What is a scientific article?

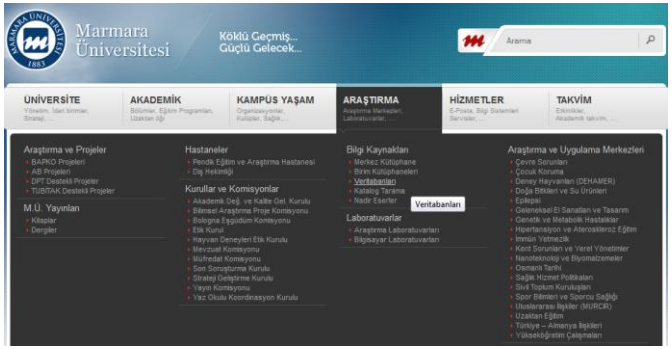
- A scientific article presents research results and is written by researchers and aimed at an academic readership.
- The article must have been reviewed by experts within the same subject area before publication (peer review).
- Scientific articles can be divided into different types:
- Original articles where the author presents empirical studies and for the first time describes the results of research work.
- Review articles are critical reviews of previously published studies.
- Theoretical articles aim at developing new theories from existing research.

Where to find scientific articles?

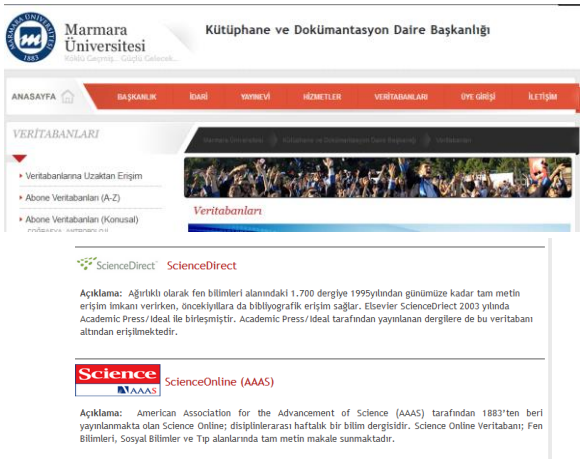
- A scientific articles published in scientific journals and can be reached by various ways.
- Journals in physical form can be purchased.
- Many academic institutions provide subscriptions to their members for the scientific journals.
- ULAKBİM (Ulusal Akademik Ağ ve Bilgi Merkezi)
- Today, most of the recent scientific articles is made online and can be reached as an electronic version of the printed articles.
- Also, some scientific journals begin to published electronic versions only and usually called as open access journals.

Where to find scientific articles?

- Our University also provide subscriptions to many scientific journal.



Where to find scientific articles?



How to read scientific articles?

Taken from : **How to Read a Scientific Article**

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Cain Project in Engineering and Professional Communication

- Reading a scientific article is a complex task.
- The worst way to approach this task is to treat it like the reading of a textbook—reading from title to literature cited, digesting every word along the way without any reflection or criticism.
- Rather, you should begin by skimming the article to identify its structure and features.
- As you read, look for the author's main points. Generate questions before, during, and after reading. Draw inferences based on your own experiences and knowledge.
- And to really improve understanding and recall, take notes as you read.

How to read scientific articles?

1. Skim the article and identify its structure.

- Most journals use a conventional IMRD structure: An abstract followed by Introduction, Methods, Results, and Discussion.
- Each of these sections normally contains easily recognized conventional features, and if you read with an anticipation of these features, you will read an article more quickly and comprehend more.
  - Features of Abstracts: Abstracts usually contain four kinds of information; purpose or rationale of study (why they did it), methodology (how they did it), results (what they found), conclusion (what it means)
- Most scientists read the abstract first. Others—especially experts in the field—skip right from the title to the visuals because the visuals, in many cases, tell the reader what kinds of experiments were done and what results were obtained.
- You should probably begin reading a paper by reading the abstract carefully and noting the four kinds of information outlined above. Then move first to the visuals and then to the rest of the paper.

