CALM1, CALM2, Gnaq, and PPP1CA. These same differentially expressed genes (Table 3) could be the

critical to the mechanism suppressing learning and memory due to LTP inducting and sustaining impairment. Moreover, other genes not typically associated with the LTP pathway or new genes could also affect learning and memory by modifying the molecules typically associated with the pathway. Notably, some important positive regulators in the LTP pathway, Sbdn, Cryab, Rabgap1, and Atp2b2 were clustered into the same subclass and coincidentally downregulated, which could play a significant role in learning and memory inhibition. Previous studies have also shown that impairment of LTP induction was essential for the disruption of spatial memory after microwave exposure[10].

Molecules Involved in the LTD Pathway

What roles of LTD in learning and memory were not as clear as that of LTP. Recent studies have suggested that LTD, similar to LTP, is the neurobiological basis of learning and memory. It participates in the consolidation of spatial memory, information deletion, plasticity, and refining. The post-learning information pruning, sculpting, and crystalizing process requires LTD-like processes to remove less relevant information from learning or disconnect contradictory knowledge already present in long-term memory[31]. LTP and LTD can overcome each other's effects, leading to a situation in which synapses operate over a dynamic range of efficacy in order to keep normal learning and memory ability. If this balance is disturbed, the cognition ability will be decreased. The exact impact of changes in LTD induction on learning and memory is complex due to different pathways and the balance between LTP and LTD. This study found that 4.17% of the differentially expressed genes belonged to the LTD pathway, to include, Gq, PPP2CA and pPP2R1A (Table 3). While Gnaq downregulating would decrease LTD induction, PPP2CA and PPP2R1A upregulating would increase its induction. In addition to balancing between LTP and LTD, the exact impact of altering these genes as it relates to learning and memory is more complex and requires further investigation.

Molecules Involved in Inflammation and AD Pathways

Evolving evidence suggests that brain inflammation and early stages of Alzheimers disease (AD) increases the risk for cognitive decline and cognitive dysfunction by positively regulating the JNK pathway[34]. As listed in Table 3, alterations in MAPK10, Cryab, etc. expression could inhibit learning and memory by the AD and inflammation pathways.

CONCLUSION

Microwave exposure is speculated to decrease LTP induction, increase LTD induction, disrupt the balance of LTD and LTP, reduce dendrite spines formation, affect the inflammatory response and impact early AD responses by the directly or indirectly modulating important pathway regulatory molecules, eventually impairing learning and memory. Among the pathways affected by microwaves, the LTP and LTD pathways were affected the most. The most notably genes were sbdn and Cryab, since they responded sensitively to microwaves at all 3 SAR levels. Microwaves are thought to inhibit learning and memory by sbdn mediating LTP and dendrite formation, while Cryab mediates inflammation and early AD reactions. These findings provide important clues into the mechanisms and possible counter measures pertaining microwave induced learning and memory inhibition (Figure 5). However, more research is required to further characterize the roles of both the known or unknown genes and how they function in microwaves affecting learning and memory.

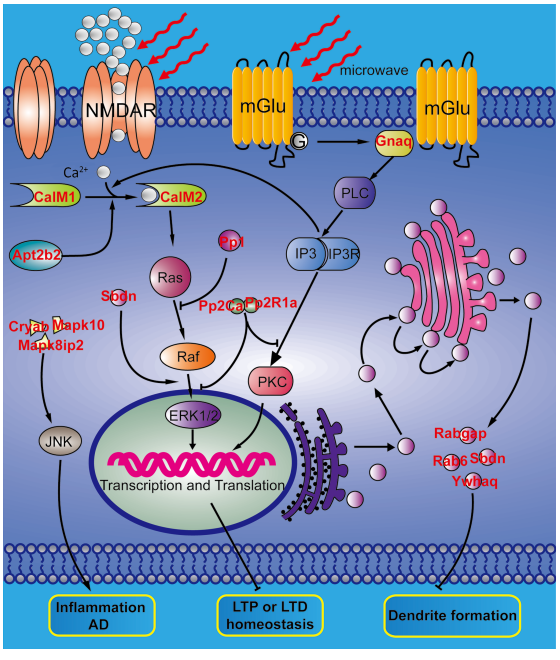

Figure 5. Schematic of the mechanisms of microwave induced learning and memory deficiencies. Differentially expressing genes are in red.

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