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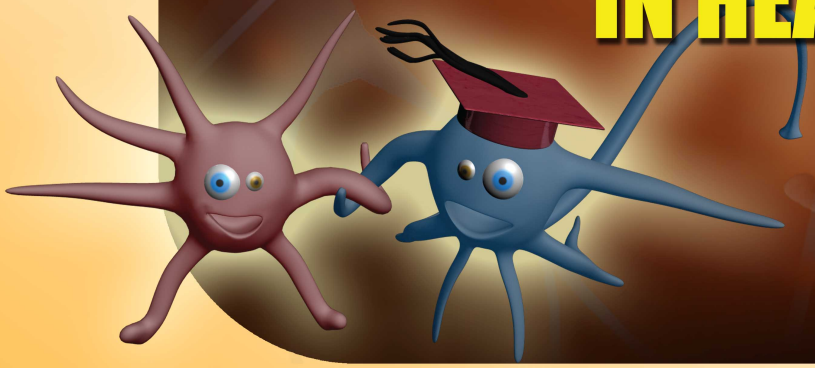
EGE Vth BIENNIAL INTERNATIONAL NEUROSCIENCE GRADUATE SUMMER SCHOOL

EGEBINGSS2009

Ege University Faculty of Medicine, Izmir, Turkey

June 29 - July 4, 2009

NEURO-GLIAL INTERACTIONS FROM WOMB TO TOMB IN HEALTH & DISEASE



PROGRAMME and ABSTRACTS

www.neurosciencesocietyofturkey.org

Old and All Time Dedicated Friends and Newcomers,
You are All Very Welcome to the V. EGE-BINGSS in Izmir, Türkiye!

It is a great pleasure and honor to see several of you again; meet, host, treat, learn from, collaborate; and have memorable time with you.

We are grateful for your peerless contributions to this initiative; most of all, your willingness in joining us for commemoration of the 10th Anniversary at the V. EGE-BINGSS which has now evolved to a prestigious tradition and genuinely altruistic international organization.

The faculty of EGE-BINGSS has always been international. For the first time this year, the V. EGE-BINGSS is very fond and proud to welcome international student participation from Greece, Azerbaijan, Pakistan, Ukraine, Romania and Tunisia.

Please help us meet the ultimate aim of EGE-BINGSS by communicating and interacting as much as possible to consolidate long term learning, to offer all that you know, and to establish new friendships as well as educational / professional bonds and networking.

Enjoy the Vth EGE-BINGSS!

Founders & Directors
Gönül Ö. Peker & Reha Erzurumlu

Coordinators
Vedat Evren, Taner Dağcı & Kenneth Moya

Organizers & Lab Assistants
Merve Uluğ, Ayşegül Keser, Emre Yıldırım, Gonca Mola, Oytun Erbaş

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Gülnur Ata, Hatice Arsoy, Aylin Suyabatmaz, Mualla Yılmaz,

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Special thanks to:

Ege University Rector's Office
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EGE BIENNIAL INTERNATIONAL NEUROSCIENCE GRADUATE SUMMER SCHOOL (EGE BINGSS)

"DEVELOPMENTAL, DEGENERATIVE AND RESTORATIVE NEURAL PLASTICITY 5"

NEURO-GLIAL INTERACTIONS FROM WOMB TO TOMB IN HEALTH AND DISEASE

Hosted by

Ege University Faculty of Medicine, Dept. of Physiology
Izmir, Türkiye

Date & Duration

End June or Beginning July, Biennially
4-6 Full Work Days & 1 Full Weekend Day for Site-Seeing & Culture Hunting

Sponsors / Organizers

EGE UNIVERSITY
NEUROSCIENCE SOCIETY OF TURKEY (NST=TÜBAS)
Turkey Chapter of the Society for Neuroscience (SfN-Turkey)

(Other (gov, org, com) National and International parties varying at each time according to theme, etc.)

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Scientific and Technical Research Council of Turkey (TÜBİTAK)

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Directors

Gönül Ö. Peker, PhD & Reha Erzurumlu, PhD

Coordinators

Taner Dağcı, MD, MSc, PhD & Vedat Evren, MD & Kenneth Moya, PhD

General Description & Aim of EGE BINGSS

The traditional EGE-BINGSS's vision is to advance the knowledge, technical skills and professional attitudes of neuroscientists in Turkey, Balkans, Eastern Europe and Mediterranean, Middle East, Black Sea Region, the Turkic States, and all other participating countries on a non-profit basis.

- EGE-BINGSS aims to introduce eminent, devoted and role-model international neuroscientists and teaching faculty to graduate (and a small minority of selected undergraduate) students for transfer of knowledge and know-how. It also creates opportunities for networking and encouragement of new international, inter-institutional, multidisciplinary and multiprofessional collaborations for research, education and translational community outreach.
- EGE-BINGSS's major target is junior neuroscientists; it optimally enrolls 50-60 graduate participants and occasionally admits several highly qualifying competent undergraduate students.
- EGE-BINGSS addresses its purpose by holding 4-6 day comprehensive theoretical and practical sessions in lively, interactive and stimulating learning environments where thematic fundamentals, state of the art methodology, very recent data, neuro-professional skills, neurophilosophy and neuroethics are covered.
- Social events related to culture-hunting and historical site-seeing constitute an important part of the educational experience.

Scientific Advisory Committee of EGE - BINGSS:

Prof. Nuran I. Hariri (TUR), Prof. Albert Aguayo (CA), Prof. Richard Morris (UK), Prof. Ranney Mize (USA), Prof. Geoffrey Raisman (UK), Prof. Mike Kuhar (USA), Prof. Robert T. Rubin (*USA)*, Prof. Susan Sara (FR), Prof. Bruce McEwen (USA), Prof. Colin Blakemore (UK), Prof. Sharon Juliano (USA), Prof. Harry W.M. Steiınbusch (NL), Prof. Pierre Magistretti (SW), Prof. Maria Bentiglovia (IT), Prof. Yucel Kanpolat (TR), Prof. Nurcan Özdamar (TUR), Prof. Nese Tuncel (TR), Prof. Turker Sahiner (TR), Prof. Gulgun Kayalioğlu (TR), Assoc.Prof. Emel Ulupinar (TR), Prof. Bayram Yilmaz (TUR)

Organizing Committee of EGE - BINGSS:

The faculty, graduate students and the staff of the Physiology Department of the Ege University Faculty of Medicine, the Executive Committees of the Neuroscience Society of Turkey and the Turkey Chapter of the Society for Neuroscience

Faculty of EGE BINGSS:

The school usually hosts 8-10 international faculty from Europe, Canada and the United States, 2-4 Turkish faculty from outside Izmir and 2-4 hosting faculty and instructing post-docs and graduate students from Izmir.

Global Learning Issues & Performance Objectives of EGE-BINGSS

Reinforce / advance the participants' basic knowledge of essential mechanisms relevant to plasticity underlying neurodevelopment, neurodegeneration and neurorepair with state of the art research data;

- Teach participants the basics, myths, restrains and the potential future capacities of "Neural & Mesenchimal Stem Cell" utilization for neurorepair, and cell / tissue engineering in the nervous system;
- Advance the participants' level of understanding and technical command on the current research findings and the clinical outcomes of the neural stem cells (building on the knowledge, skills and attitudes acquired at the previous EGE BINGSS);
- Expose participants to - and enable them – to acquire and to advance their dissection, derivation, isolation, and preparation skills in embryonic rat brain and adult rat bone marrow for neuronal and mesenchimal stem cell culture methodologies (including cell identification);
- Expose / challenge participants to / with psycho-social issues; enable them to acquire and advance their perceptions and attitudes related to neuro –philosophical and –ethical aspects / dimensions; and help them develop behavioral and technical survival skills fundamental to (neuro-) scientific and academic life styles.

Organization

Venue:
Ege University Faculty of Medicine, Muhittin Erel Hall; Teaching and Research Labs (AREL), Dept. of Physiology Labs, Izmir, Turkey

Language: English

Weather:
30-34 C, humid, very sunny. Bring very light casual clothes, sunglasses, hat, swimming suit, tennis racket, camera and sunscreen products.

Accommodation:
*Hotel Anemon Ege Sağlık (***/****)* is very conveniently located at 5 min walking distance from the venue. Facilities include Indoor and Outdoor Cafeterias, Outdoor Swimming Pool, Turkish Bath, Gym, Sauna, Tennis Courts, and Parking. Rates include tax and breakfast and are 60E and 75E for AC, wireless internet access, and cable TV equipped single and double rooms, respectively. Usually, only complimentary reservations for the invited faculty are arranged by the organizers.
Tel: +90 232 3734862 & 3734934
Fax: +90 232 3734863
E-mail: ayse.kaya@anemonege.com

*Ege University Campus Guest House (**/***)* is located at 5-10 min drive from the venue. A courtesy shuttle runs to/from the venue every morning and evening. Single, double and quadruple room (with bathroom, TV, telephone and AC) rates include tax and continental breakfast, and range between 20-40 E. Participants are usually requested to arrange their own reservations.
Telefax: +90 232 3881447 & 3399993

Logistics:
The venue and the major accommodations are only 25 km from the Izmir A. Menderes International Airport. Faculty are met there and picked for transfers. Ground transportation by Municipal and Havaş buses and cabs is very convenient. The organizers may arrange collective courtesy transfers to/ from the airport also for the participants if arrivals and departures coincide and yield to reasonable groups. The venue is accessible also by metro-railway, by boat and by bus.

Registration:
Undergraduate and Graduate Students and Residents: 100-150 €
Post-docs, Specialists, Fellows, Faculty: 150-200 €
Fee includes access to all lecture sessions, SIGs, roundtables, video workshops, wet hands-on practices; certificate, teaching material, bags / folders, 8-10 coffee-tea, 4-5 lunches, Opening Cocktail Prolongé, admission to the Museums and the Turkish Barbeque Dinner.

E-banking (swift) is possible @ Garanti Bankasi, Bornova
(Ege Tip: 524) Branch
Account No: 6670677 (TL) or
6490747 (USD), Izmir

IMPORTANT: Students' status should definitely be assured by written and undersigned documents from their advisors / mentors.
A-priori Registration Fee Payment is a very strict mandatory prerequisite for poster abstract submissions.

