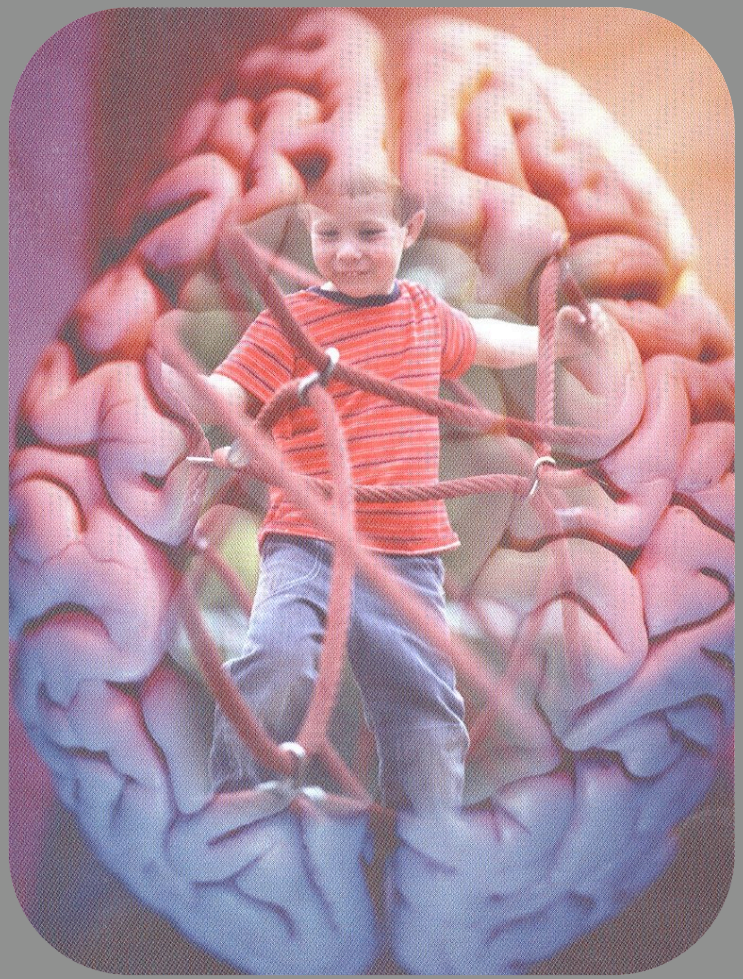
The modification of biocellular chemical reactions by physicochemical stimulants in the environment

Masami Ishido

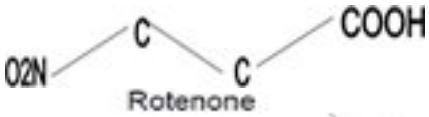
Environmetnal Risk Res Programme, National Institute for Environmental Studies