How to read scientific articles?

- Features of Introductions
- Introductions serve two purposes: creating readers' interest in the subject and providing them with enough information to understand the article.
- Generally, introductions accomplish this by leading readers from broad information (what is known about the topic) to more specific information (what is not known) to a focal point (what question the authors asked and answered).
- Thus, authors describe previous work that led to current understanding of the topic (the broad) and then situate their work (the specific) within the field.

How to read scientific articles?

- Features of Methods
- The Methods section tells the reader what experiments were done to answer the question stated in the Introduction.
- Methods are often difficult to read, especially for graduate students, because of technical language and a level of detail sufficient for another trained scientist to repeat the experiments.
- However, you can more fully understand the design of the experiments and evaluate their validity by reading the Methods section carefully.

How to read scientific articles?

- Features of Results and Discussion
- The Results section contains results—statements of what was found, and reference to the data shown in visuals (figures and tables).
- Normally, authors do not include information that would need to be referenced, such as comparison to others' results.
- Instead, that material is placed in the Discussion—placing the work in context of the broader field.
- The Discussion also functions to provide a clear answer to the question posed in the Introduction and to explain how the results support that conclusion.

How to read scientific articles?

- Atypical Structure
- Some articles you read will deviate from the conventional content of IMRD sections. For instance, Letters to Nature appear to begin with an abstract, followed by the body of the article.
- Upon reading, however, you will see that the "abstract" is a summary of the work filled with extensive introduction (for the purpose of catching the attention of a wide audience), and the next paragraph begins a description of the experiments.
- Therefore, when you begin to read an article for the first time, skim the article to analyze the document as a whole. Are the sections labeled with headings that identify the structure?
- If not, note what the structure is. Decide which sections contain the material most essential to your understanding of the article.

How to read scientific articles?

- 2. Distinguish main points.
- Because articles contain so much information, it may be difficult to distinguish the main points of an article from the subordinate points.
- Fortunately, there are many indicators of the author's main points:
  - Document level: Title, Abstract, Keywords, visuals (especially figure and table titles), first sentence or the last 1-2 sentences of the Introduction.
  - Paragraph level: words or phrases to look for; we hypothesize that, we propose, we introduce, we develop, the data suggest.

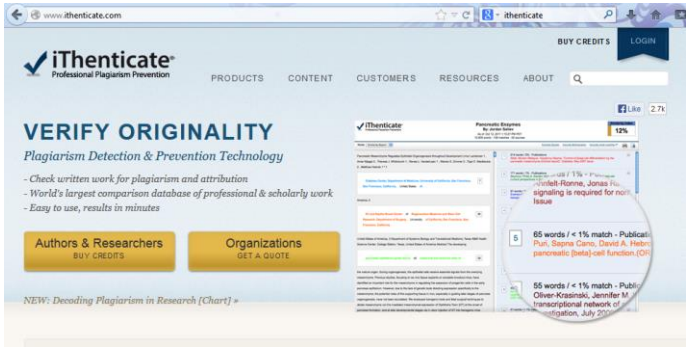
How to read scientific articles?

- Generate questions and be aware of your understanding
- Reading is an active task. Before and during your reading, ask yourself these questions:
  - Who are these authors? What journal is this? Might I question the credibility of the work?
  - Have I taken the time to understand all the terminology?
  - Have I gone back to read an article or review that would help me understand this work better?
  - Am I spending too much time reading the less important parts of this article?
  - Is there someone I can talk to about confusing parts of this article?