INFORMATION & APPLICATION:

www.neurosciencesocietyofturkey.org

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Global Learning Issues & Performance Objectives of the V. EGE-BINGSS

At the end of this summer school, the participants should be able to:

- Describe the macro and micro morphological features of the nervous system.
- Perceive the basic and state of the art knowledge of cell biology and metabolism in neurons and glia.
- Assimilate the basic knowledge of the primary and secondary injury mechanisms in the central and peripheral nervous system with respect to topography and neuro-glial interactions.
- Define the basic and recently proposed mechanisms favoring and inhibiting developmental, degenerative and restorative neuro-plasticity.
- Conceptualize the knowledge on well established and especially, recently proposed roles for different types of glial cells in the CNS and PNS.
- Merge their existing knowledge and know how on lab animal ethics with the up-to-date understandings and practices in the area.
- Remember, translate and transfer the methodological and practical know-how they were introduced to during the wet lab sessions they have participated.
- Take home at least a global understanding of the novel approaches in imaging, especially in-vivo neuroimaging.
- Reevaluate their views and reorganize their gains acquired at the psycho-social issues and professional survival skills roundtable or SIG .

PROGRAMME

Day 1 **June 29, Monday**

HOUR	TOPIC	FACULTY
10:00-10:30	Welcome, Opening Remarks, Orientation to the V. EGE BINGSS	Gönül Ö. Peker, Reha Erzurumlu
10:30-11:30	Basic Anatomy and Development of the Spinal Cord and Brain	Mahendra Rao
11:30-11:50	**Coffee**	
11:50-12:50	Basic Histology & Cell Biology of Neurons and Glial Cells	Gülgün Kayalıoğlu, Emel Ulupınar
12:50-13:50	Molecular Biologist's Insight into Neuro-Glial Interactions: An Overview of Genetic and Metabolic Events in Neuron-Astrocyte Interactions	Işıl Kurnaz
13:50-14:30	**Lunch**	
14:30-15:30	An Overview of Neurotrophic Factors Involved in Neuro-Glial Interactions: Glia Derived Neurotrophic Factor	Emel Ulupınar
15:30-16:30	How do Astrocytes Induce Synapse Formation?	Çağla Eroğlu
16:30-16:50	**Coffee**	
16:50-17:50	Role of Astrocytes in Peripheral Nerve Injury Induced Synaptic Plasticity in the Developing Brain	Reha Erzurumlu
17:50-19:00	Poster Viewing & Discussion ***Beverages will be available during the session.***	(All Participants are expected to attend. A Faculty Team will be assigned to evaluate the posters.)
20:00-22:00	**Opening Reception Cocktail Prolongé**	

Day 2 **June 30, Tuesday**

09:00-10:00	Development of the Peripheral Nervous System: Schwann Cell Development and Biology and its Role in Regeneration	Ahmet Höke
10:00-11:00	An Overview of Primary and Secondary Injury Processes in the Nervous System	Gönül Ö. Peker
11:00-11:30	**Coffee**	
11:30-12:30	Progenitor Biology and Response After Injury	Mahendra Rao
12:30-14:00	**Lunch**	
14:00-17:30	Group A: Lab 1: Session 1: Immunofluorescence Labeling, IHC and HC for Myelin and General Histology to Study Injured Spinal Cord	Samuel David et al.
14:00-17:30	Group B: Lab 2: Session 1: Culture of Human Glial Precursors and IHC and PCR Analysis	Mahendra Rao et al.
14:00-17:30	Group C: Lab 3: Session 1: Astroglial Cell Culture (Rat) 1	Özlem A. Yılmaz et al.
	Lab Groups are limited to 6-8. ***Lab 1, 2 and 3 last for 4 afternoons.*** ***SIG Groups are limited to 15 and last for a single afternoon.*** ***No definite coffee break; beverages will be available during the labs.***	
14:00-17:30	Group E: SIG 1: How to Prepare / Perform Effective Presentations; How to Transform Your Data to an Impressive Article; How to Critically Review a Scientific Manuscript	Kenneth Moya et al.
14:00-17:30	Group F: SIG 2: Neuroethics	Gönül Ö. Peker et al.
14:00-17:30	Group G: SIG 3: Good Balance of Demand and Supply in Mentoring and Trouble Shooting	Mary Bunge et al.
	Dinner on your own	

Day 3 **July 1, Wednesday**

09:00-10:00	Glutamate Transporter EAAT2 Featuring a Major Role in Astroglial-Neuronal Homeostasis	Oğuz Gözen
10:00-11:00	The Role of Axon-Schwann Cell Interaction in Peripheral Neuropathies	Ahmet Höke
11:00-11:20	**Coffee**	
11:20-12:20	Exogenous Transcription Factors as Survival Promoting Factors for Damaged Adult Neurons	Kenneth Moya
12:20-13:30	**Lunch**	
13:30-15:30	Group A: Lab 1: Session 2: Immunofluorescence Labeling, IHC and HC for Myelin and General Histology to Study Injured Spinal Cord	Samuel David et al.
13:30-15:30	Group B: Lab 2: Session 2: Culture of Human Glial Precursors and IHC and PCR Analysis	Mahendra Rao et al.
13:30-15:30	Group C: Lab 3: Session 2: Neuron / Glia Progenitor Cell Culture (Rat) 1	Taner Dağcı et al.
	Lab Groups are limited to 6-8 participants. Lab 1, 2 and 3 last for 3 afternoons. NO SIG SESSIONS TODAY. No definite break for coffee; refreshments will be available during the practicals	
16:00-19:30	**Site Seeing in Izmir, Guided Tour to the Izmir Art & History Museum or Free Time for Leisure Activities.**	
	Dinner on Your Own	

Day 4 **July 2, Thursday**

09:00-10:00	Good Conduct of Experimental Research; New Perspectives, Codes and Practices in Animal Research	Pedro Maldonado
10:00-11:00	Molecular Mechanisms Underlying Secondary Damage After Spinal Cord Injury	Samuel David
11:00-11:20	**Coffee**	
11:20-12:20	Combinatorial Strategies with Schwann Cell Transplantation to Repair the Injured Spinal Cord	Mary Bunge
12:20-12:50	Neuroprotective Therapy Strategies After Stroke: The Roles of Intercellular Interactions, Drug Delivery and VEGF	Ertuğrul Kılıç
12:50-14:00	**Lunch**	
14:00-15:30	Mini Symposium on Neuro-Imaging of Neuro-Glial Interactions: From DTI in the Rat to Novel Approaches in Neurology and Psychiatry. "Imaging in Vivo the Myelination Abnormalities and the Recovery in Animal Models: Fine Microstructural Analysis of Brain White Matter"	Ali Saffet Gönül, Laura Harsan
15:30-18:00	Group A: Lab 1: Session 3: Immunofluorescence Labeling, IHC and HC for Myelin and General Histology to Study Injured Spinal Cord	Samuel David et al.
15:30-18:00	Group B: Lab 2: Session 3: Culture of Human Glial Precursors and IHC and PCR Analysis	Mahendra Rao et al.
15:30-18:00	Group C: Lab 3: Session 3: Neuron / Glia Progenitor Cell Culture (Rat) 2 & Astroglial Cell Culture (Rat) 2	Taner Dağcı et al. Özlem A. Yılmaz et al.
	Lab Groups are limited to 6-8. (Lab 1, 2 and 3 last for 3 afternoons) SIG Groups are limited to 15. All SIGs last for 1 afternoon. No definite break for coffee; refreshments will be available during the sessions.	
15:30-18:00	Group H: SIG 4: Lab Animal Handling and Ethics	Pedro Maldonado et al.
15:30-18:00	Group I: SIG 6: In-Vivo Neuro-imaging: Diffusion Tensor Imaging in the Rat	Laura Harsan, Ali Saffet Gönül
20:00-23:00	**Mangal (Barbeque) Party**	

Day 5 **July 3, Friday**

HOUR	TOPIC	FACULTY
09:00-10:00	Glial Regulation of Iron Homeostasis in the CNS in Injury and Disease	Samuel David
10:00-10:30	Protective effect of Erythropoietin on neuron and glial cells	Şermin Genç
10:30-10:50	**Coffee**	
10:50-11:50	Neuron-Glia Interactions in Neurology and Psychiatry	Robert T. Rubin
11:50-12:50	The Role of Axonal Transport in Alzheimer's Disease	Kenneth Moya
12:50-14:00	**Lunch**	
14:00-16:00	Group A: Lab 1: Session 4: Immunofluorescence Labeling, IHC and HC for Myelin and General Histology to Study Injured Spinal Cord	Samuel David et al.
14:00-16:00	Group B: Lab 2: Session 4: Culture of Human Glial Precursors and IHC and PCR Analysis	Mahendra Rao et al.
	FINAL DAY of Lab 1 and Lab 2 **NO SIGs TODAY** ***No definite break for coffee; refreshments will be available during the practicals***	
16:00-17:30	Summary and Evaluation of the V. EGE-BINGSS Poster Awards, SIG Awards, Adjurn	Reha Erzurumlu, Gönül Ö. Peker
	Dinner on your own	

Day 6 : July 4, Saturday

09:00-19:00

Full Day Guided Tour to Ephesus (including the Selcuk Museum)

Faculty Lecture Abstracts, Learning Objectives, and Recommended Reading Lists

(*Lecture material was prepared by Gülgün KAYALIOĞLU, but it will be presented by Emel ULUPINAR)

BASIC HISTOLOGY & CELL BIOLOGY OF NEURONS, GLIAL CELLS:

Summary of the lecture:

This lecture provides a general overview of the structural and functional properties of the nervous system cells, both neurons and glia, describing the main functions of neurons and glial cells, various parts of the neuron and how neurons communicate synapses.

Learning objectives:

1. Identify the types of cells found in the nervous system and describe their general functions and characteristics
2. Describe a typical neuron and describe the general functions of each componen
3. Describe the structural and functional classifications of neurons
4. Identify the types of supporting cells/glia found in the central nervous system (CNS) and peripheral nervous system (PNS). Describe the structure and general functions of each cell type

Recommended pre-reading list:

1. Ronald A. Bergman, Ph.D., Adel K. Afifi, M.D., Paul M. Heidger, Jr., Ph.D. Atlas of Microscopic Anatomy - A Functional Approach: Companion to Histology and Neuroanatomy: Second Edition.
2. Bear, Connors, and Paradiso (2007) Neuroscience: Exploring the Brain, 3rd Ed, Lippincott Williams & Wilkins
3. Zigmond, Bloom, Landis, Roberts, Squire (1999). Fundamentals of Neuroscience. Academic Press

Işıl KURNAZ

MOLECULAR BIOLOGIST'S INSIGHT INTO NEURO-GLIAL INTERACTIONS

Summary

The dynamic interaction between developing and adult neurons and glia will be discussed in this lecture, from a molecular biology perspective. Specifically gene expression, signaling and cell-cell intarction molecules will be summarized. The contribution of glia to brain tumors will also be analyzed from a molecular biologist's point of view.