16-2 Onogawa, Tsukuba 305-8506, Japan

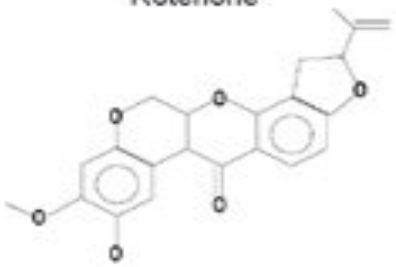


Health risk assessment

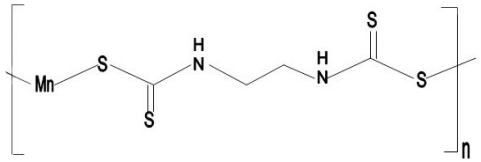
Environmental physicochemicals

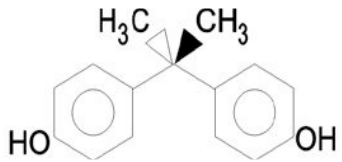


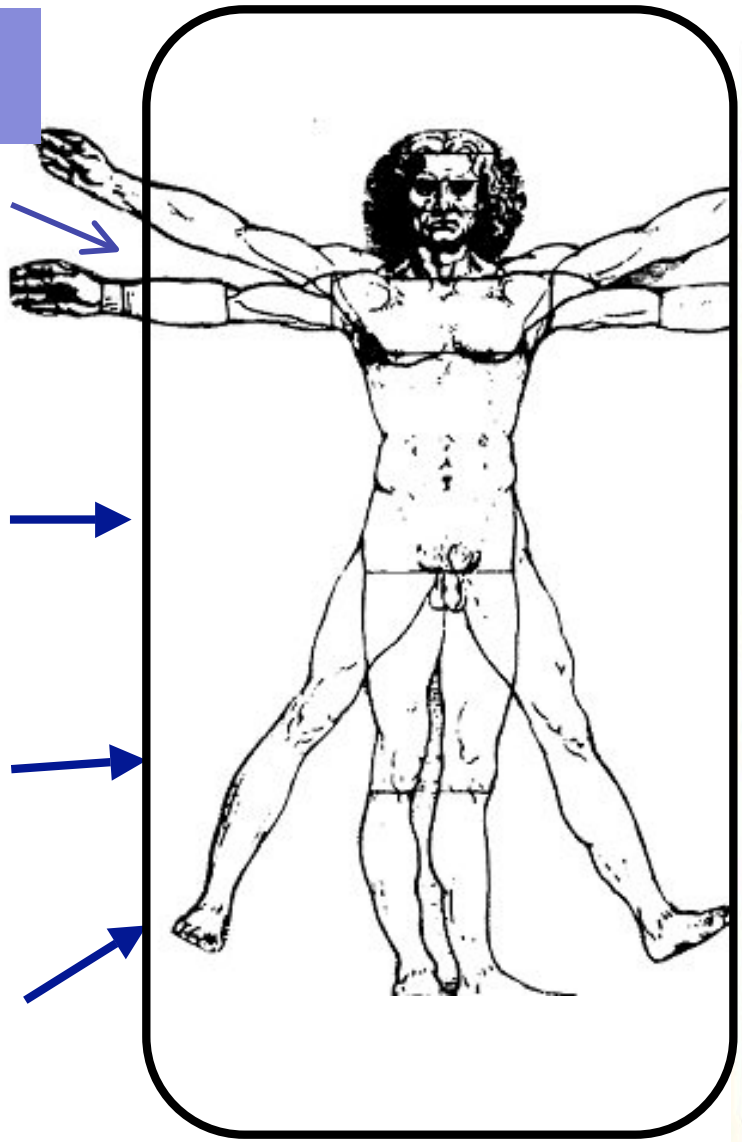
Rotenone

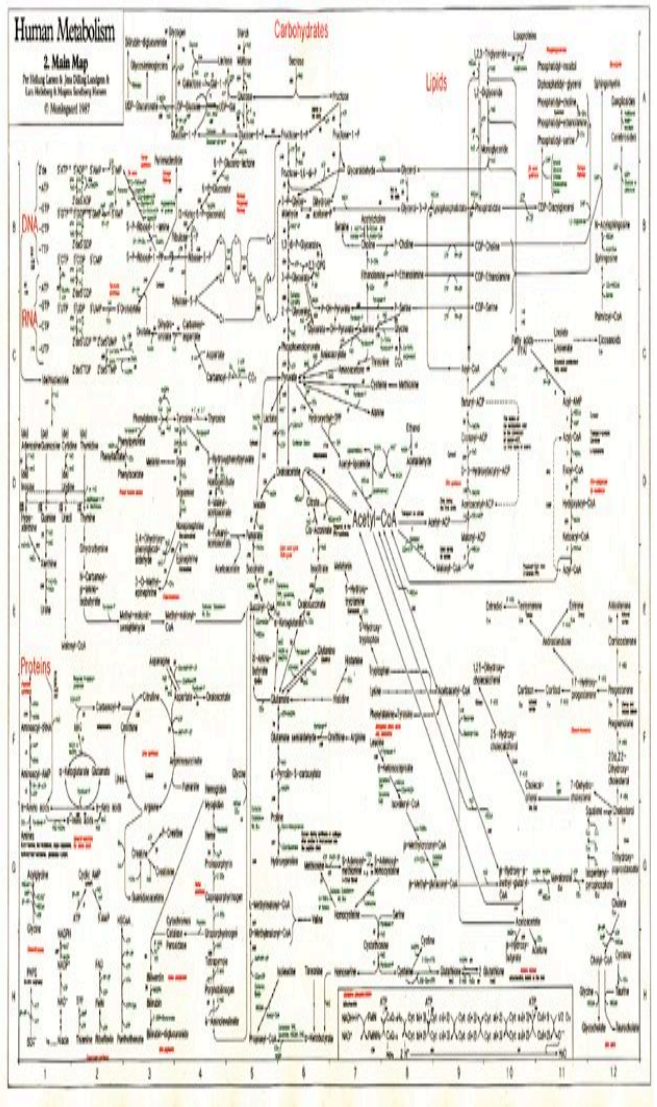


Maneb









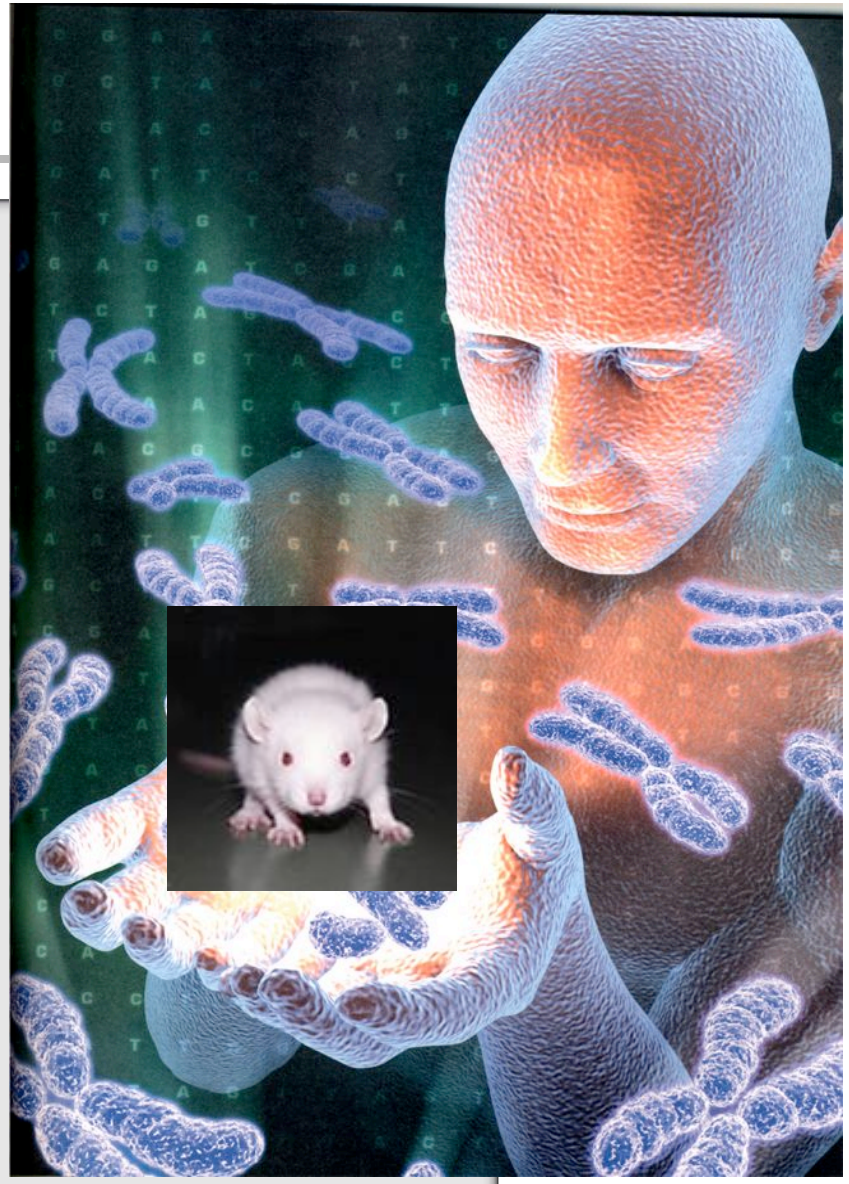
CONTENTS

1. Renal LLC-PK1 cells
   cadmium cytotoxicity

2. Human breast cancer MCF-7 cells
   1) Electromagnetic fields
   2) Estrogenic chemicals

3. Neural stem cells
   Developmental toxic and
   neurodegenerative chemicals

4. Model rats
   Environmental chemicals
   1) Hyperkinesia (Hyperactivity disorder)
   2) Hypokinesia (Parkinsonism)



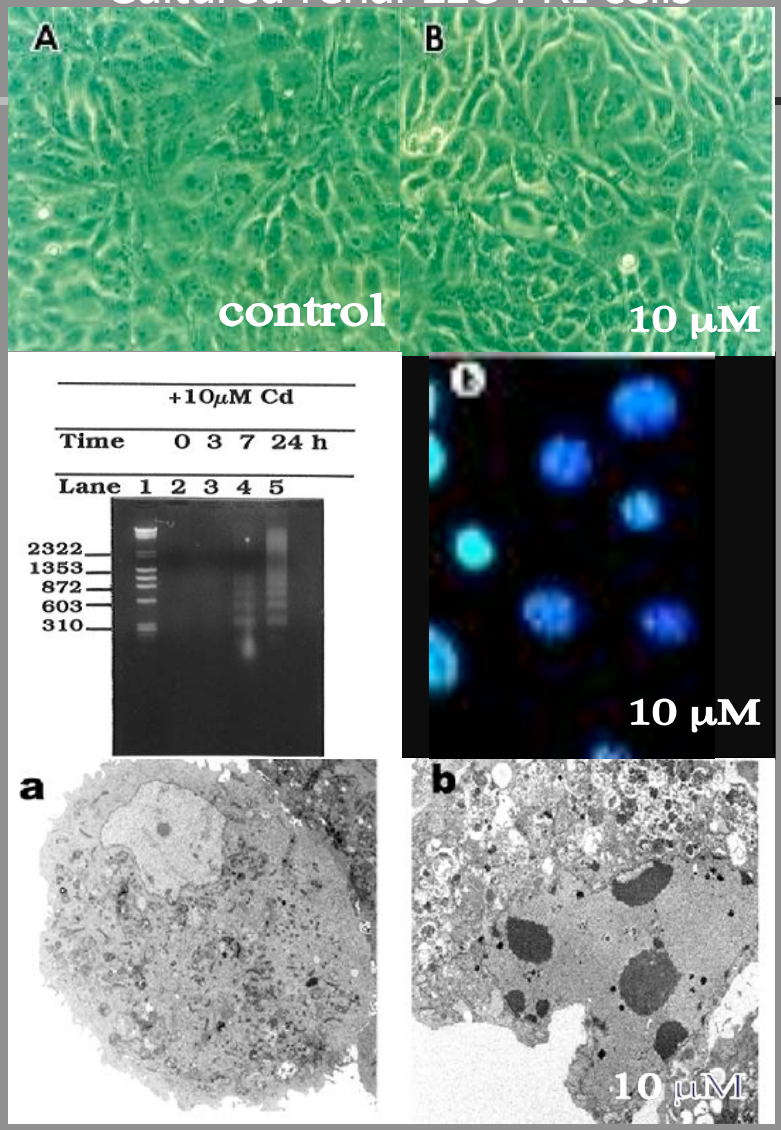
I. In the past, Japan had met serious pollution diseases caused by heavy metals.

Itai-Itai Disease (1960's~)

Apoptogenic nature of cadmium was discovered in cultured renal cells.

M. Ishido, *et al.* (1995) Life Sci 17: 351-356
M. Ishido, *et al.* (1998) Life Sci 63: 1195-1204
M. Ishido, *et al.* (1998) J. Toxicol. Environ. Health 55:1-12
M. Ishido, *et al.* (1999) Life Sci 64: 797-804
M. Ishido, et al. (1999) JPET 290: 923-928
M. Ishido, *et al.* (2001) J. Health Sci 47: 9-13
M. Ishido, et al. (2002) Environ Health Pers 110: 37-42
M. Ishido, (2004) Recent Res Devel. Life Sci 2: 57-67
M. Ishido, (2007) Cell Apoptosis 141-156

Cultured renal LLC-PK1 cells



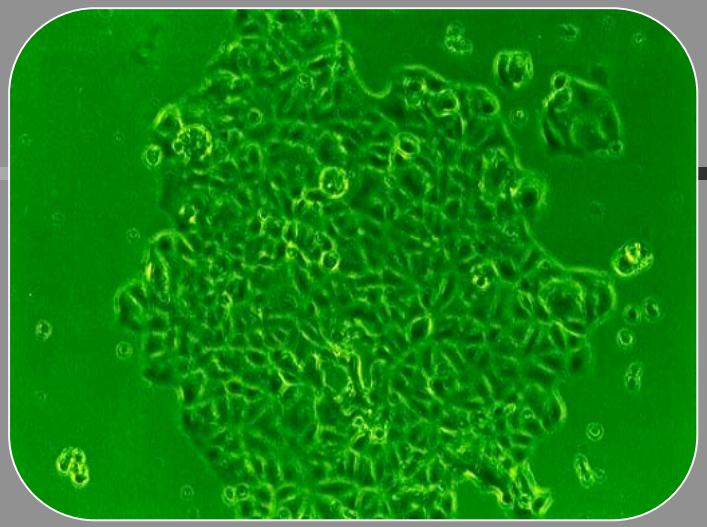
Today's Worldwide Concerns about Environmental Factors

I. Electrocmagnetic Fields(EMF)
   1) Children leukemia?
   2) Breast Cancer?

II. Environmental Chemicals
   1) Neurological Disease
   2) Endocrinological Disease
   3) Immunological Disease
   4) Reproductive System Disease

II. Effects of electromagnetic fields (EMF) on human breast cancer MCF-7 cells

WHO workshop at 桂林 in 2003



Environmnetal Health Criteria (238)

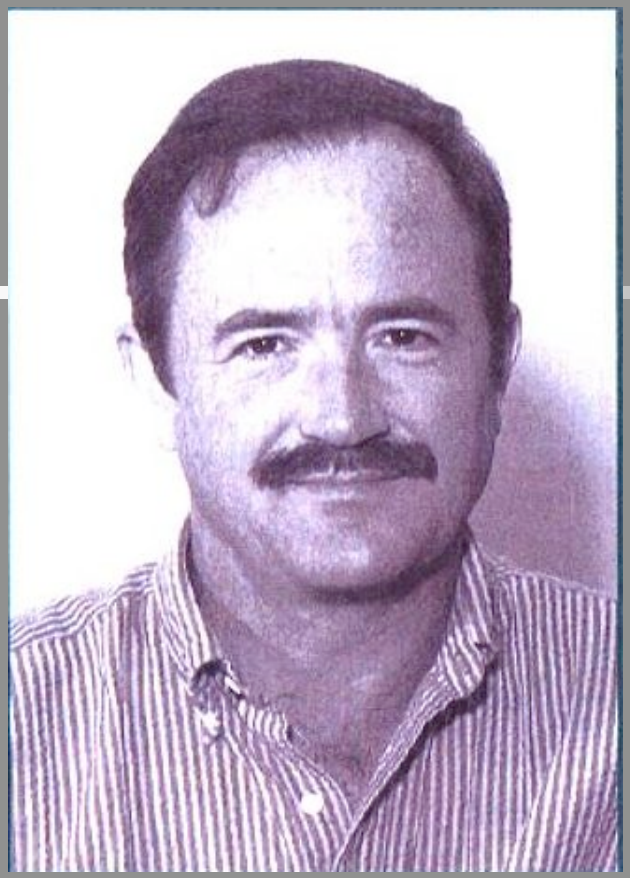
World Health Organization

1. Summary and recommendations for future study
2. Sources, measurements and exposure
3. Electric and magnetic fields inside the body
4. Biophysical mechanisms
5. Neurobehaviour
6. Neuroendocrine system
   - 6-1 Volunteer studies
   - 6-2 Animal studies
   - 6-3 In vitro studies
     - 6-3-1 Effects on melatonin production in vitro
     - 6-3-2 Effects on the action of melatonin in vitro
7. Neurodegenerative disorders
8. Cardiovascular disorders
9. Immune system and haematology
10. Reproduction and development
11. Cancer
12. Health Risk Assessment
13. Protective measures