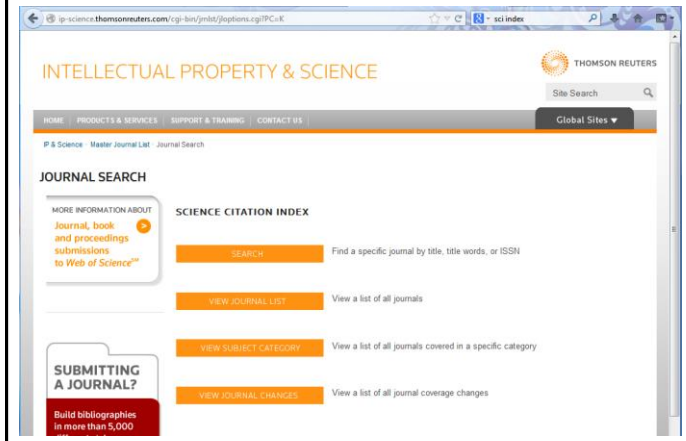
How to read scientific articles?

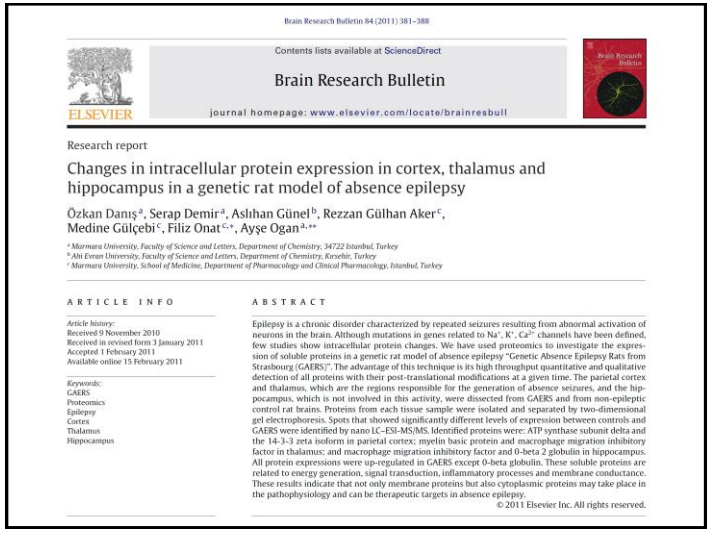
- After reading, ask yourself these questions:
  - What specific problem does this research address? Why is it important?
  - Is the method used a good one? The best one?
  - What are the specific findings? Am I able to summarize them in one or two sentences?
  - Are the findings supported by persuasive evidence?
  - Is there an alternative interpretation of the data that the author did not address?
  - How are the findings unique/new/unusual or supportive of other work in the field?
  - How do these results relate to the work I'm interested in? To other work I've read about?
  - What are some of the specific applications of the ideas presented here?
  - What are some further experiments that would answer remaining questions?

- Plagiarism; an act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author:
- 2. a piece of writing or other work reflecting such unauthorized use or imitation: "These two manuscripts are clearly plagiarisms," the editor said, tossing them angrily on the floor.
- Plagiarism is the "wrongful appropriation" and "purloining and publication" of another author's "language, thoughts, ideas, or expressions," and the representation of them as one's own original work. The idea remains problematic with unclear definitions and unclear rules.
- Plagiarism is considered academic dishonesty and a breach of journalistic ethics. It is subject to sanctions like expulsion. Plagiarism is not a crime per se but in academia and industry it is a serious ethical offense, and cases of plagiarism can constitute copyright infringement.



- The impact factor (IF) of an academic journal is a measure reflecting the average number of citations to recent articles published in the journal.
- It is frequently used as a proxy for the relative importance of a journal within its field, with journals with higher impact factors deemed to be more important than those with lower ones.
- The impact factor was devised by Eugene Garfield, the founder of the Institute for Scientific Information.
- The Science Citation Index (SCI) is a citation index originally produced by the Institute for Scientific Information (ISI) and created by Eugene Garfield.
- It was officially launched in 1964. It is now owned by Thomson Reuters.
- The larger version (Science Citation Index Expanded) covers more than 6,500 notable and significant journals, across 150 disciplines, from 1900 to the present. These are alternately described as the world's leading journals of science and technology, because of a rigorous selection process.