Aim

To gain insight into the molecular mechanisms operating within neurons and glia of the nervous system.

Learning Objectives

This lecture intends to give the audience a brief introduction to some of the molecular pathways involved major cellular events within neurons as well as glial cells of the nervous system, and the relation and contribution of these pathways to healthy system vs disorders of the nervous system.

Methods/Procedures

Recent molecular biology tools such as microarrays, RNA interference etc will be introduced within this context.

References

- Allen, N.J and Barres, B.A (2005). Signaling between glia and neurons: focus on synaptic plasticity. Curr. Opin. Neurobiol. 15: 542-548
- Barres, B.A (2008). The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60: 430- 440
- Chao DL, Ma L and Shen K (2009). Transient cell-cell interactions in neural circuit formation. Nat Rev Neurosci 10: 262 – 271.

GENETIC AND METABOLIC EVENTS IN NEURON-ASTROCYTE INTERACTIONS

Summary

Glycolysis and aerobic respiration and energy metabolism in astrocytes and neurons will be introduced. Various alternative hypotheses on neuron-astrocyte lactate shuttle vs conventional metabolic theories will be discussed, with examples from both modeling and experimentation. Genetic regulation of metabolic enzymes in neurons during normoxia and ischemia/hypoxia will be shown by some examples from our own laboratory and the transcriptional mechanisms behind this regulation will be discussed.

Aim

To introduce alternative hypotheses on metabolic events within the brain, and to introduce computational research conducted in the area of brain energy metabolism.

Learning Objectives

This lecture intends to give the audience an introduction to the field, and to present them with different opinions and methods to address these issues.

1- To gain insight into the metabolic coupling and interactions between neurons and glia
2- To obtain some background on the genetic regulation of metabolic events in neurons
3- To gain the ability to correlate oxygen and glucose supply to gene regulatory events
4- To have a molecular biology perspective to cellular events within neurons and glia
5- To have a computational biology perspective to cellular events within neurons and glia

Methods/Procedures

Experimental as well as computational methods in our laboratory as well as other labs will be introduced.

EGE BINGSS

References

- Genc, S, Bal, E, Aksan Kurnaz, I, Ozilgen, M (2008). A preliminary Biochemical Model for Glucose-Lactate Metabolism in Neuron. Oral presentation, International Health Informatics and Bioinformatics, Turkey (HIBIT)'08, Istanbul.
- Chih, C-P, Lipton, P, and Roberts, El, Jr. (2001). Do active cerebellar neurons really use lactate rather than glucose? Trends in Neurosci. 24(10): 573 – 578.
- Dienel, G.A and Cruz, N.F (2003). Neighborly interactions of metabolically-activated astrocytes in vivo. Neurochemistry Intl. 43: 339 – 354.
- Magistretti, P.J (2006). Neuron-glia metabolic coupling and plasticity. The Journal of Experimental Biology 209: 2304-2311

Suggested pre-reading list

- Allen, N.J and Barres, B.A (2005). Signaling between glia and neurons: focus on synaptic plasticity. Curr. Opin. Neurobiol. 15: 542-548
- Almeida, A, Delgado-Esteban, M, Bolanos, J.P ve Medina,J.M (2002). Oxygen and glucose deprivation induces mitochondrial dysfunction and oxidative stres in neurons but not in astrocytes in primary culture. Journal of Neurochemistry. 81: 207-217
- Barres, B.A (2008). The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60: 430- 440
- Chih, C-P, Lipton, P, and Roberts, El, Jr. (2001). Do active cerebellar neurons really use lactate rather than glucose? Trends in Neurosci. 24(10): 573 – 578.
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- Gladden, L.B (2004). Lactate metabolism: a new paradigm for the third millenium. J. Physiol. 558.1: 5 – 30.
- Gordon, G.R.J, Choi, H.B, Rungta, R.L, Ellis_Davies, G.C.R ve MacVicar, B.A (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature. 456: 745-749
- Hertz L, ve Dienel G.A (2004). Lactate transport and transporters: General principles and functional roles in brain cells. J Neurosci Res. 79: 11-18.
- Itoh, Y, Esaki, T, Shimoji, K, Cook, M, Law, MJ, Kaufman, E, Sokoloff, L (2003). Dicholoracetate effects on glucose and lactate oxidation by neurons and astroglia *in vitro* and on glucose utilization by brain *in vivo*. PNAS 100(8): 4879- 4884.
- Magistretti, P.J (2006). Neuron-glia metabolic coupling and plasticity. The Journal of Experimental Biology 209: 2304-2311
- Munns,S.E, Meloni, B.P, Knuckey N.W, Arthur, P.G (2003). Primary cortical neuronal cultures reduce cellular energy utilization during anoxic energy deprivation. J.Neurochemistry, 87: 764-772
- Phatak, G.H.M, Patel, A.B, Xia,Y, Hong, S, Chowdhury, G.M.I, Behar, K.L, Orina, I.A, Lai, J.C.K (2008). Effects of continuous hypoxia on energy metabolism in cultured cerebro-cortical neurons, Brain Research 1229:147-154
- Pelerin, L (2003). Lactate as a pivotal element in neuron-glia metabolic cooperation. Neurochem 43: 331 – 338
- Seyfried, T.N, ve Mukherjee, P (2005). Targeting energy metabolism in brain cancer: review and hypothesis. Nutrition and Metabolism 2: 30 – 39
- Takata, T, Sakurai, T, Yang, B, Yokono, K ve Okada, Y (2001). Effect of lactate on the synaptic potential, emergy metabolism, calcium homeostasis and extracellular glutamate concentration in the dentate gyrus of the hippocampus from guinea-pig. Neuroscience 104(2): 371 – 378

Emel ULUPINAR

AN OVERVIEW OF NEUROTROPHIC FACTORS INVOLVED IN NEURO-GLIAL INTERACTIONS: GLIA DERIVED NEUROTROPHIC FACTOR:

Summary of the lecture

Normal development and formation of the vertebrate nervous system crucially depends on tightly regulated survival and death signals. Trophic stimuli ensure survival of preferred developing neurons, whereas apoptotic signals function to match the number of neurons to the target size and to refine target innervation. The closely related neurotrophic factors are essential for this developmental process, as they promote survival, differentiation and death of neurons.

The best known neurotrophic factors are those of the neurotrophin family which, in mammals, comprises four highly related polypeptides both functionally and structurally: Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3), and Neurotrophin 4/5 (NT 4/5). These molecules are first encoded as neurotrophin precursors and then the "pro-region" is proteolytically cleaved to form the mature neurotrophins. The core structure of all family members is remarkably similar, formed by a cysteine knot connecting six hydrophobic domains; but, their exposed regions vary to provide receptor-binding specificity. The different actions of neurotrophins are dictated via a complex receptor system consisting of two classes of cell surface receptors; the Trk family of tyrosine kinases and the p75 neurotrophin receptor. Unlike Trk receptors that exhibit selectivity for binding to neurotrophin ligands, p75 binds to all neurotrophins with similar nanomolar affinity. These interactions then activate several signal transduction pathways responsible for most of the survival and growth properties of the neurotrophins (for comprehensive review of neurotrophin signaling, please refer to Huang and Reichardt 2001). Paradoxically, the p75 receptor can mediate survival versus apoptotic outcomes. A number of other binding partners for p75 receptor indicate that this receptor also plays critical roles in vascular, glial and tumor biology.

GDNF (Glial cell-line Derived Neurotrophic Factor), on the other hand, is the founding member of the GDNF family of neurotrophic factors which additionally includes three other members, neurturin, persephin, and artemin. They belong to the transforming growth factor (TGF)-β superfamily and signal through a multicomponent receptor complex comprising the transmembrane Ret

tyrosine kinase and a member of a family of glycosyl-phosphatidyl-inositol (GPI)-anchored cell surface proteins, the GFRα. In the Ret/GFRa receptor complex, ligand binding is performed by the GFRα and the intracellular signalling by Ret. Additionally, there is a Ret-independent signalling pathway, using GFRα1-associated Src kinase activation, and GFRα has been shown to use also neural cell adhesion molecules (NCAM) as signalling receptors for GDNF family ligands (reviewed by Sariola and Saarma, 2003). Although GDNF was initially identified as a trophic factor for midbrain dopaminergic neurons and received much attention as a potential therapeutic agent for the treatment of neurodegenerative diseases; GDNF family ligands promote survival and maintenance of many types of neurons.

Neurotrophic factors are, now, known to have surprisingly diverse roles, not only during development; but also in functioning of the nervous system. They have been shown to regulate cell fate, axon growth and guidance, dendrite structure and pruning, synaptic function, and synaptic plasticity. Analysis of mice carrying mutations in neurotrophic factor or their related receptor genes has revealed their essential actions on the different neural populations.

Learning objectives

1.) Summarize the principles that modulate neuronal number in the developing nervous system and the role of neurotrophic factors.
2.) Classify the members of neurotrophic factor family and their receptors.
3.) Outline the endogenous expression pattern of neurotrophic factors and their receptors.
4.) Describe the developmental deficits seen in NGF, NT-3, BDNF and GDNF mutant mice.
5.) Summarize the roles of neurotrophic factors during development and normal activity of the nervous system.
6.) Identify the endogenous and pharmacological agents driving GDNF expression in the nervous system.

Recommended pre-reading list

1.) Huang EJ and L F Reichardt. NEUROTROPHINS: Roles in neuronal development and function. Annu. Rev. Neurosci. (2001) 24:677–736.
2.) Paratcha G and Ledda F. GDNF and GFRα: A versatile molecular complex for developing neurons. Trends in Neurosci. (2008) 31:384–391.
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Çağla EROĞLU

HOW DO ASTROCYTES INDUCE CENTRAL NERVOUS SYSTEM SYNAPTOGENESIS?