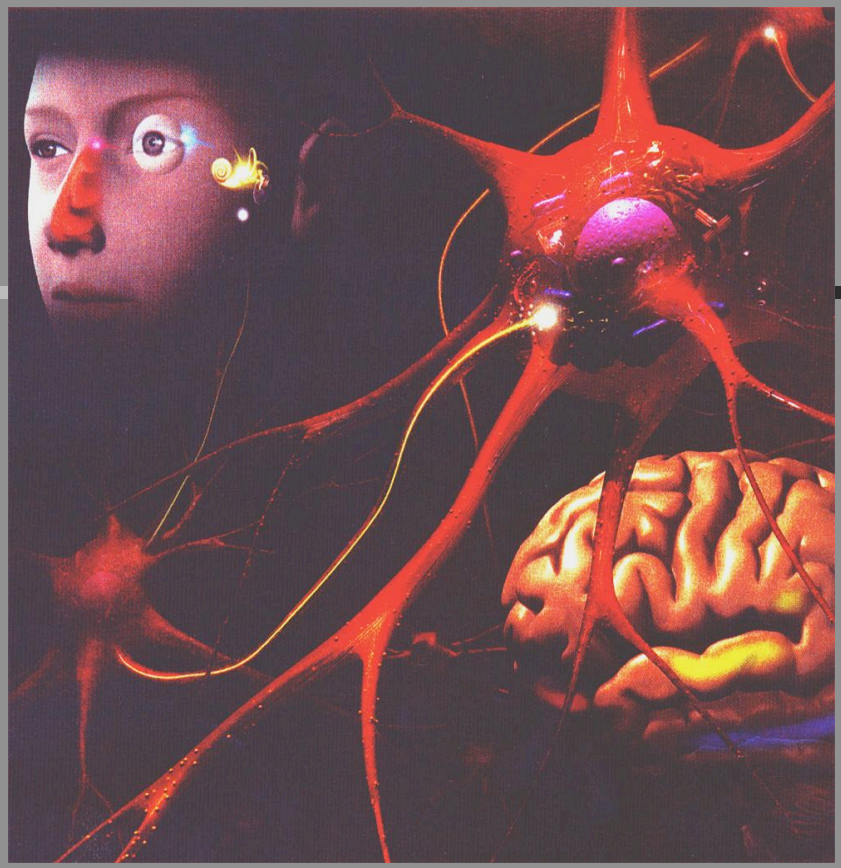
Effects on cell responses to melatonin or tamoxifen *in vitro*

Melatonin inhibition of MCF-7 cell growth	60 Hz 1.2 uT 7 days	EMF exposure reduced growth inhibition	Liburdy 1993
Tamoxifen inhibition of MCF-7 cell growth	60Hz 1.2 uT 7 days	EMF exposure reduced growth inhibition by tamoxifen	Harland & Liburdy 1997
Melatonin or Tamoxifen inhibition of MCF-7 cell growth	60 Hz 1.2 uT 7 days	EMF exposure reduced growth	Blackman 2001
Melatonin inhibition of cAMP and DNA Synthesis in MCF-7 cells	50 Hz 1.2 or 100 uT 7 days	Reduction of melatonin induced inhibition	Ishido 2001