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Research report

Changes in intracellular protein expression in cortex, thalamus and hippocampus in a genetic rat model of absence epilepsy

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ABSTRACT

Epilepsy is a chronic disorder characterized by repeated seizures resulting from abnormal activation of neurons in the brain. Although mutations in genes related to Na^+, K^+, Ca^{2+} channels have been defined, few studies show intracellular protein changes. We have used proteomics to investigate the expression of soluble proteins in a genetic rat model of absence epilepsy "Genetic Absence Epilepsy Rats from Strasbourg (GAERS)". The advantage of this technique is its high throughput quantitative and qualitative detection of all proteins with their post-translational modifications at a given time. The parietal cortex and thalamus, which are the regions responsible for the generation of absence seizures, and the hippocampus, which is not involved in this activity, were dissected from GAERS and from non-epileptic control rat brains. Proteins from each tissue sample were isolated and separated by two-dimensional gel electrophoresis. Spots that showed significantly different levels of expression between controls and GAERS were identified by nano LC–ESI-MS/MS. Identified proteins were: ATP synthase subunit delta and the 14-3-3 zeta isoform in parietal cortex; myelin basic protein and macrophage migration inhibitory factor in thalamus; and macrophage migration inhibitory factor and 0-beta 2 globulin in hippocampus. All protein expressions were up-regulated in GAERS except 0-beta globulin. These soluble proteins are related to energy generation, signal transduction, inflammatory processes and membrane conductance. These results indicate that not only membrane proteins but also cytoplasmic proteins may take place in the pathophysiology and can be therapeutic targets in absence epilepsy.

1. Introduction

Epilepsy is a common serious neurological condition that is characterized by recurrent seizures and affects more than 0.5% of the world population [7]. Although earlier studies have defined mutations and polymorphisms in genes related to Na^+, K^+ and Ca^{2+} ion channels and to neuronal signalling in some types of epilepsy, there are few studies showing intracellular protein changes [24,51]. Proteomics that is a technique enables one to find protein changes responding to different states in cells [17], may be useful to understand the mechanisms underlying the diseases [10].

Absence epilepsy is a particular epileptic syndrome in which patients show generalized non-convulsive seizures characterized by a brief unresponsiveness to environmental stimuli and a cessation of motor activity [31,46]. Spike-and-wave discharges in the electroencephalogram (EEG) are the hall mark of the seizures. Several studies have pointed out that hypersynchronization in thalamo-cortical circuits is the major mechanism underlying absence epilepsy [47]. Recently, experimental studies of genetic rat models of absence epilepsy have indicated that the perioral region of somatosensory cortex initiates the seizure activity in the first milliseconds of a seizure and then entrains the thalamus to sustain the activity in the thalamo-cortical circuit and produce generalized spike-and-wave activity [36,48]. One of the most studied genetic rat models are the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a fully inbred strain of rats, with 100% of animals displaying the EEG and behavioural characteristics similar to those observed in human absence epilepsy [12]. No structural changes were observed in these animals but several changes at the subcellular level have been shown, such as changes in receptor subunits and ion channel expressions. For example, the mRNA of the alpha1G subunit of low-voltage activated calcium channel was elevated in the neurons of ventral posterior relay nuclei of the thalamus in GAERS compared to control animals [56], mRNA and protein

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2. Materials and methods

2.1. Materials

Immobilized pH-gradient (IPG) strips, tributyl phosphine, ampholyte pH 3–10 were purchased from BioRad (BioRad Laboratories, Hercules CA, USA). Dithiothreitol (DTT), acrylamide, *N,N*′-methylenebisacrylamide, TEMED (*N,N,N*′,*N*′-tetramethyl-ethane-1,2-diamine), iodoacetamide, protease inhibitor cocktail, Tris, urea, thiourea, ASB-14, alfa-naphtol, anti-rabbit IgG (whole molecule) – alkaline phosphatase antibody produced in goat, fast red, nitrocellulose membranes were all from Sigma Chemical Co., St. Louis, MO, USA. Myelin basic protein antibody was from Abcam Inc., Cambridge, MA, USA. Anti-14-3-3 zeta was from Anaspec, San Jose, CA, USA. All the chemicals used were analytical grade.