Synapses are asymmetric cellular adhesions that are critical for nervous system development and function, but the mechanisms that induce their formation are not well understood. Thrombospondin (TSP) is a large oligomeric astrocyte-secreted extracellular matrix protein that is sufficient to induce synapse formation in the central nervous system and is necessary for astrocyte-enhanced synaptogenesis *in vitro*. In this study we identify the thrombospondin receptor involved in synapse formation as the calcium channel subunit $\alpha 2\delta 1$, which is also the receptor for the anti-epileptic and analgesic drug gabapentin. We show that $\alpha 2\delta 1$ interacts with the epidermal growth factor-like repeats common to all thrombospondins. $\alpha 2\delta 1$ overexpression increases synaptogenesis *in vitro* and *in vivo* and $\alpha 2\delta 1$ is required for thrombospondin and astrocyte-induced synapse formation *in vitro*. We found that gabapentin is a potent inhibitor of excitatory CNS synapse formation *in vitro* and *in vivo*. These findings identify $\alpha 2\delta 1$ as a novel signaling receptor that induces synapse formation and suggest that gabapentin may mediate its therapeutic function by blocking new synapse formation.

EGE BINGSS

Reha ERZURUMLU

ROLE OF ASTROCYTES IN PERIPHERAL NERVE INJURY INDUCED SYNAPTIC PLASTICITY IN THE DEVELOPING BRAIN

Ischemic brain injury, hypoxia or mechanical damage results in new synapse formation in the brain. In the hippocampus, deafferentation results in rapid synaptic loss followed by a prolonged new synapse formation, which has been termed "reactive synaptogenesis." Not much is known about reactive synaptogenesis in the CNS following peripheral nerve injury, particularly during development when synapses are being built and refined through activity-dependent mechanisms.

We have recently shown that neonatal damage to the infraorbital branch of the trigeminal nerve (ION) results in rapid synaptic plasticity and reactive synaptogenesis in the principal trigeminal sensory nucleus (PrV) in the brainstem. This nucleus is the first relay station of the trigeminal nerve and provides the main input to the upstream thalamic and cortical centers. We have estimated the number of trigeminal fibers that innervate each neuron in the postnatal PrV with electrophysiological techniques. Single barrelette neurons are innervated by an average of 4-5 trigeminal fibers during the first postnatal week. The number of trigeminal afferent inputs to each barrelette neuron decreases to 3 during the second postnatal week. In the deafferented PrV trigeminal synaptic inputs to individual barrelette cells double and are stable during the second postnatal week. These results indicate that neonatal deafferentation results in convergence of more trigeminal fibers onto single barrelette neurons. The underlying mechanisms of this reactive synaptogenesis are not known. *In vitro* studies indicate that astrocytes actively promote synapse formation and function following damage. The following sequence of events is envisioned for the role of astrocytes in reactive synaptogenesis: Brain injury leads to an increase in extracellular Adenosine 5’triphosphate (ATP) released by damaged cells. ATP activates purinergic receptors, especially P2Y4 receptors abundant in astrocytes, so that astrocytes release soluble factors such as thrombospondins, especially TSP-1 and cholesterol. These extracellular signaling molecules promote synapse formation. In this presentation, various pharmacological manipulations of astrocyte function and their effects on peripheral nerve injury-associated synaptic plasticity in the PrV will be discussed

Ahmet HÖKE

TALK 1: Development of the Peripheral Nervous System: Schwann Cell Development and Biology and its Role in Regeneration

Abstract

Schwann cells are the primary glial cells of the peripheral nervous system (PNS). They originate from the neural crest during development and form one-to-one relationship with their axons before myelination takes place. Non-myelinating Schwann cells wrap around multiple axons. Both myelinating and non-myelinating Schwann cells are important in normal maintenance of the axonal health and when peripheral axons are injured they assume the role of "temporary target" and secrete a variety of growth factors and extracellular matrix proteins to promote regeneration. However, this process is not efficient in humans and chronic denervation in Schwann cells hamper nerve regeneration in humans.

Learning objectives:

- To learn about the developmental origin of the PNS
- To learn the differences and similarities between myelinating and non myelinating Schwann cells
- To learn about the role of myelination and axon-glia interactions in the PNS
- To learn about the role of Schwann cells in regeneration

Articles to read:

The origin and development of glial cells in peripheral nerves. Jessen KR, Mirsky R. Nat Rev Neurosci. 2005 Sep;6(9):671-82. Review.

Development of the Schwann cell lineage: from the neural crest to the myelinated nerve. Woodhoo A, Sommer L. Glia. 2008 Nov 1;56(14):1481-90. Review.

Mechanisms of Disease: what factors limit the success of peripheral nerve regeneration in humans? Höke A. Nat Clin Pract Neurol. 2006 Aug;2(8):448-54. Review.

EGE BINGSS

TALK 2: Axon-Schwann Cell Interaction in Peripheral Neuropathies

Abstract:
Axon-Schwann cell interaction is very important for the maintenance of axonal health. Perturbations in Schwann cell function or axon-Schwann cell interaction can lead to PNS diseases. These include inherited disorders like Charcot-Marie-Tooth disease type I (CMT-I) and acquired disorders such as Guillain-Barre syndrome (GBS), chronic inflammatory demyelinating neuropathies (CIDP) or diabetic neuropathies.

Learning objectives:

- To learn about molecules relevant to axon-Schwann cell interaction
- To learn about inherited disorders with Schwann cell pathology
- To learn about acquired peripheral neuropathies with Schwann cell dysfunction
- To learn about the role of axon-Schwann cell interaction in development of peripheral neuropathies and potential therapies

Articles to read:

- Molecular mechanisms of inherited demyelinating neuropathies. Scherer SS, Wrabetz L. Glia. 2008 Nov 1;56(14):1578-89. Review.
- Negative regulation of myelination: relevance for development, injury, and demyelinating disease. Jessen KR, Mirsky R. Glia. 2008 Nov 1;56(14):1552-65. Review.
- Neuroprotection in the peripheral nervous system: rationale for more effective therapies. Höke A. Arch Neurol. 2006 Dec;63(12):1681-5. Review.

Gönül PEKER

NÖROETİK

Kendi içinde zaten çoğul disiplinli bir düşün, eğitim ve araştırma alanı bulunan "sinirbilim" veya "beyin bilimi", günümüzde, kendine özgü felsefi, etik, estetik, hukuksal ve politik açılımları ile sınırları zorlamakta ve iyice özgül bir etik alt disiplini olarak, "Nöroetik" kavramını önermektedir.

ABDde, özellikle beyin ve kanser araştırmalarını destekleyen saygın ve güçlü Dana Vakfı'nın daveti üzerine, yüzelliyi aşkın yetkin ve ünlü temel ve klinik sinirbilimci, biyoetikçi, psikiyatrist, psikolog, sosyolog, felsefeci, hukukçu ve kamu yöneticisi, Mayıs 2002de San Francisco kentinde buluşarak, ortak bir dil bulma ve nöroetik alanını betimleme çabasına giriştiler. Başlangıçta, nöroetik, "beyine ilişkin bilimsel gelişmelerin, tıp uygulamaları, hukuksal yorumlar, hatta yasal düzenlemeler ile sosyal ve sağlık politikalarına taşınması sonucu ortaya çıkan ve çıkabilecek olan etik, hukuksal ve toplumsal soru ve sorunları inceleyen bir bilim alanı" olarak görece yalın bir biçimde tanımlandı. Böylesi soru ve sorunların doğabileceği ilk akla gelen alanlar, kuşkusuz, genetikbilim, beyin görüntüleme, beyin hastalıklarının tanısı ve yönetimidir. Aynı yaklaşımla, hekim, biliminsanı, eğitimci, yargıç, hukukçu, sigorta yöneticisi ve yasa koyucular ile kamunun, bu soru ve sorunlara karşı tutum, uygulama ve çözümlemeleri, nöroetiğin temel konularını oluşturmalıdır.

Nöroetik sorunsalına somut ve didaktik ama aynı zamanda sansasyonel bir çıkış noktası örneği, en güzel anlatımını A. Damasio'nun "Descartes'ın Yanılgısı" adlı yapıtında bulduğumuz, tarihsel Phineas Gage iş kazası olgusudur. Tüm sinirbilimcilere tanıdık olan bu demiryolu işçisi, beyin ön lobunun yaşamla bağdaşmayacak derecede zedelenmesine karşın, "yaşamış" (yaşatılmış), ancak, yaşamını, önceki kişiliğinden çok farklı biçimde, "ahlaki karar alma ve uygulama özürlü" olarak sürdürmek zorunda kalmıştır. Damasio, etik düşünce ve davranışı, evrimle ilişkilendirir ve biyodüzenlemenin bir yüzü olarak tanımlar; etiğin biyolojik temelleri için, frenolojiyi ve genlerin egemen rolünü red, çoğul sistemli nöronal örüntüleri, genlerin yatkınlaştırıcılığını, kültürün önemini, durumsal ve bağlamsal ögelerle beyin sağlığının belirleyiciliğini savunur.

Nörofelsefeci P.S. Churchland, davranışı beyin olaylarının sonucu olarak tanımlar. Ona göre, nöron ve nöron ağları düzeyinde beyin, nedensel bir makine olsa bile, ceza hukuku bağlamında bu "nedensellik", cezanın etkililiği, toplumun güvenliği ve affın toplumsal önemi gibi etkenlerin biçimlendirdiği sorumluluğu ortadan kaldırmaz. Churchland, "kendi" kavramını nörobiyolojik açıdan ele

aldığında, mutlak çözümleme için, “özgür irade”, “toplumsal uyumluluk” ve “kontrol-dışılık” halleri ve kişilikleri için nöral farklılıkların tanımlanması gereğini vurgular.

Biyofelsefeci K.F. Schaffner, özellikle, “aydınlatılmış ve özgür seçim ve onam” kavram ve uygulamalarına, “açık veya kapalı indirgemecilik” ve “açık veya kapalı determinizm” ölçüleri içinde yaklaşır. Hastalık yönetimine ilişkin klinik uygulamalar ve hukuksal düzenlemelerde, konu beyin sağlığı olduğunda, aydınlatılmış onamın, “seçimin anlaşılması, takdiri, akıla vurulması ve ifadesi” ögelerini içeren, “MacCat” ve benzeri ölçüm araçlarının ne denli yetersiz, uzak ve anlamsız kalabildiğini vurgular.