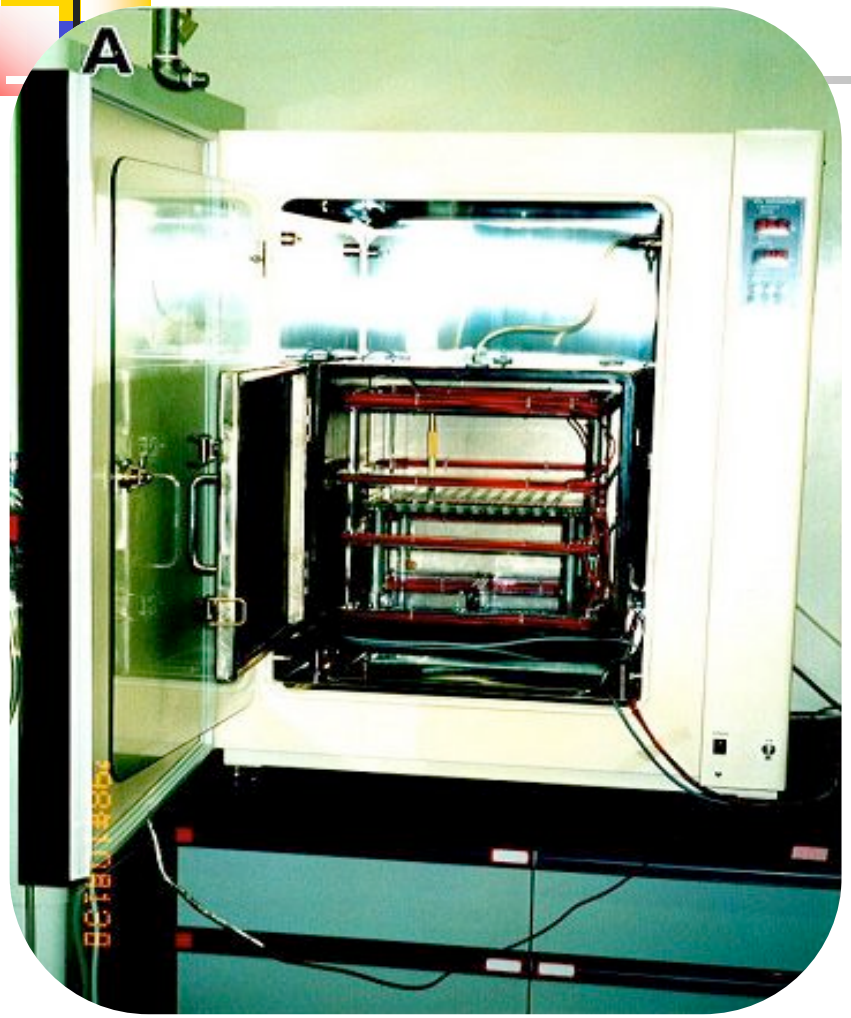
The Melatonin Hypothesis



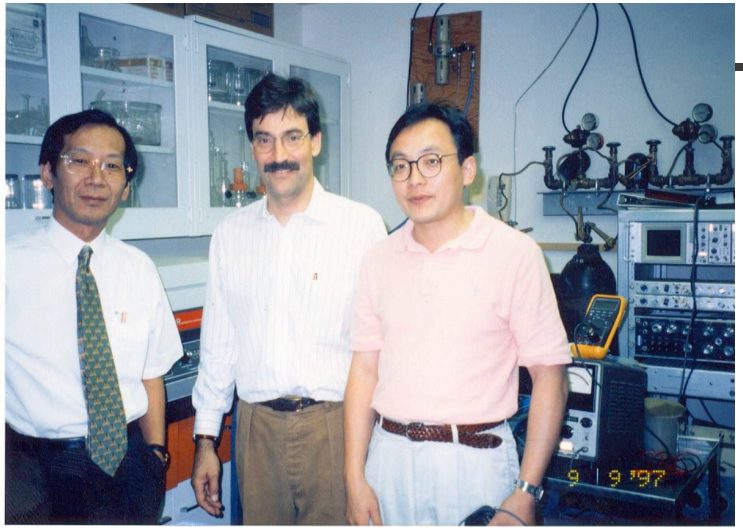
Dr. Stevens



In Vitro EMF Exposure System



National Institute for Environmental Studies

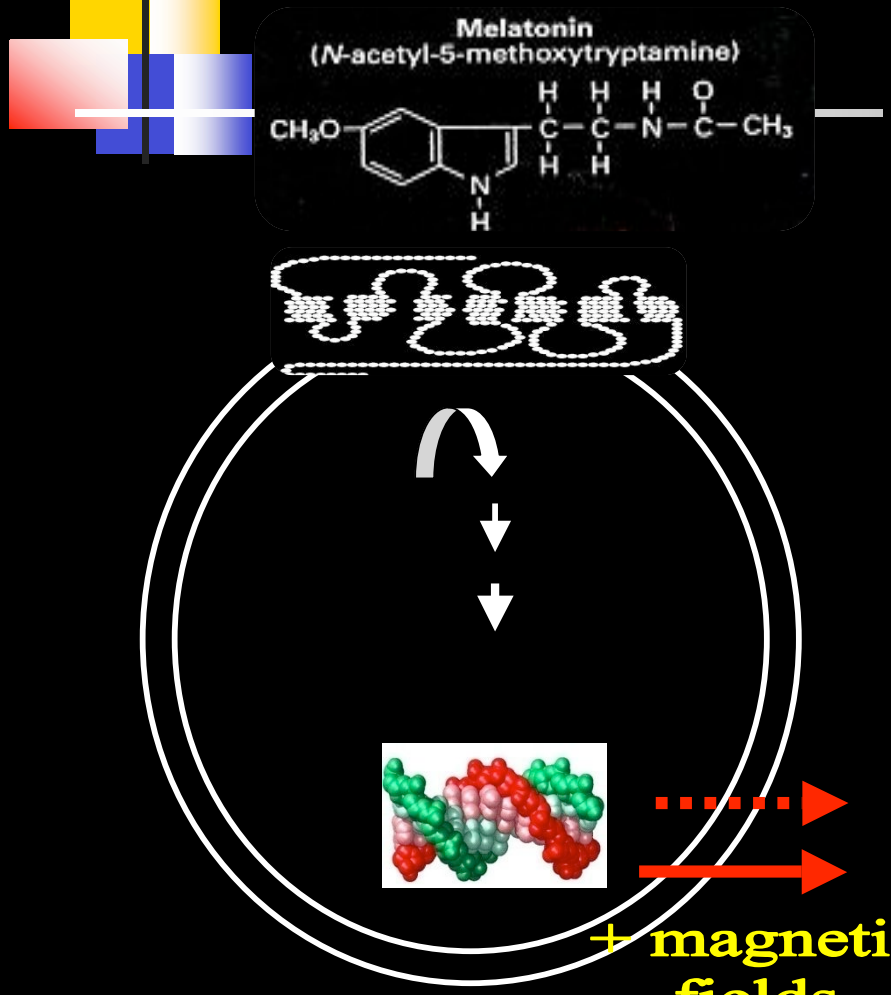


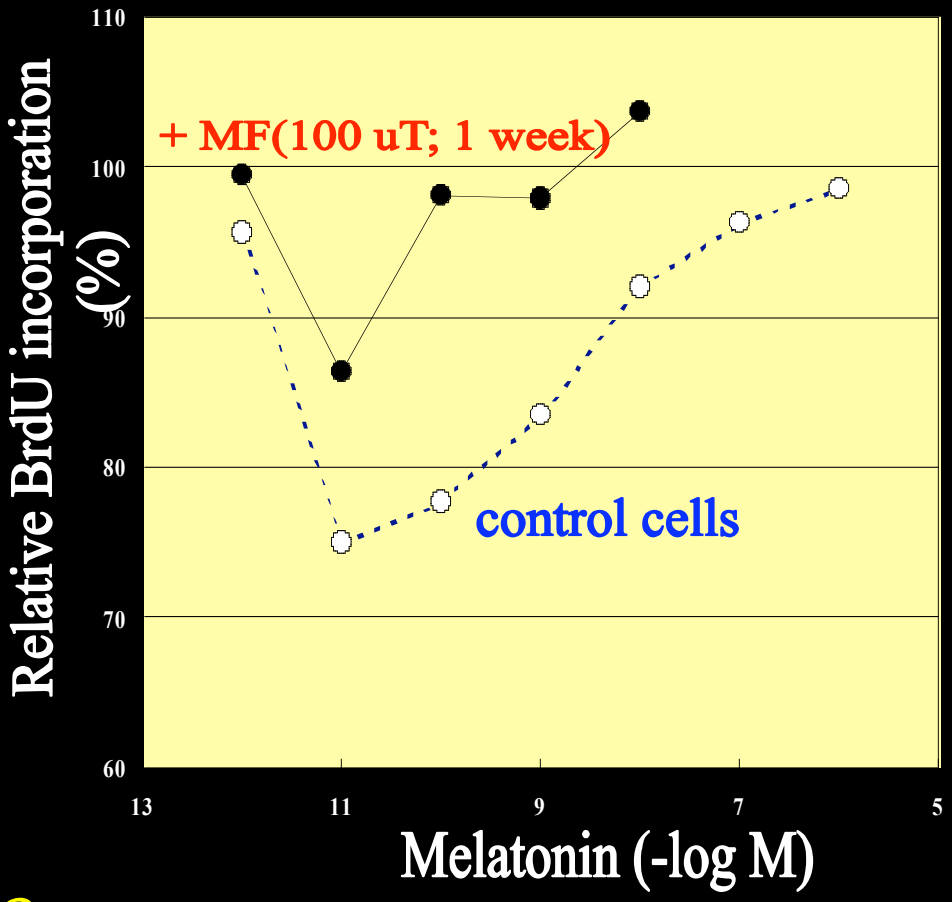
Dr. Liburdy (center)

MCF-7 cells

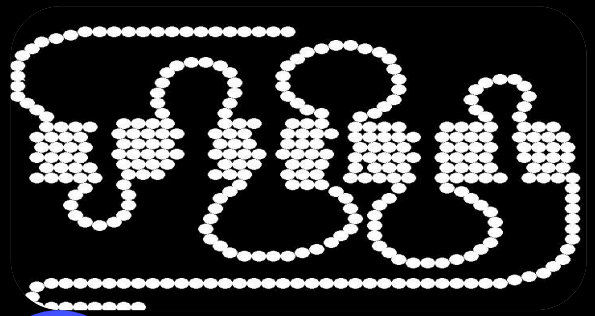
EMF exposed(100 uT; 1 week)

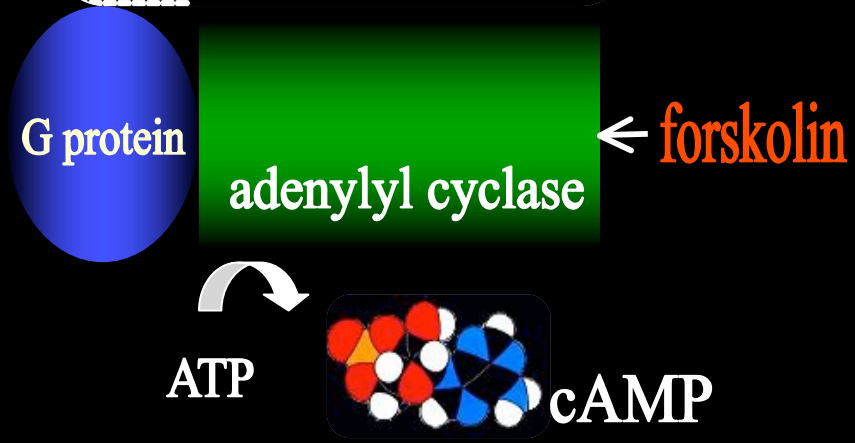
EMF might have a biological effect on the signal transduction pathway of melatonin

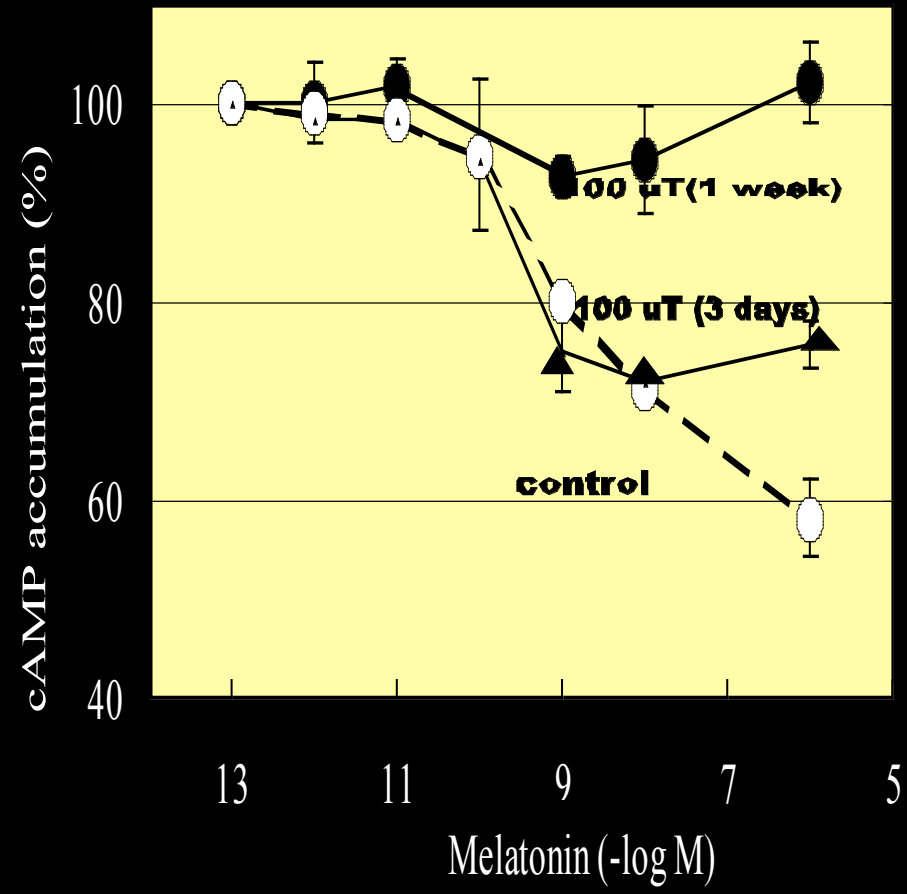




Uncoupling by EMF-exposure of melatonin-mediated inhibitory pathway of adenylyl cyclase

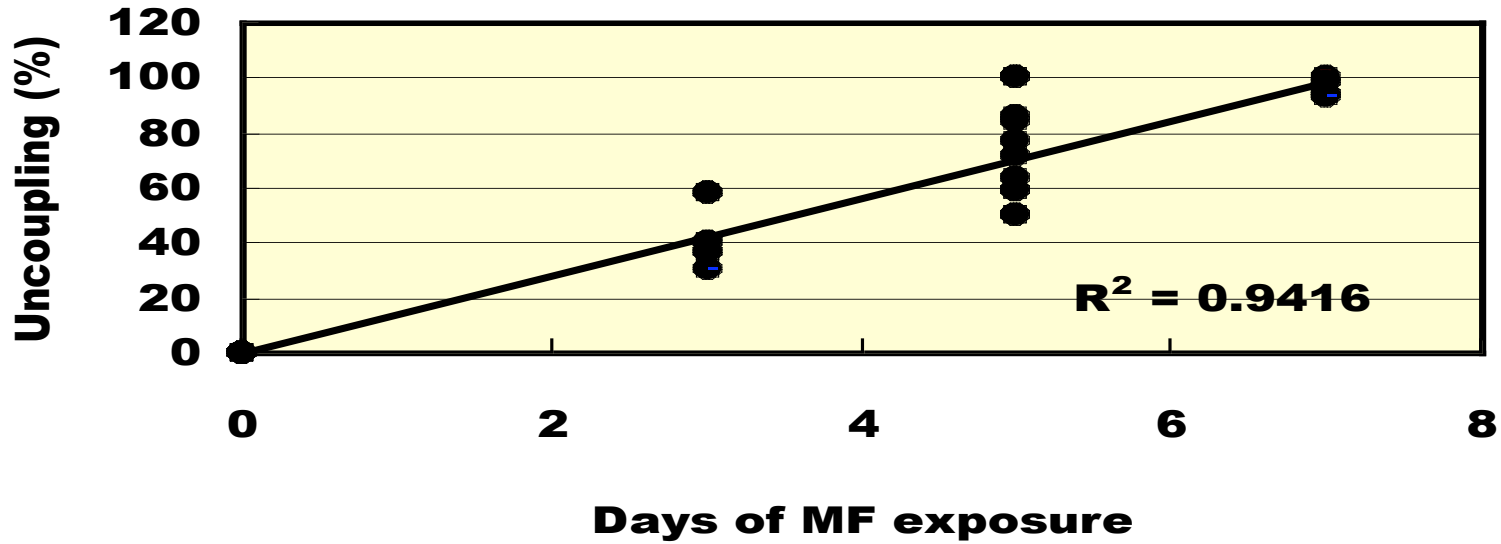






M. Ishido, *et al.* Carcinogenesis (2001)22:1043-1048

The rate of uncoupling by 1 μM melatonin of forskolin-stimulated cAMP accumulation