2.2. Experimental animals

Four to six months old male non-epileptic control Wistar ($n = 6$) and GAERS ($n = 8$) rats weighing 250–300 g were used in the study. All the animals were housed in a temperature-controlled room (20 ± 3 °C) with a 12-h light–dark cycle and were allowed free access to commercial rat pellets and tap water. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the experimental protocol was approved by the Animal Care and Use Committee of Marmara University (Protocol number: 43.2004.Mar).

2.3. Sample preparation

Animals were decapitated under ether anaesthesia, brains were quickly removed and washed twice in ice cold homogenization buffer consisting of 7 M urea, 2 M thiourea, 1% (w/v) ASB-14, 40 mM Tris base, 0.001% (w/v) bromophenol blue, 1% (v/v) tributylphosphine, 5% (v/v) protease inhibitor cocktail, 0.5% (w/v) ampholyte pH 3–10. After the brain was placed in a dish on ice, parietal cortex and hippocampal tissue were dissected from one hemisphere, thalamic tissue was dissected from the other hemisphere.

For each experimental animal about 40–60 mg wet weight of tissue from the hippocampus, thalamus and parietal cortex were separately grounded up in liquid

by adding 0.3 volumes of 2% formic acid. After incubation for 1 h the supernatant was transferred to a new reaction tube or directly applied to nano-LC–ESI-MS/MS analysis.

2.7. Nano-LC–ESI-MS/MS analysis

Protein identification using Nano LC–ESI-MS/MS was performed by Proteome Factory (Proteome Factory AG, Berlin, Germany). The MS system consists of an Agilent 1100 NanoLC system (Agilent Technologies, Waldbroon, Germany), a PicoTip emitter (New Objective, Woburn, MA, USA) and an Esquire 3000 plus ion trap MS (Bruker, Bremen, Germany). Protein spots were in-gel digested by trypsin (Promega, Mannheim, Germany) and applied to nano LC–ESI-MS/MS. After trapping and desalting the peptides on an enrichment column (Zorbax SB C18, 0.3 mm × 5 mm, Agilent Technologies, Waldbroon, Germany) using 1% acetonitrile, 0.1% formic acid solution for 5 min, peptides were separated on a Zorbax 300 SB C18, 75 μm × 150 mm column (Agilent Technologies, Waldbroon, Germany) using an acetonitrile, 0.1% formic acid gradient from 5% to 40% acetonitrile within 40 min. MS spectra were automatically taken by Esquire 3000 plus according to the manufacturer's instrument settings for nano-LC–ESI-MS/MS analyses. Proteins were identified using the MS/MS ion search of Mascot search engine (Matrix Science, London, UK) and nr protein database (National Center for Biotechnology Information, Bethesda, MD, USA). Search parameters used for MS/MS ion search of the Mascot Search engine are as follows: variable modifications: oxidation, mass values: monoisotopic, protein mass: unrestricted, peptide mass tolerance: ±0.1%, fragment mass tolerance: ±0.5 Da, maximum missed cleavages: 1. Instrument type is ESI-QUAD-TOF, number of queries is 300. Probability-based Mowse scores above the calculated threshold value ($p < 0.05$) were considered for protein identification.