Biyoetikçi J.D. Moreno, “kendi kaderini belirleme” kavram ve uygulamalarını, nöroetik platformunda, beyin-beden sorunsalı çerçevesinde ele alır. Kararların, koşulluluk, bağlamsallık ve durumsallık nitelikleri nedeniyle, ne denli “özgür” olabileceğini sorgular ve bu konudaki Amerikan yaklaşımını “indirgemeci ve kolaycı” olarak niteler.

“Belleğin Yedi Günahı” adlı yapıtın yazarı, psikolog D.L. Schacter, özellikle dört bellek durumunu nöroetik ile ilişkilendirerek, beyine etkili ilaçların farklı kişilere uygulanmasının yaratabileceği sorunları vurgular. Ona göre, “anılara erişimi engelleyen” ilaçlar bir işyeri veya okulda eşitsizliğe yolaçabilir, “dalgınlık veya dikkat boşlukları” yasal sorunlar doğurabilir, “karıştırma” gerçek ve düzmece bellek birbirinden ayrılamadığından yanlış suçlamalara neden olur ve istenmedik anılara “takıntılar” da ilaçların yardımı ile yokedilebilir. Bu durumda, bir “şiddet mağduru” veya “felaket gönüllüsü”ne bu ilaçların uygulanması ne kadar doğru olacaktır.

Tıp felsefecisi ve hukukçu W.J. Winslade, travmatik beyin hasarı ve cezai ehliyet - yasal sorumluluk bağıntısına dikkat çeker. Ceza hukuku uygulamalarında, suçlanan kişinin beyin travması geçirmiş olması, hatta bunun tekrarı ve sıklığı ögelerinin hiç dikkate alınmamasını çok önemli bir evrensel kusur olarak ortaya koyar.

Hukukçu ve onursal genetikbilimci H. Greely, uzmanı olduğu insan genetiğinin etik, yasal ve toplumsal boyutları ile nöroetiği karşılaştırır ve özellikle, “sağkalım ve esenliğe ilişkin öngörü”, “insan klonlama” ve “determinizm/özdecilik” sorunsallarının nöroetik bağlamındaki önemini vurgular. Greely’ye göre, sinirbilimsel gelişmelerin hızı, öncül deneysel yaklaşımların yakında alınacak olan sonuçları ve karar vericilerin inanç sistemleri ile belirlenecek olan nöroetiği yakın gelecekte çok önemli ve zorunlu kılacaktır.

Beyin biliminin uygulamalarına bakıldığında, psikofarmakoloji araştırma ve uygulamaları, Alzheimer ve Huntington hasta ve yakınlarındaki genetik

bilgilenmeler, bunların gizliliği, farklı paydaşlar tarafından farklı amaçlar doğrultusunda kullanılması, dikkat bozukluğu ve hiperaktivitenin "sağaltımı" konuları, nöroetiğin en ön saflarında yer almaktadır.

Teknoloji ve tıp felsefecisi E. Parens, Fukuyama'ya atıfta bulunarak, "İnsan-sonrası Gelecek" uzgörüsü ile "sağlıksızlıkta sağaltım ve koruyuculuk" ile "sağlıklılıkta süperleştirme" arasında çok kesin bir ayırım yapılması gereğine işaret etmekte ve bu iklemin özellikle nöroetik alanında çok önemli olacağını vurgulamaktadır.

Biyoetikçi P.R. Wolpe, "organik dünyayı teknolojize ederken, teknolojik dünyayı da organikleştirmekteyiz; bu yeniliklerin, bir parçamız durumuna gelerek, tüm yaşamımızı, hatta insan evrimini etkilemesi işten değildir" diyerek, "biyonik" teknolojilere, özellikle nöronal yongaları içeren "fizyoteknolojiler"e ve "siborglara" dikkat çekerken, "bilimi izlemek yerine, yönlendirmek ve yönetmek" farkındalığı ve bilinci uzerinde durmaktadır.

Moleküler, genetik, hücresel ve elektronik bilimlerden çok önemli destek alan sinirbilim, son 20 yıldır giderek artan bir ivme ile önlenemeyen bir gelişme göstermektedir. Bu "sunum" (arz) bolluğu, elbette, kendi "pazarının" (talebinin) da mühendisliğine soyunmaktadır ve bunu daha da ileriye taşıyacaktır. "İnsan-sonrası" veya "insan-ötesi" bir döneme evrilmemek üzere, bilimi de dizginlemek ve denetleyebilmek gerekir. Bu önerme, şimdilerde çok septik ve aşağılayarak baktığımız, bir zamanların "yararcılığı"ndan (pragmatizm) farklı bir durumu irdelemektedir. Genelde, bilim ve bilimsel gelişmeler ve özelde sinirbilim, etik ve insancıl ögelerin dikkatle izlediği; yine özelde, nöroetiğin soluksuz peşinden koşturduğu ve denetlemeye çalıştığı bir süreçler bütünüdür.

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Şermin GENÇ

PROTECTIVE EFFECT OF ERYTHROPOIETIN ON NEURONAL AND GLIAL CELLS

Erythropoietin is a hematopoietic cytokine, which has recently been shown to be expressed in the nervous system (1). The expression of Erythropoietin and its receptors in the nervous system is modulated by hypoxia and metabolic insult. Erythropoietin receptor signaling is required for normal brain development. Erythropoietin reveals protective effects in experimental models of brain injury including hypoxia-ischemia, stroke, trauma, and inflammation (1-2). The advantages of Erythropoietin are the use in clinical situation with anemia indication for many years and the ability to pass across the blood-brain barrier. In recent studies, anti-apoptotic, anti-oxidant and anti-inflammatory effects of Erythropoietin have been demonstrated. However, the mechanisms of its effects were not fully clarified. I will present our data about the protective effect of erythropoietin in vitro models of neuronal and glial injury. I will also discuss possible intracellular signaling mechanism of Erythropoietin in these models (3-5).

Keywords: Erythropoietin; glia, protection; Neuron

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Kenneth L. MOYA

Abstract

We have transplanted cultured adult olfactory ensheathing cells into lesions of intraspinal long tracts and spinal root avulsions in adult rats. The grafted cells encourage the growth of the cut nerve fibres, and suppress the excessive neuromatous branching found in untreated lesions. The grafted cells take up an elongated shape, and form a tightly aligned bridge between the ends of the cut fibre tract. The regenerating nerve fibres enter the graft and follow this new, aligned bridge pathway. Within the bridge the nerve fibres are intimately ensheathed by the Schwann-like cells, and enclosed in an outer, perineurial-like sheath of fibroblasts. In the case of the spinal tracts, once they reach the end of the graft they re-enter the host spinal cord, and continue along the distal part of the corticospinal tract to form terminal arborisations. The effect is to put a patch over the lesion, restoring the integrity of the original pathway, and results in the functional recovery of some specific functional tasks. In the case of the dorsal roots the fibres re-enter the spinal cord, arborise in the dorsal horn, and ascend in the dorsal columns. In the case of the ventral roots there is a 4-5 fold increase in the numbers of fibres entering the proximal part of the root.

Kenneth L. MOYA

THE HOMEOPROTEIN OTX2 PROMOTES SURVIVAL OF DAMAGED ADULT RETINAL GANGLION CELLS.

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The degeneration of the axons and the loss of RGCs lead to profound and irreversible blindness. The leading cause of RGC degeneration is glaucoma. A 2002 World Health Organization survey estimated that 37 million people are afflicted with frank blindness and that glaucoma is the primary cause for about 4.5 million of these individuals (Resnikoff *et al*., Global data on visual impairment in the year 2002. *Bull World Health Organ* 82: 844-851, 2004, doi: 10.1590/S0042-96862004001100009). The identification of factors that increase RGC survival would be of paramount therapeutic value.

Homeoprotein transcription factors can translocate between cells and regulate transcription and translation in the recipient cell. One of these proteins, engrailed 2 has been shown to contribute to neuronal survival in vivo. In mice lacking one allele of engrailed 1 ventral mesencephalic dopaminergic neurons progressively degenerate. This neuronal degeneration can be attenuated by infusion of Engrailed 2. More recently, it has been shown that Engrailed promotes the survival of mesencephalic dopaminergic neurons in culture (ref).

While engrailed is expressed in central visual structures it is not expressed in the retina. The homeoprotein Otx2 is expressed in the retina where its mRNA is detected in photoreceptors and bipolar cells. At the protein level, Otx2 is detected in photoreceptors, bipolar cells and RGCs. The presence of Otx2 in RGCs raised the possibility that Otx2 can be taken up by RGCs. Recently, it has been demonstrated that Otx2 can be taken up by RGCs, trans-synaptically transferred to parvalbumin cells in visual cortex

where the protein initiates the opening and closure of the critical period for ocular dominance plasticity.

Here we tested the hypothesis that Otx2 promotes the survival of adult RGCs after damage. In cultures of dissociated adult mouse retina Otx2 promoted the survival of RGCs at 6 days in vitro in a dose-dependent manner with a maximal effect at 1.65nM. Since cultures of dissociated retina contain all cell types that are in the retina we purified RGCs and cultred them. We found that Otx2 significantly increases RGC survival demonstrating that Otx2 acts directly on RGCs.

To test the effects of Otx2 on RGC survival in vivo we used the excitotoxic model of intraocular injection of NMDA. 4 days after intraocular injection of NMDA, levels of Brn3A mRNA (a protein specific to RGCs) was significantly decreased compared to the noninjected eye consistent with massive RGC death. When Otx2 was injected at the same time as NMDA, the Otx2 completely protected against the decrease in Brn3A indicating a sparing of the RGCs.

Our results show that Otx2 stimulates the survival of axotomized adult RGCs in vitro and protects RGCs against NMDA-induced toxicity in vivo and suggest that Otx2 may offer a therapeutic approach for glaucoma.