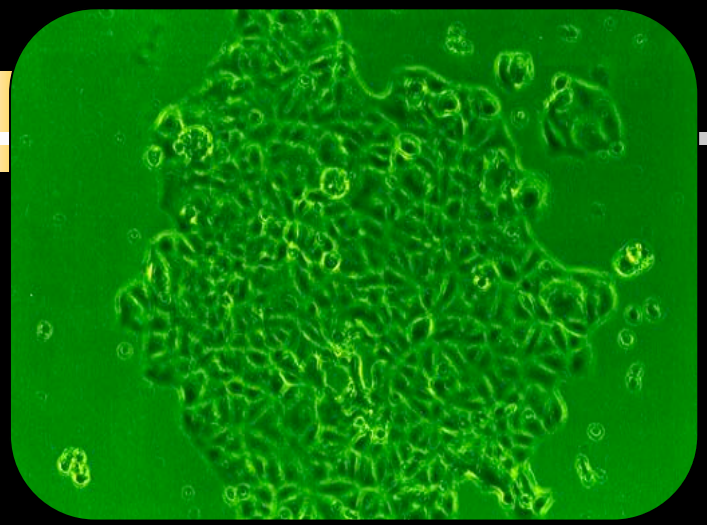


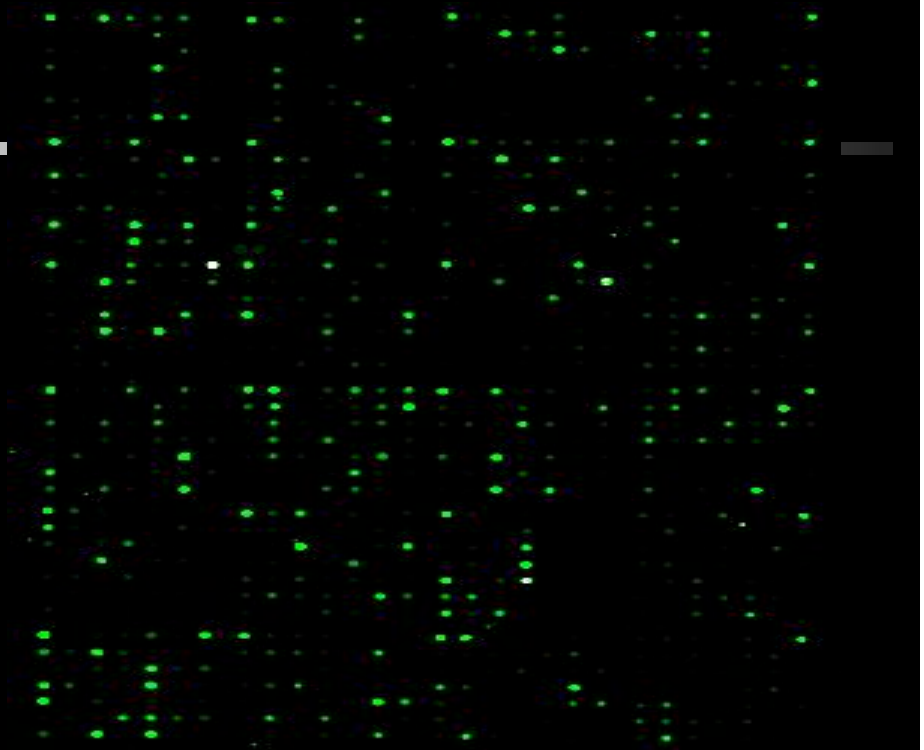
The rate of uncoupling by 1 μM melatonin of forskolin-stimulated cAMP accumulation was calculated using the following formula:

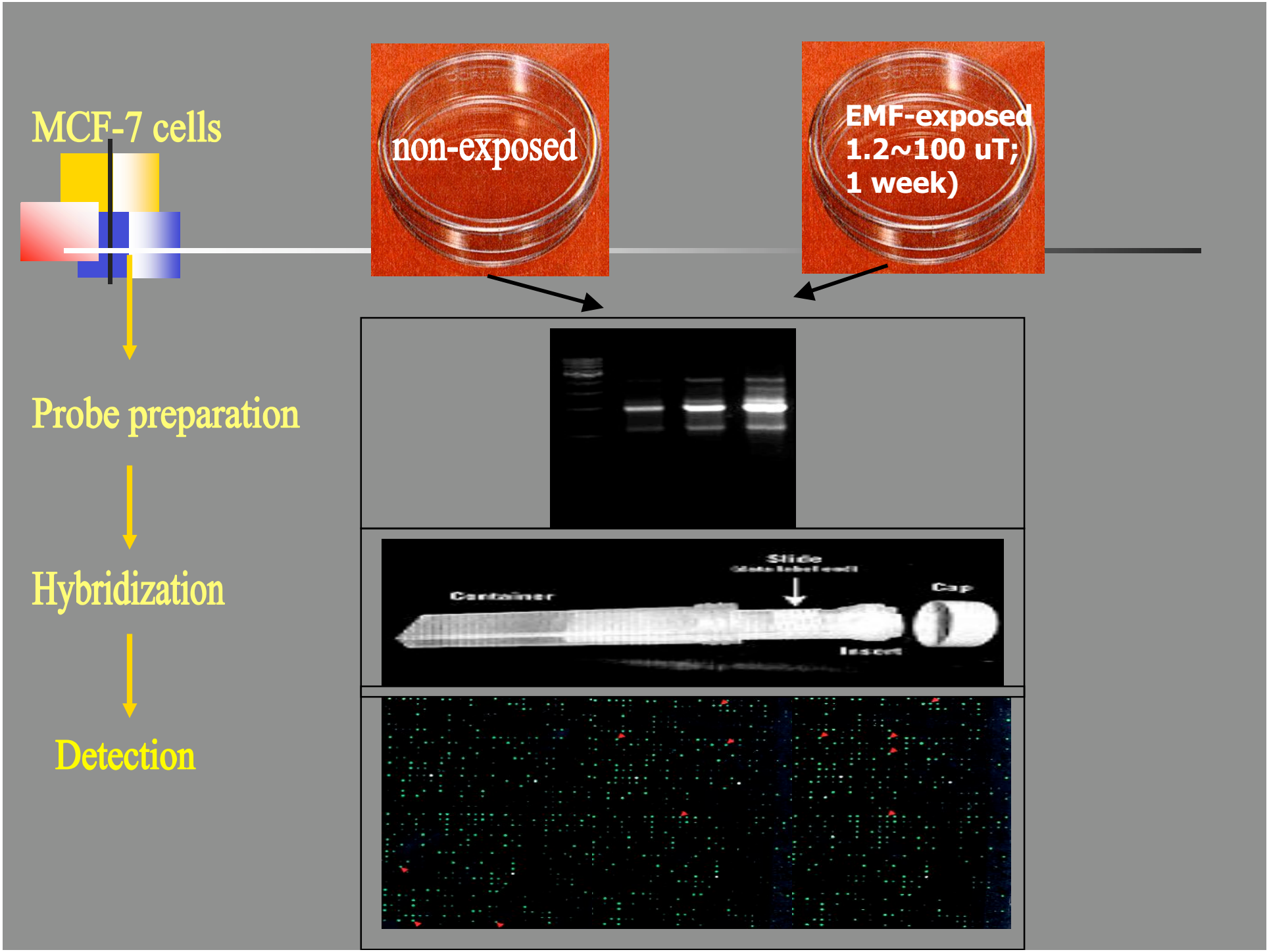
Uncoupling(%)=(A-B)/A x 100

where A= percentage inhibition by 1 μM melatonin of forskolin-stimulated cAMP accumulation in control cells, whose value was 41.9%; and B= percentage inhibition by 1 μM melatonin of forskolin-stimulated cAMP accumulation in MF(100 μT)-exposed cells.

Gene expression profiling exerted by magnetic fields of 50 Hz at 1.2 μT and 100 μT in MF-sensitive MCF-7 cells.