2.8. Western blotting

Western blotting was carried out according to Towbin et al. [57]. After completion of the 2-DE, the polyacrylamide gels were soaked in transfer buffer (100 mM Tris, 135 mM glycine) and then transferred onto nitrocellulose membranes (Sigma Chemical Co., St. Louis, MO, USA). The membranes were washed three times in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20) and then blocked in 2% BSA in TBS (50 mM Tris, pH 7.5, 150 mM NaCl) for at least 2 h. The membranes were

The passive rehydration was carried out for 12 h. Isoelectric focusing was performed by using a Protean IEF cell (BioRad Laboratories, Hercules, CA, USA). Focusing was started at 250 V, and after 20 min the voltage was gradually increased to 10,000 V in a linear mode during 150 min and, finally, 10,000 V was applied until 52 kV·h was reached. The temperature was kept at 20 °C. After isoelectric focusing the strips were equilibrated in equilibration buffer I and equilibration buffer II for 15 min each sequentially according to the manufacturer's instructions. The equilibrated strips were then placed onto second dimension 12.5% SDS–PAGE gels. The SDS–PAGE was conducted in a standard Tris–Glycine–SDS buffer in Protean II xi Cell (BioRad Laboratories, Hercules, CA, USA) at a constant current setting of 20 mA/gel for 1 h, then at 40 mA/gel until the bromophenol blue dye reached the end of the gel. Gels were stained by the colloidal Coomassie staining method [41].

3. Results

3.1. 2-DE of proteins in parietal cortex, thalamus and hippocampus

Protein extracts from the parietal cortex, thalamus and hippocampus of GAERS ($n=8$) and control animals ($n=6$) were separated by 2-DE and the protein spots were visualized by colloidal Coomassie staining and compared between the GAERS and the controls using the PDQuest 2D-gel analysis software as

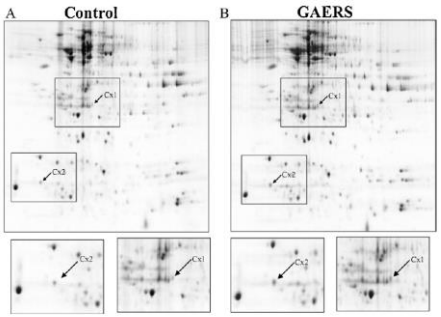

Fig. 1. Two-dimensional gel electrophoresis patterns of parietal cortex tissue of control (A) and GAERS (B) groups. Up-regulated spots in the GAERS are shown as Cx1 and Cx2 with arrows in the figures. Magnified images represent the regions defined in the rectangular boxes of each gel. Spots Cx1 and Cx2 were identified as subunit delta of ATP synthase and 14-3-3 zeta isoform, respectively, by nano LC–ESI-MS/MS analysis.

3.2.2. Thalamus

Fig. 2 shows the comparison of 2D gel patterns obtained from the thalamus of control (Fig. 2A) and GAERS (Fig. 2B). Two differentially expressed spots (T1 and T2) were identified as myelin basic protein and macrophage migration inhibitory factor, respectively. In the Mascot search, 7 queries matched with this T2 protein and 3 of these are unique peptides for more details see supplementary Table S1. T1 was predicted to be localized in the cytoplasm and nucleus, whereas T2 in the cytoplasm. Both were upregulated in GAERS ($p<0.05$). The results related to these spots are shown in Table 1.

4. Discussion

This proteomics study showed alterations in the expression of intracellular proteins obtained from the parietal cortex, thalamus and hippocampus in rats with genetic absence epilepsy. The identified proteins were the delta subunit of ATP synthase and the 14-3-3 zeta isoform in the parietal cortex, myelin basic protein and macrophage migration inhibitory factor in the thalamus, and macrophage migration inhibitory factor and 0-beta 2 globulin in the hippocampus.

Table 1
Differentially expressed proteins in GAERS identified by Nano LC–ESI-MS/MS.