Suggested reading

Sonnier L, Le Pen G, Hartmann A, Bizot JC, Trovero F, Krebs MO, Prochiantz A. (2007) Progressive loss of dopaminergic neurons in the ventral midbrain of adult mice heterozygote for Engrailed1. J Neurosci. 27:1063-71

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EGE BINGSS

Kenneth L. MOYA

LOOKING THROUGH THE EYE INTO THE ALZHEIMER BRAIN

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Alzheimer's disease (AD) is a neurodegenerative disease with severe and progressive memory loss and dementia. The neuropathological hallmarks of AD are the presence of senile plaques and neurofibrillary tangles. Interestingly, a number of studies have reported that synapse loss is an early event in AD and that it correlates with cognitive decline suggesting that AD is a disorder of synaptic function. Senile plaques are deposits of amyloid with a 41-42 amino acid peptide at their core. This peptide is a fragment of the amyloid precursor protein (APP) produced by a series of proteolytic cleavage events mediated by β and γ secretases. An important role for APP in AD pathology is further supported by familial cases of the disease, which are linked to mutations in APP or in presenilin, a component of the γ secretase. APP is heavily expressed in neurons where the protein is axonally transported and is localized to synaptic terminals, although its precise function is not known.

The eye can be used as a window onto synaptic function in the brain. *In vivo* metabolic labeling studies of the primary visual projection from the retina to the midbrain have shown that APP is developmentally regulated and that the expression of some isoforms is correlated with synaptogenesis. The APP that arrives at the presynaptic terminal is *N*- and *O*-glycosylated, and carries chondroitin sulfate, posttranslational modifications that are consistent with the protein functioning as an adhesion molecule at the synaptic terminal. The full-length transmembrane form of APP is cleaved and rapidly eliminated from the synapse *in vivo*. The half-life of APP at the synaptic terminal is 2-3 hours and the turnover is not dependent on retinal ganglion cell activity.

Based on these results we proposed a model of AD in which synaptic dysfunction due to an alteration of APP metabolism is the precipitating

cellular event in the disease. We hypothesize that APP plays a fundamental role in normal synaptic function, perhaps by contributing to synaptic integrity. We tested this hypothesis using RNA interference *in vivo* to knockdown presynaptic APP in the brain. Twenty four hours after intraocular injection, siRNA targeted against APP accumulates in retinal cells and the APP in retinal terminals in the superior colliculus is significantly reduced. Surprisingly, the amyloid precursor-like protein 2 (APLP2) was reduced as well. Functional imaging experiments in rats during visual stimulation showed that knockdown of presynaptic APP/APLP2 showed that reducing APP/APLP2 in the axon terminal had a profound effect on physiological activity. Namely, APP/APLP2 knockdown significantly decreased stimulation-induced cerebral glucose uptake, a measure of synaptic activation.

Our results suggest that in AD, subtle changes in the amount of APP delivered to the synapse or in its rate or turnover lead to a change in synaptic function. Such changes could arise sporadically from alterations in axonal transport rate or from familial mutations in APP itself or the proteins essential for its proteolytic processing. Prolonged synaptic dysfunction would destabilize synapses leading to their loss and contribute to the early cognitive changes observed in patients with AD.

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EGE BINGSS

Mary Bartlett BUNGE

NOVEL COMBINATION STRATEGIES TO REPAIR THE INJURED MAMMALIAN SPINAL CORD

Summary

Due to the varied and numerous changes in spinal cord tissue following injury, successful treatment for repair may involve strategies combining neuroprotection (pharmacological prevention of some of the damaging intracellular cascades that lead to secondary tissue loss), axonal regeneration promotion (cell transplantation, genetic engineering to increase growth factors, neutralization of inhibitory factors, reduction in scar formation), and rehabilitation. Our goal has been to find effective combination strategies to improve outcome after injury to the adult rat thoracic spinal cord. Combination interventions tested have been implantation of Schwann cells (SCs) plus neuroprotective agents and growth factors administered in various ways, olfactory ensheathing cell (OEC) implantation, chondroitinase addition, or elevation of cyclic AMP. The most efficacious strategy in our hands for the acute complete transection/SC bridge model, including improvement in locomotion [Basso, Beattie, Bresnahan Scale (BBB)], is the combination of SCs, OECs, and chondroitinase administration (BBB 2.1 vs 6.6, 3 times more myelinated axons in the SC bridge, increased serotonergic axons in the bridge and beyond, and significant correlation between the number of bridge myelinated axons and functional improvement). We found the most successful combination strategy for a subacute spinal cord contusion injury (12.5–mm, 10–g weight, MASCIS impactor) to be SCs and elevation of cyclic AMP (BBB 10.4 vs 15, significant increases in white matter sparing, in myelinated axons in the implant, and in responding reticular formation and red and raphe nuclei, and a significant correlation between the number of serotonergic fibers and improvement in locomotion). Thus, in two injury paradigms, these combination strategies as well as others studied in our laboratory have been found to be more effective than SCs alone and suggest ways in which clinical application may be developed.

Ertugrul KILIÇ

NEUROPROTECTIVE THERAPY STRATEGIES AFTER STROKE: THE ROLES OF INTERCELLULAR INTERACTIONS, DRUG DELIVERY AND VEGF

Neuroprotection therapies have made limited progress in recent years. Several compounds shown to be efficacious in animals were tested in humans in cost-expensive trials. Unfortunately none of these studies were able to demonstrate efficacy under clinical conditions in patients. In order to establish treatments that are of benefit not only in animals but also humans, new strategies are clearly needed, comprising (i) new factors mimicking intrinsic mechanisms that the brain itself makes use of, (ii) novel delivery techniques allowing drugs to pass the blood-brain barrier more efficaciously than before, (iii) better, functionally relevant readouts of brain recovery and (iv) strategies that are of usefulness not only in the acute, but also post-acute stroke phase. In this presentation, our recent studies will be reviewed.

References

Kilic et al., 2008 Brain 131: 2679-89;
Kilic et al., 2006 *FASEB J* 20: 1185-7 ;
Kilic et al., 2006 *J.Neurosci.* 26: 12439-46
Spudich et al.,2006 *Nature Neuroscience* 9: 487-8

Learning Objectives

1. General pathophysiology of stroke
2. ATP binding cassette transporters and their roles in brain pharmacotherapy after stroke
3. The effects of endogenously expressed- and exogenously administrated-Vascular Endothelial Growth Factor after Retinal ganglion cell- and Brain-injury

Prereading list

1. Kilic E, Spudich A, Kilic U, Rentsch KM, Vig R, Wunderli-Allenspach H, Fritschy JM, Bassetti CL, Hermann DM. ABCC1: A gateway for drugs to the ischemic brain. *Brain* 131: 2679-2689 2008
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4. Kilic E, Kilic U, Wang Y, Bassetti CL, Marti HH, Hermann DM. The phosphatidylinositol-3 kinase/ Akt pathway mediates VEGF's neuroprotective activity and induces blood brain barrier permeability after focal cerebral ischemia. *FASEB J* 20: 1185-7.2006.

Oğuz GÖZEN

PRESYNAPTIC REGULATION OF ASTROGLIAL EXCITATORY NEUROTRANSMITTER TRANSPORTER GLT1/EAAT2

The core elements of the central nervous system (CNS) functional unit include presynaptic axons, postsynaptic dendrites, along with the perisynaptic astroglia which ensheathe the vast majority of all synapses. The neuron-astrocyte synaptic complex is a fundamental operational unit of the nervous system. Astroglia play a vital and active role in synaptic transmission, by regulating extracellular K+ concentration, by releasing gliotransmitters, and by modulating glutamate receptor (GluR) activation through glutamate transporter (GluT)-mediated control of synaptic and extra-synaptic glutamate clearance. The astroglial specific plasma membrane glutamate transporter subtype GLT1 and its human homology, EAAT2 are the dominant functional/physiologically active transporter in the CNS. GLT1/EAAT2 is typically concentrated in perisynaptic membranes. GluTs directly contend with GluRs for glutamate, modulating the intensity and duration of postsynaptic activation and preventing the interference (‘‘spillover’’) neighboring synapses. Importantly, astroglial GluTs also prevent accumulation of extracellular glutamate and subsequent over-stimulation of GluRs, thus preventing possible excitotoxic neuronal death. Despite of the importance of astroglial GluTs in synaptic function, the normal molecular regulation of the neuron-astrocyte functional unit is not known. . We showed that presynaptic terminals regulate astroglial synaptic functions, GLT1/ EAAT2, via kappa B-motif binding phosphoprotein (KBBP), the mouse homolog of human heterogeneous nuclear ribonucleoprotein K (hnRNP K), which binds the GLT1/EAAT2 promoter. Neuron-stimulated KBBP is required for GLT1/EAAT2 transcriptional activation and is responsible for astroglial alterations in neural injury. Denervation of neuron-astrocyte signaling by corticospinal tract transection or neurodegeneration in amyotrophic lateral sclerosis all result in reduced astroglial KBBP expression and transcriptional dysfunction of astroglial transporter expression. Presynaptic elements dynamically coordinate normal astroglial function and also provide a fundamental signaling mechanism by which altered neuronal function and injury leads to dysregulated astroglia in CNS disease.

LAB & SIG Readings

Immunofluorescence Labeling, IHC and HC for Myelin and General Histology to Study Injured Spinal Cord

LABORATORY GUIDE AND PROTOCOL NOTES

Dr. Sam Davis, V. Ege BINGSS, 29 June – 4 July 2009, Izmir, TURKEY

Recipes of basic histochemistry solutions:

- **0.1M Phosphate Buffer**

Solution A= Dissolve 2.76g NaH2PO4.H2O (Sodium Phosphate Monobasic FW: 137.99) in 100ml distilled H_20.

Solution B= 2.84g Na_2HPO_4 in 100ml (Sodium Phosphate Dibasic Anhydrous FW 141.96)

Mix 19 ml of A + 81 ml of B = 100ml of 0.2 Molar Phosphate Buffer. To 100ml of 0.2 M Phosphate Buffer add 100ml of distilled water to make final solution of **0.1M phosphate buffer.** Check pH with pH meter and adjust **to pH 7.4** if necessary

- **Phosphate Buffered Saline (PBS)**

To prepare 1 liter of PBS, place magnetic stirring bar in beaker with 800 ml distilled H_20 and dissolve each of the following one at a time while stirring constantly on magnetic stir plate:

- 8.0g NaCl (Sodium Chloride)
- 0.2g KCl (Potassium Chloride)
- 1.44g Na_2HP0_4 (Sodium Phosphate Dibasic Anhydrous)
- 0.24g KH_2P0_4 (Potassium Phosphate Monobasic)

Adjust the pH to 7.4 using a pH meter. Remove stirring bar and adjust the volume to 1 liter with distilled H_20.