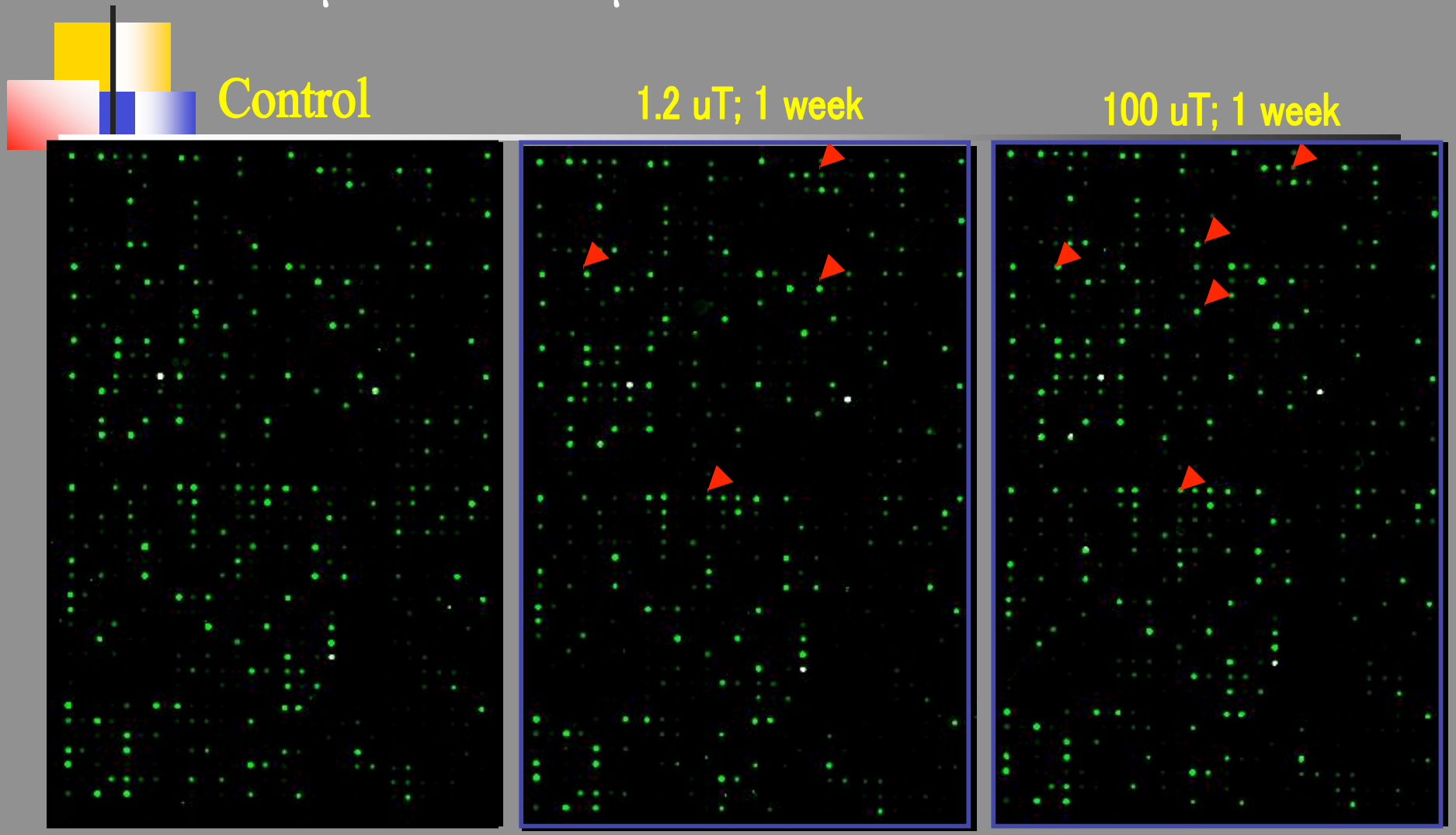

MCF-7 cells
non-exposed
EMF-exposed
1.2~100 uT;
1 week)
Probe preparation
Hybridization
Detection
Container
Slide
Cap

Atlas Glass Human 1.0 Microarray Gene Category (BD Biosciences Clontech)

1,081 genes

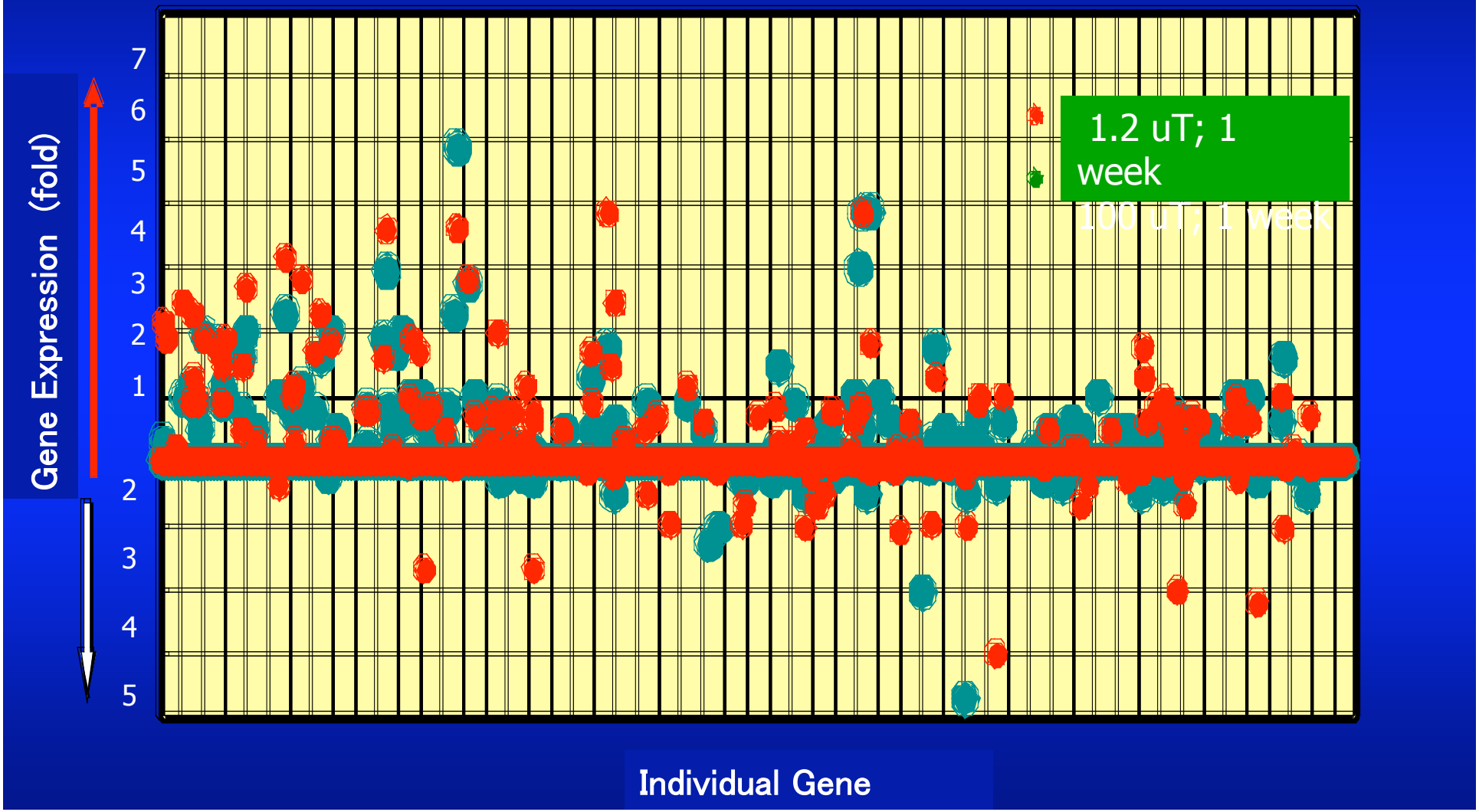
Gene Classification	Numbers (ca.)
1. Oncogenes & Tumor Suppressors	83
2. Cell cycle-related genes	45
3. Channel & Transporter	46
4. Cell Signaling	329
5. DNA Damage Repair-related genes	55
6. Ligands	106
7. Miscellanea	

Alteration of gene expression by MF of 50 Hz at 1.2 μT and 100 μT in MF-sensitive MCF-7 cells



▲：発現変動が見られる代表的な遺伝子

Gene Expression Profiling by MF-exposure in MF-sensitive MCF-7 human breast cancer cells.



Typical alterations of gene expression by EMF in MCF-7 cells

1. A-myb proto-oncogene
2. c-jun proto-oncogene
3. myc proto-oncogene
4. c-rel –proto-oncogene
5. ets1 proto-oncogene
6. B-raf-proto-oncogene
7. c-kit proto-oncogene
8. met proto-oncogene
9. myt 1 kinase
10. JAK 3 kinase
11. Phosphorylase B kinase
12. MAR kinase
13. B-MYB
14. ETR101
15. IGF-binding proteins 5
16. Notch 4

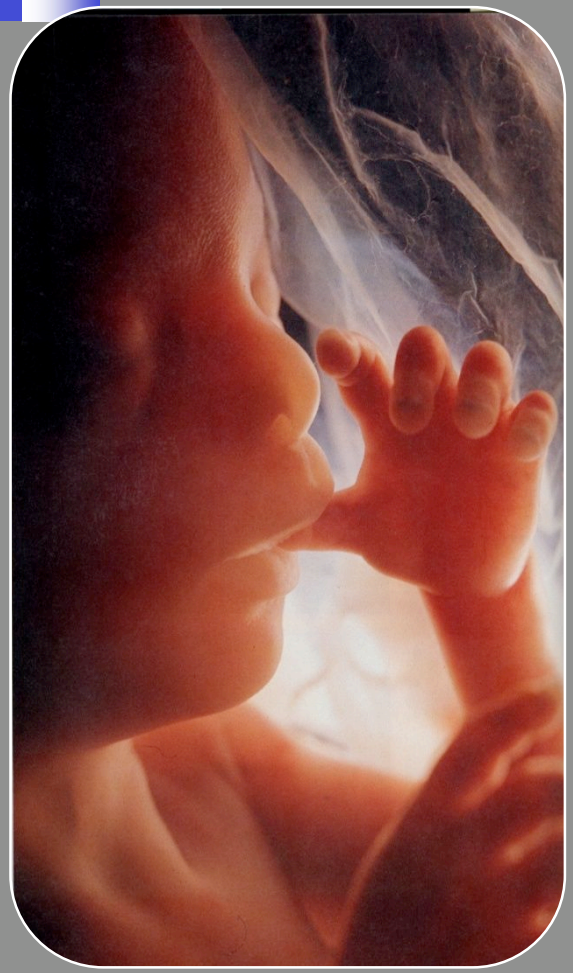
Reports: Ets gene and MF exposure

1. Biological effects of EMF exposure on Ets gene
   Radiats Biol Radioecol (2003) 43(5): 528-530

2. In vivo modulation of ETS genes induced by electromagnetic fields
   In vivo (2001) 15(6) 489-494

3. Ets1 oncogene induction by ELF-modulated 50 MHz radiofrequency
   Bioelectromagnetics (2000) 21(1) 8-18.

III. Effects of environmental chemicals on CNS



Some psychiatric disorders and sporadic neurodegenerative
diseases have well-documented environmental causes.