Spot no	Protein identity	Accession no (NCBI–swissprot)	Taxonomy	Subcellular localization	Function	PM*/SC/Mascot score	Change in epilepsy	Two-tailed p value
Cx1	ATP synthase subunit delta, mitochondrial	BAB27577–P35434	*Mus musculus*	Mitochondria	H^+ ion transport and ATP synthase	1/8%/72	↑	0.01
Cx2	14-3-3 zeta isoform	AAA80544–P63102	*Rattus norvegicus*	Cytoplasm	Protein complex binding	7/3%/402	↑	0.01
T1	Myelin basic protein	AAB59712–P04370	*Mus musculus*	Cytoplasm and nucleus	structural constituent of myelin sheath	2/14%/91	↑	0.05
T2	Macrophage migration inhibitory factor	AAA62644–P30904	*Rattus norvegicus*	Cytoplasm and nucleus	Cytokine	3/33%/115	↑	0.05
H1	Macrophage migration inhibitory factor	AAA62644–P30904	*Rattus norvegicus*	Cytoplasm and nucleus	Cytokine	2/15%/112	↑	0.01
H2	0-Beta 2 globin	CAA47877–Q62670	*Rattus norvegicus*	Cytoplasm	Heme binding	3/23%/180	↓	0.05

Spot numbers correspond to 2D gels in Figs. 1–3. Proteins were identified by MS/MS analysis and MASCOT search of MS/MS spectra with BLAST. All identifications met statistical confidence criteria according to MASCOT and BLAST scoring schemes. ↑: upregulated and ↓: downregulated. Subcellular localization is predicted by WoLF-PSORT search engine. PM: peptides matched and SC: sequence coverage.

increased Ca^{2+} sensitivity led to a greater potassium conductance so that enhancement of inhibitory currents can switch neurons into a bursting mode as seen in absence epilepsy. Similarly, upregulation of myelin basic protein in the thalamus of GAERS can play a role in the hyperpolarization in the thalamic relay cells that are responsible for the generation and maintenance of spike-and-wave activity. Hyperpolarization of thalamo-cortical neurons and a subsequent rebound low-threshold Ca^{2+} spike are involved in the spike-and-wave oscillatory activity and physiopathology of absence epilepsy [35].

We found that the macrophage migration inhibitory factor (spots H1 and T2) is upregulated in both hippocampal and thalamic regions of GAERS relative to controls. The migration inhibitory factor is universally expressed in immune and nonimmune tissues and has extensive actions in the immune, endocrine, and nervous systems [2,42]. In the nervous system it was shown that it is constitutively expressed in neurons in the hippocampus, cortex, hypothalamus and pons [2] playing a role in the modulation of nitric oxide and prostaglandin production, catecholamine metabolism, regulation of neuronal sensitivity to glucocorticoids [19] and increases in neuronal delayed rectifier K^+ currents [34]. Migration inhibitory factor, as a proinflammatory cytokine, plays a pivotal regulatory role in the immune response and is implicated in the pathogenesis of many acute and chronic inflammatory diseases such as sepsis, acute respiratory stress syndrome, multiple sclerosis, neuro-Behcet's disease, and romatoid arthritis [9,14]. Involvement of inflammation and inflammatory cytokines in the

entific Research Project under grants FEN-DKR-130206-0016 and FEN-DKR-130206-0017. The authors thank Ray Guillery for his criticism of an earlier draft of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.brainresbull.2011.02.002.

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5. Conclusions

This study showed changes in the intracellular protein expression by proteomics techniques 2-DE combined with MS in GAERS relative to non-epileptic control rats. The changes at the level of ion channels and receptors are thought to play a principal role in the generation of spike-and-wave activity and intracellular proteins are not primarily responsible for the altered neuronal excitability during seizures. Nevertheless, there are significant experimental data suggesting these soluble proteins play an essential role in the generation of energy (delta subunit of ATP synthase), ion channel localization and signal transduction (14-3-3 zeta), inflammatory processes (macrophage inhibitory factor), membrane K^+ conductance (myelin basic protein, 0-beta globulin) that are important in neuronal function and excitability. Yet, the definite function of these proteins and their relation to the mechanisms of absence epilepsy need to be investigated in future studies.

Conflicts of interest

The author declares that there are no conflicts of interest.

Acknowledgments

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