- **4% Paraformaldehyde Fixative**

To make 200 ml 4% paraformaldehyde fixative solution, weigh out **8 grams** of Paraformaldehyde and place in beaker containing **100ml** of distilled H_20 with magnetic stirrer. Stir on a magnetic stirrer/hot plate until the temperature reaches **60^o C.** *(Be careful not to over shoot the temperature as this will breakdown the paraformaldehyde).*

Add 10N NaOH drop wise until the solution clears. It usually requires approximately 1 drop per 100ml of water being used. Once the solution clears, remove it from heat. Add 100ml of 0.2 Molar Phosphate Buffer solution *(see protocol for preparing phosphate buffer)* and stir to mix. This will bring the final solution to **4% paraformaldehyde in 0.1M phosphate buffer.**

Filter solution using Whatman filter paper and funnel into another beaker. Check pH using Litmus Paper *(do not use pH meter as the fixative will damage your electrode).* The pH should be between 7.2 and 7.4, you should not need to have to adjust.

Hematoxylin and Eosin Staining

Ammonia Water: Stir 3 ml of Ammonium Hydroxide 20-22% into 1000 ml Tap Water

Eosin Preparation

Stock Solution:

1) Dissolve 1g of Y-Eosin *(Fisher # E511-25)* in 20 ml distilled water (dH_2O).
2) Add 80ml of 95% Ethanol
3) Stir to mix

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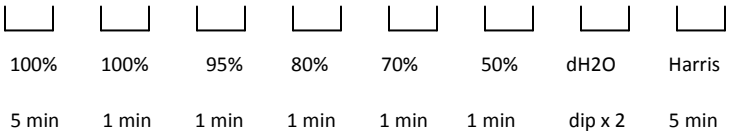
Final Eosin Solution:

1) Dilute stock solution (above) 1:6 with 80% Ethanol (1 part eosin and 6 parts ethanol).
2) Example: Mix 50ml Eosin Stock Solution + 250ml Ethanol = 300ml total. Add 0.5ml Glacial Acetic Acid for every 100 ml Eosin. For the above example you would add 1.5 ml glacial Acetic Acid
3) Stir to mix

Harris Hematoxylin Solution:

Purchased in solution (Fisher # 23-245-677)

Prepare in advance all solutions including the different percent ethanol solutions and water and place in staining dishes. Begin by placing microscope slides *(Fisher # 12-550-15)* with tissue into glass racks to fit your staining dishes.



Protocol

1) Fix slides in 100% Methanol for 5 minutes.
2) Hydrate sequentially in 100%, 95%, 80%, 70%, and 50% Ethanol for 1 minute each.
3) Wash slides in distilled water (dH_2O) by dipping several times for 2 minutes.
4) Stain slides by incubating in Harris Hematoxylin for 5 minutes
5) Dip slides in fresh dH_20 until clear (approximately 1 minute).
6) Dip slides in Ammonia Water until bright blue (about 5 dips).
7) Wash slides in dH_2O for 2 minutes, place in fresh dH_20 and rinse for another 2 minutes.
8) Dip slides in final Eosin solution for 20 seconds.
9) Wash slides in dH2O for 1 minute.
10) Dehydrate slides sequentially in 50%, 70%, 80%, 95%, 100% Ethanol for 1 minute each.
11) Incubate slides in Hemo-De *(Scientific Safety Solvents)* 2 times for 1 minute each. (*Hemo-De is used instead of Xylene*).
12) Coverslip with Entellan.

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Cresyl Violet Staining

Preparation of 0.1% Cresyl Violet Solution

- Add 0.5 grams of Cresyl Violet into 500 ml distilled water.
- Add 10 drops of Glacial Acetic Acid.
- Add magnetic stirring bar and stir well on magnetic stirring plate (15 min or until well dissolved).
- Filter solution using Whatman filter paper and funnel.
- Store in glass bottle in the dark at room temperature.
- Stain may be re-used, as it gets better with age but must always be returned to a tightly capped bottle each time.

Protocol: Prepare ahead of time 1 glass staining dish for each of the following: distilled water, Cresyl violet solution, 50%, 70%, 95% ethanol, and 3 dishes of 100% ethanol and 3 dishes of Hemo-De.

H_2O Cresyl H_2O x 2 50% Eth 70% Eth 95% Eth 100% Eth x 3

1. Place slides in glass racks and wash with distilled water (dH_2O) in staining dish 2 times for 5 minutes each by replacing water each time.
2. Incubate in 0.1% Cresyl Violet solution for 10 minutes. *(Staining time is variable between tissues and thickness of sections. It can vary from between 2-10 minutes, with 10 minutes being the longest tissues should be left in the stain).*
3. Wash with dH_2O for 2 minutes.
4. Wash with 50% Ethanol for 2minutes.
5. Wash with 70% Ethanol for 2minutes.
6. Wash with 95% Ethanol for 2minutes.
7. Wash with 100% Ethanol 3 times for 1minute each. *(At this point you may check 1 slide for staining intensity. If staining is too dark or too light you can rehydrate slides by incubating in the alcohols in reverse order and washing longer in the water or staining longer in the Cresyl Violet and then repeating the dehydration process).*
8. Incubate in Hemo-De *(Scientific Safety Solvents)* 3 times for 5 minutes each.
9. Coverslip and mount with a permanent mounting media such as Entallan

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Immunofluorescence

- Collect 10-14µm cryostat frozen tissue sections onto Super Frost Plus or gelatin-coated microscope slides.
- Sections must be dried completely for at least 1hour in a dessicator before placing in -20^{o}C freezer for storage if not using on same day.
- When removing from freezer allow slides to come to room temperature before using.
- Slides should not be warmed on heating plate.
- You will need a slide box that can be used to incubate slides flat with the antibody solutions.

Preparation of Blocking Solution:

In 5ml of PBS (see preparation of PBS protocol) add:

100 µl of Normal Serum *(serum that your secondary antibody is made in. For example if you would be using Fluorescein conjugated Goat anti Rabbit IgG then you would be adding Normal Goat Serum (e.g. from Jackson Immunoresearch # 005-000-121) in your blocking solution).*

0.05 grams of Ovalbumin

5 µl of 10% Tween-20

*Note: If tissue sections are from mouse tissue and the primary antibody is a mouse monoclonal then you must also add unlabelled anti mouse IgG to your blocking solution at a dilution of 1 in 100. Therefore for 5ml blocking solution add 50 µl.

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Preparation of Primary Antibody (example: Rabbit anti-Serotonin (5-HT))

Prepare antibody to desired concentration or dilution using blocking solution. Each slide will require 300 µl of antibody solution. For example if staining 5 slides you will require 1500 µl antibody solution. Rabbit Anti- Serotonin for example is used at a 1 in 5000 dilution so you would need 3 µl of antibody and 1497 µl of blocking solution - mix well using a vortex.

Preparation of Secondary Antibody

Prepare fluorescent secondary antibody to desired concentration or dilution using blocking solution. Cover tube with foil to keep in dark. Again each slide will require 300 µl of solution. For example if Rabbit anti-Serotonin was the primary antibody that was used you will need to use the Fluorescein conjugated Goat anti- Rabbit IgG secondary antibody at a 1 in 200 dilution. For example you would use 8 µl of secondary antibody and 1592 µl of the blocking solution for a total of 1600µl

PROTOCOL

Day 1

1. Remove slides from freezer and allow them to come to room temperature.
2. Place slides in a Coplin jar containing PBS and wash gently for 10 minutes on a clinical rotator (approximately 80 rpm).
3. Remove slides from PBS and carefully wipe the excess buffer.
4. Place slides flat in a slide box and add 300 µl of Blocking Solution and incubate covered for 3 to 5 hours at room temperature. To prevent the slides from drying, place wet tissue paper in the slide box.
5. Drain blocking solution from slides (except for negative control, that will not receive primary antibody) and gently wipe around sections again.
6. Add 300 µl of primary antibody solution to each of the remaining slides.
7. Cover box and incubate overnight at 4^{o} C (make sure that box is placed on a flat surface and will not be disturbed).

Day 2

1. Remove antibody solution and place slides in coplin jar containing 0.05% Tween-20 in PBS *(50µl of 10% Tween-20 in 100 mls of PBS).*
2. Incubate while shaking on clinical rotator (80 rpm) for 15 min at room temperature.
3. Replace wash solution with fresh 0.05% Tween-20 and incubate as above for 15 minutes.
4. Replace wash solution with PBS and incubate as above for 15 minutes.
5. Remove slides from wash solution gently wipe excess buffer around sections and place flat in slide box.
6. Add 300µl of the secondary antibody solution onto each slide and cover box (protect from light).
7. Incubate slides in dark at room temperature for 1 hour.
8. Remove antibody solution and place slides in coplin jar containing PBS. During wash steps protect from light by covering coplin jar with aluminum foil each time.
9. Incubate while shaking on clinical rotator (80rpm) for 15 minutes at room temperature.
10. Replace wash solution with fresh PBS and incubate as above for 15minutes 2 times.
11. Wipe excess buffer carefully around tissue sections and coverslip with Vectashield Anti-fading mounting media*)* containing DAPI nuclear stain.
12. Wipe away excess mounting media and nail-polish along the sides of the coverslip to seal.
13. Let dry for 15 minutes always in the dark before viewing under microscope or storing in -20^{o}C freezer.

Brain Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) in Small Animals

WORKSHOP-ROUNDTABLE DISCUSSION

DT-MRI is an exciting technique in neuroimaging that affords a unique opportunity to quantify the diffusion of water in brain tissue. Taking advantage of the fact that diffusion is not uniform throughout the brain (differing, for example, between gray matter, white matter, and cerebrospinal fluid), one can employ DT-MRI to evaluate tissue characteristics. The technique is particularly useful in the study of white matter tracts in the brain since the mobility of water is restricted perpendicular to the axons and oriented along the fiber tracts (anisotropic diffusion).