It has been believed that children are much sensitive to environmental insults, leading to the deficit in development , and that they might be predictors of disease in later life.

Neurodevelopmental Disorders

1. Attention Deficit Hyperactivity Disorder (ADHD)
2. Autism

   impulsivity, inattention, hyperactivity, antisociality
3. Rett syndrome
4. Tourette syndrome
5. Fragile X syndrome
6. Down's syndrome
7. Phenylketonuria
8. Cretinism

Neonatal endocrine disruptors lesion

Birth

Oral administration of Endocrine disruptor

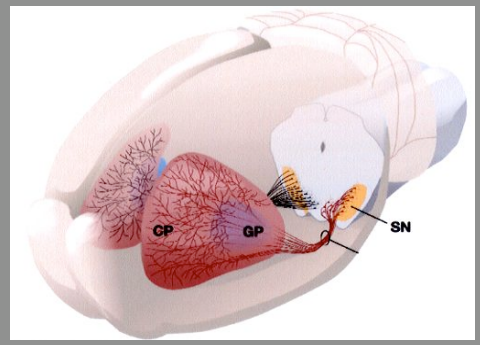
Measurement of spontaneous motor activity

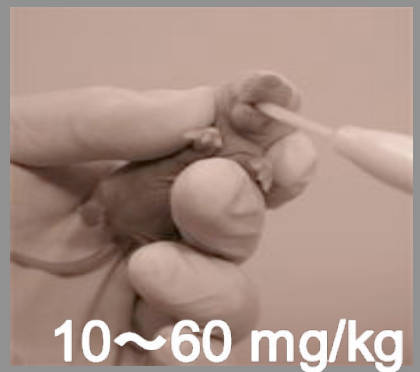
Biochemical analyses

4 ~ 5

7 ~ 8

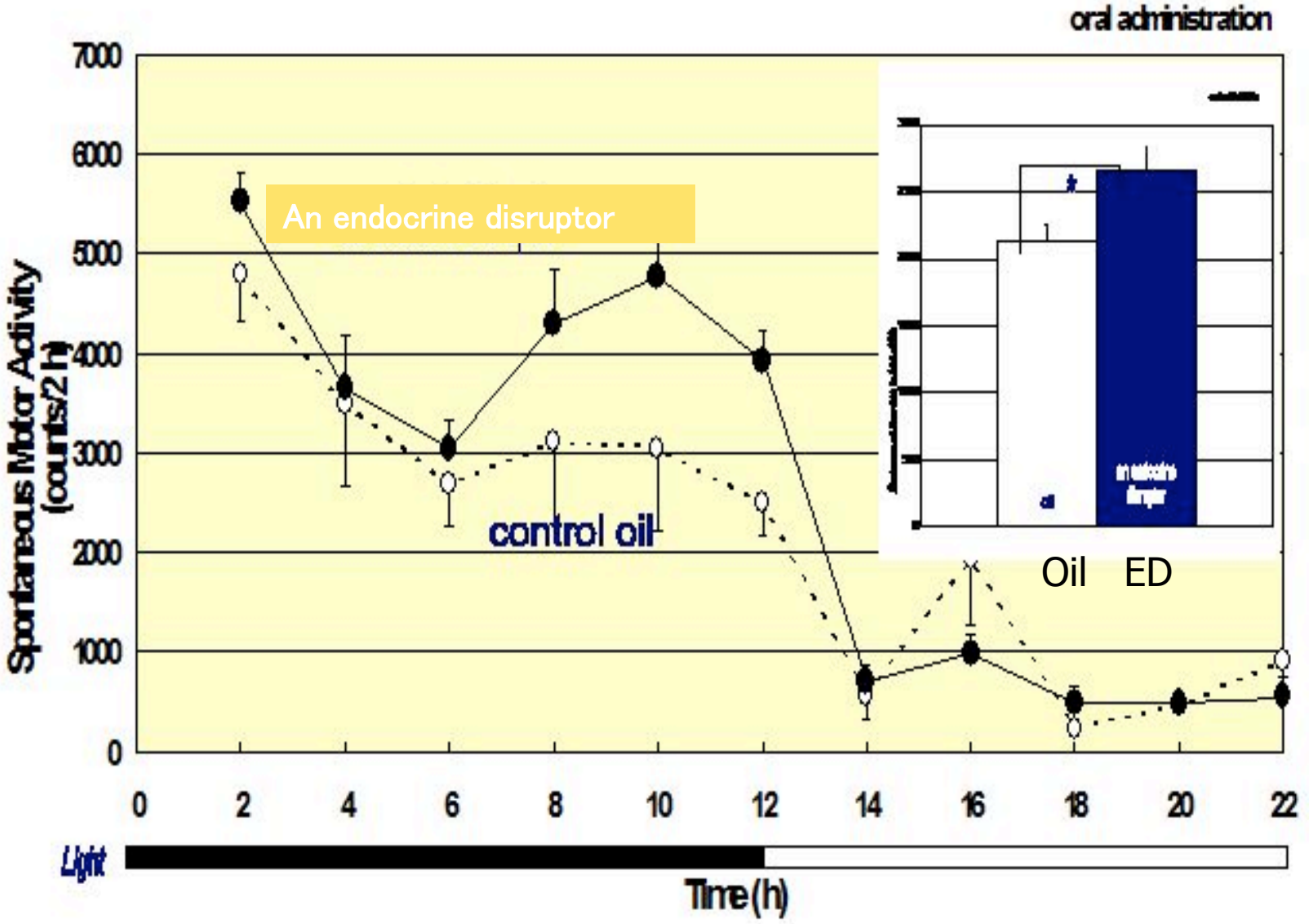
age (week)





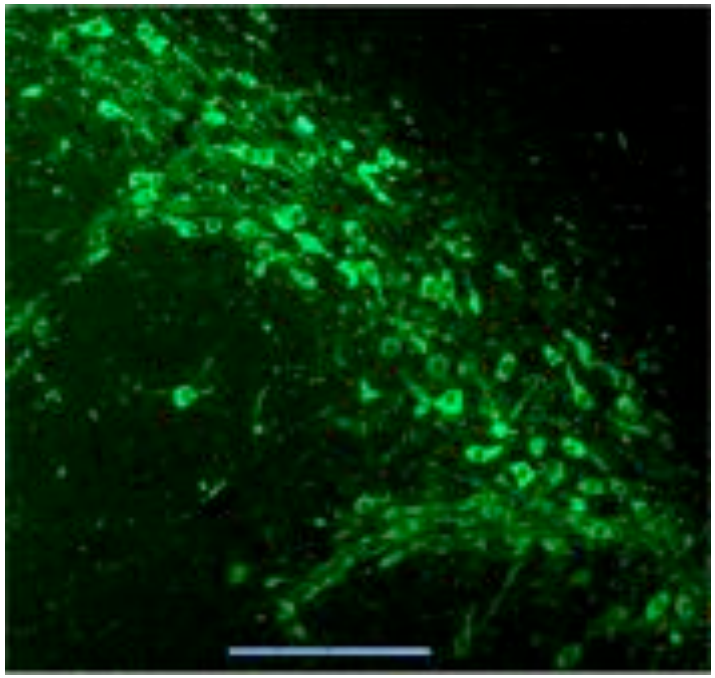
1. Immunostaining
2. Cell death

Oral administration of endocrine disruptors into neonatal rats causes hyperactivity at juvenile.

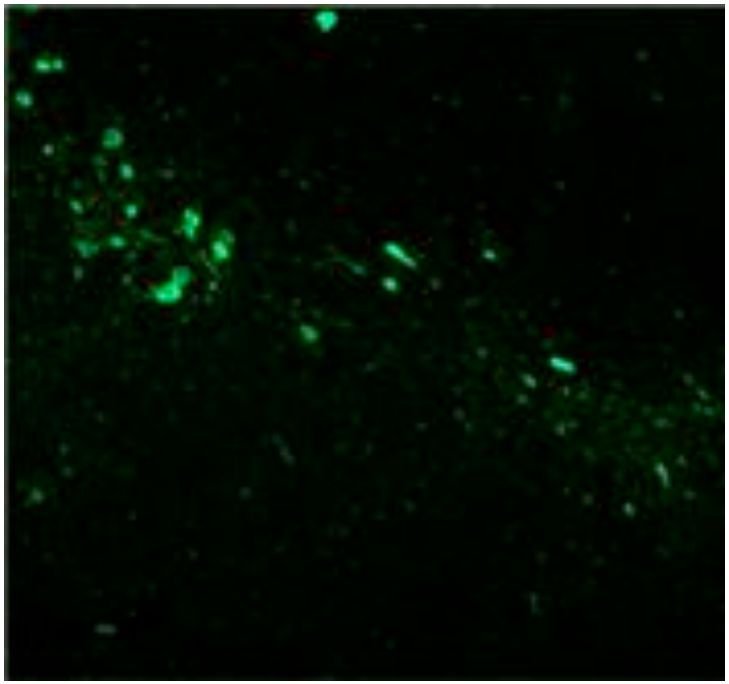


Neonatal endocrine disruptors lesion causes developmental deficit in dopaminergic neurons, resulting in hyperactivity

A: control

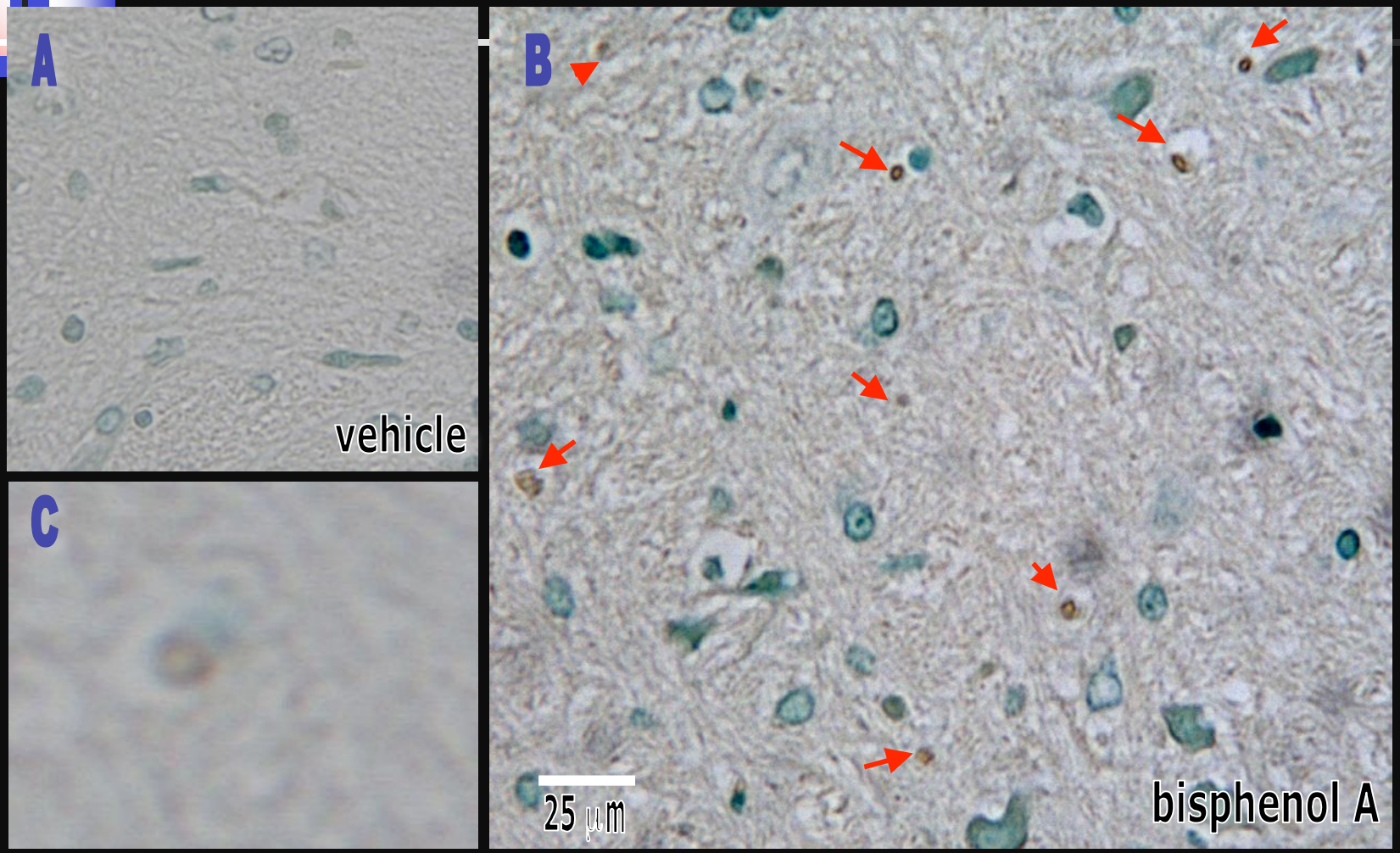


B: endocrine disruptor



Tyrosine hydroxylase immunohistochemistry in substantia nigra at 7 weeks of age

TUNEL-positive cell death seen in endocrine disruptor-induced hyperactive rats at 8 weeks of age



Historical view of Hyperactivity

1. Hyperactivity among children was first described by Dr. von Economo in case of encephalic lethargica.

   Hyperactivity , sleep disorders and antisocial personality disorder are all associated with this disease in children and Parkinsonism is observed in adult cases.

2. In 1937, Bradley found that amphetamine works on the children with hyperactivity.

3. In 1959, Knolbock & Pasamanick proposed the concepts MBD（Minimal Brain Dysfunction）as a etiology of hyperactivity.

4. WHO and ASPR clarify the diagnostic criterion for ADHD or autism.

The Expanded Barker's Hypothesis

Early Environmental Origins of Neurodegenerative Disease in Later Life

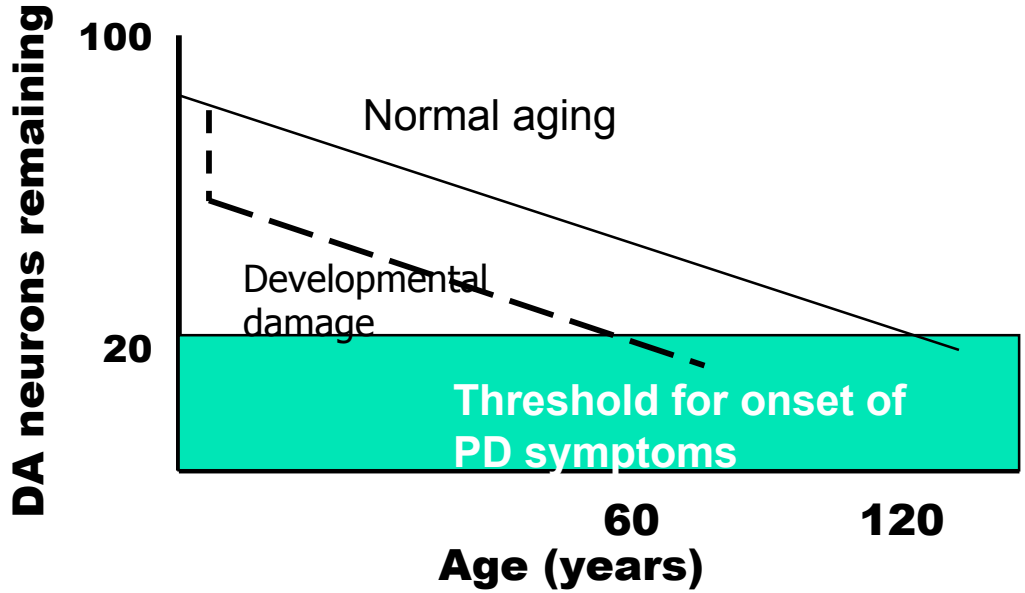
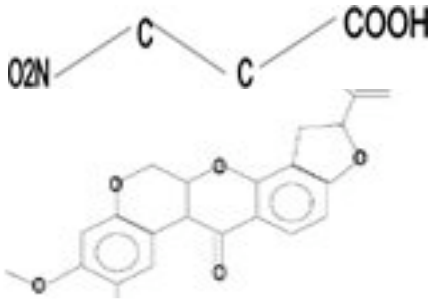


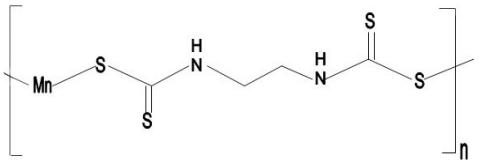
Fig. Long-term consequences of early loss of critical neurons after developmental damage. The impact of early developmental damage is not immediately evident but produces disease years or decades later as the number of neurons decreases with advancing age.

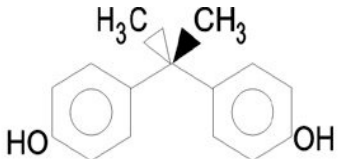
Environmental enrichment mitigates cognitive deficits in mouse model

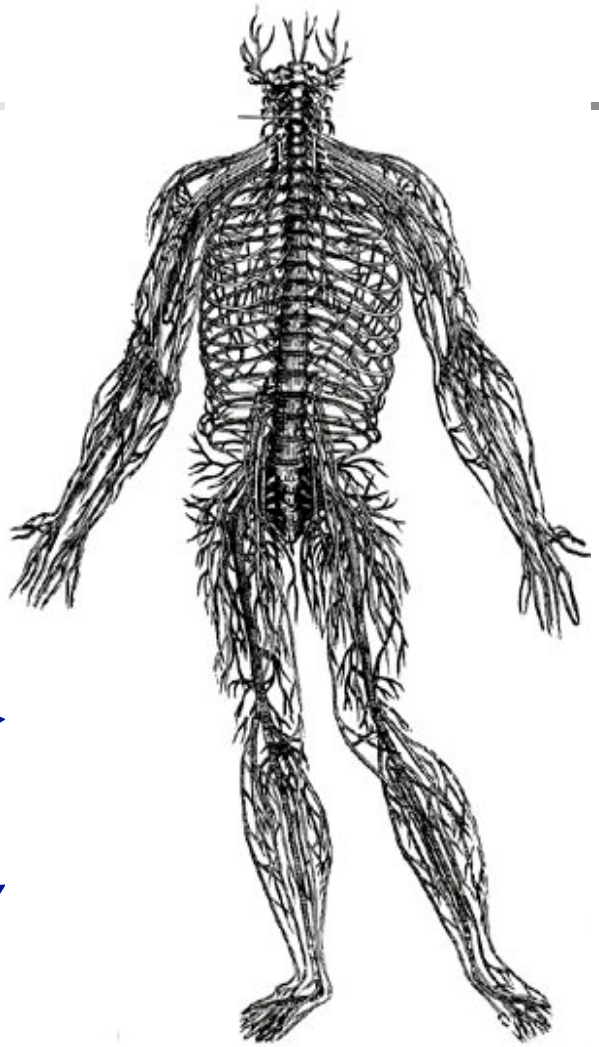
Environmental physicochemicals



Maneb







Environmental enrichment