DT-MRI at high magnetic fields provides a unique opportunity to investigate the brain anatomy and the microstructure in animal models of brain disorders. A close comparison and correlation between DT-MRI data sets obtained in human patients and brain imaging data of the corresponding animal models requires the use of comparable acquisition schemes. Even though DTI has been routinely performed in the clinic, DT-MRI of the mouse brain remains a challenging task. A mouse brain is approximately 1000 times smaller than a human brain in term of the total volume. The current resolution of human brain DT-MRI is about 1 2 mm per pixel. In order to achieve the same relative resolution, we need to achieve a resolution of 0.1 - 0.2 mm per pixel for mouse brain DT-MRI by using special techniques.

During the roundtable discussion the principles of DT-MRI methodologies and the technical challenges related to mouse will be explained and several applications in mouse models will be introduced. One of the primary technical challenges in DT-MRI of the mouse brain is to achieve high spatial resolution while preserving satisfactory signal to noise ratio (SNR). DTI is known as a poor SNR technique because the signal magnitude in diffusion weighted images is attenuated by diffusion sensitizing gradients. To achieve satisfactory SNR, most mouse brain DT-MRI experiments have been performed on high field systems with custom made coils. However the disadvantage of high field systems is that have more severe field in homogeneity than 1.5 Tesla or 3 Tesla magnets. The field inhomogeneity makes difficult the implementation of echo planar imaging (EPI)

type of acquisition, commonly used for clinical DT-MRI. In addition to the resolution challenge, DT-MRI data are often marred by artifacts caused by physiological motion or gradient eddy current. Subject motion during *in vivo* experiment can be minimized by better animal constrains and respiratory triggering. Even with these challenges, DT-MRI of mouse brain has many advances in recent years and these advances will be presented during the workshop.

Several applications of the DT-MRI technique in animal models of neurodegeneration will be exemplified. Particular importance will be given to the brain imaging of mouse models of demyelination (e.g. mouse models of Multiple Sclerosis - MS). Commonly employed rodent models of MS include the experimental autoimmune encephalomyelitis (EAE), the viralinduced models of Theiler's murine encephalitis virus (TMEV) infection, and the toxininduced models of the cuprizone or lysolecithin administration. Our experience examining mouse models of both cuprizone induced demyelination-remyelination and EAE using in vivo DT-MRI will be described in details. Quantitative validation of DTI findings will be presented with neurological and histological correlations in cuprizone treated mice.Visualization of axonal tracts in myelination disorders will be exemplified as well. The strengths and limitations of DT-MRI in neurodegenerative disorders will be also discussed. The fiber tracking procedures and the modalities of data interpretation will be discussed depending on the specific research applications.

Learning objectives

After this round table discussion the participants should be able to:

- Identify hardware/software configurations that are needed to perform anatomical imaging and diffusion tensor imaging of the rodent brain;
- Recognize the constraints associated with the small size of the mouse body and brain and how to achieve stable physiology of the animals during the DT-MRI experiments;
- Describe the anatomical or physiological properties that can be measured with MRI and DT-MRI in mouse brain.
- Define how to perform anatomical imaging and diffusion tensor imaging in the mouse brain, identify and give solutions for the technical challenges, chose the optimal acquisition methodologies
- Identify significant biomedical findings that have been obtained through mouse brain neuroimaging using anatomical imaging and DT-MRI.

- Be able to perform data interpretation: tools to be used, brain atlases and correlation with the histology
- Have basic knowledge about the fiber tracking procedures.

Recommended pre-readings:

Beaulieu, C., *The basis of anisotropic water diffusion in the nervous system - a technical review.*NMR Biomed, 2002. **15**(7-8): p. 435-55.
Le Bihan D. Looking into the functional architecture of the brain with diffusion MRI. Nat Rev Neurosci 2003; 4: 469-480.
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Primary Culture of Rat Cerebral Astrocytes

Dr. Ozlem Yilmaz

MATERIALS AND METHODS

Brain Dissection

Primary cultures of rat brain astrocytes were prepared from prefrontal cortices of 2–4-day-old Sprague–Dawley rat pups. The procedure was adapted from *A Dissection and Tissue Culture Manual of the Nervous System* (*Shahar, de Vellis, Vernadakis & Haber, 1989).*

Using microdissecting scissors, the skin was opened at the midline of the head, cutting from the base of the skull to the mid-eye area. After folding back the skin flaps with the 6 scissors, the skull was cut at the midline fissure, without cutting into the brain tissue. The raised skull cap was removed with the curved forceps, applying slight pressure. The brain was then released from the skull cavity by running a microspatula underneath and along the length of the brain from the olfactory lobes to the beginning of the spinal cord. After gently transferring the brain to a 60 mm Petri dish, it was rinsed with a squirt of modified DMEM/F12 culture medium, containing 10% FBS, 1% glutamine and gentamicin antibiotic, from a syringe to remove the adhering blood and moisten the tissue. An identical procedure was performed for the second brain. The brains were then moved to a second Petri dish and placed in a tissue culture hood. Using micro-dissecting forceps, each brain was transferred to the inverted lid of a 35 mm Petri dish that was resting on a pad of sterile gauze. While steadying the brain with the forceps, the cerebrum was separated from the cerebellum and brain stem, and the cerebral hemispheres were separated from each other by gently teasing along the midline fissure with the sharp edge of a second forceps. The cortex (grey matter containing the cell bodies) was deflected and peeled away, leaving behind the white matter. The meninges were gently peeled from the individual cortical lobes that

were immediately placed into a fresh 60 mm Petri dish containing modified DMEM/F12 culture media.

Tissue Digestion

Cortices were dissociated into a cell suspension using mechanical digestion. After digestion, any remaining undigested tissue was plated as an explant in modified DMEM/F12 culture medium. Cells were plated in 75 cm2 tissue culture flasks at a concentration of 15x106 cells in 11 ml of medium. Incubating the flasks at 37oC in a 7 moist 5% CO2, 95% air atmosphere for 48-72 hours before moving, allowed the cells sufficient time to adhere and begin multiplying. The medium was changed at this time and every 48-72 hours until the cells were ready to be used for culture on slides.

Culture purification

After incubating the primary cultures for 7-9 days, the medium was changed completely (11 ml), and the caps were tightened. Flasks were wrapped in plastic, placed on a shaker platform in a horizontal position with the medium covering the cells, and were shaken at 350 rpm for 6 hours at 35o C to separate the oligodendrocytes from the astrocytes. The flasks were changed with 10 ml of medium and replaced on the shaker for 18 hours. After removing the flasks, the contents were aseptically poured into a new 75 cm2 flask, and placed in the incubator. The shaken flasks were again changed with 10ml of fresh medium and the shaking process was repeated for an additional 24 hours. Contents were then aseptically poured into a new 75 cm2 flask, and placed in the incubator. When confluent, cells were passed from the flasks into sterile Petri dishes containing glass slides. The secondary cultures were grown to confluency, rinsed in Dulbecco's Phosphate Buffered Saline, and the slides were fixed for 10 minutes in methanol. Concurrently, secondary cultures were grown in plastic chamber slides and 16-well slides and then fixed in methanol. Lastly, a sample of the cells was frozen in liquid nitrogen and thawed and re-grown one month later.

Workshop: Critical review of a scientific manuscript -B.

Workshop leader: **Ken Moya,** Centre Nationale de Recherche Scientifique UMR 8542, Ecole Normale Supérieure, Paris France.

In addition to planning experiments, carrying them out, writing up research results and managing research projects, scientists are often called upon to review manuscripts submitted by other scientists for publication in scientific journals. However, many scientists receive little or no training in the review process. The goal of this workshop is to help you establish the context for scientific review, develop a global evaluation of the question posed in a manuscript, evaluate the appropriateness of the experimental approach and judge if the results support the conclusions reached.

Following you will find an invented manuscript submitted to the *World's Best Journal of Neurobiology*. The Editorial board of the Journal has decided to review this submission and has contacted experts (you!) to review the manuscript. Your task is to read the manuscript carefully, determine if the question posed is interesting, judge if the methods are appropriate, if the results are of good quality and if the interpretation is correct. You should include in your valuation the quality of the figures, statistical analyses and methods used as well as how well written the manuscript is.

Fill in the Manuscript Score Sheet and at least outline your comments to the authors and editors in terms of criticisms and evaluation.

During the workshop each of you will be asked to discuss the strong and weak points you may find in this manuscript.

RAT E13.5 NRP/GRP DISSECTION

DISSECTION / DISSOCIATION

1) Start dissection afternoon so embryos will be the right age.
2) Measure 'Crown to Rump' distance: should be 8.5-9 mm for E13.5 (8.0 is E13).
3) Dissect and clean spinal cords from E13.5 rat embryos in DMEM/F12.
   a. After dissecting cord from embryo, peel off as much of meninges as possible.
   b. The lumbar section is often too sticky at this age to clean well.
   c. Cut off cleaned section and place in a 50 ml centrifuge tube of ice-cold DMEM/F12.
   d. Place sticky section in collagenase/dispase for 8 min at RT.
   e. After dissection, remove from collagenase/dispase, rinse in DMEM/F12, put back in normal dissecting dish and peel off the rest of meninges.
   f. Transfer cleaned sections to the 50 ml centrifuge tube of ice-cold DMEM/F12.
4) Centrifuge pooled cords at 800 RPM for 5 minutes, decant medium to 50 ml tube.
5) Add 1 ml of 0.05% Trypsin-EDTA to cords; incubate 20 minutes at 37^0C.
6) Using a sterile cotton-plugged 1000 µl pipette tip, set at 800 µl, gently triturate cells until chunks of tissue look dissociated by eye.
7) Add 10 ml NRP Basal Media to quench trypsin, and gently triturate with 10 ml pipette.
8) Pass cell suspension through a 40 µm filter into a fresh 50 ml tube.
9) Rinse the filter with another 10 ml NRP Basal Media.
10) Centrifuge cell suspension at 800 RPM for 5 minutes to get rid of the trypsin, decant medium, re-suspend in 1 ml NRP Basal Media.
11) Dilute 10 µl cell suspension 1:1 in trypan blue and count 10 µl with a hemocytometer.
12) Plate cells overnight to PLL/LM coated T-75's at $3x10^6$ cells/flask in NRP basal medium, supplemented with 10 ng/ml bFGF and 20 ng/ml NT3.

Collagenase/Dispase Solution: Collagenase Type 1 (10 mg/ml of 178U/mg, Worthington Biochemical's) and Dispase II (20 mg/ml, Boehringer Mannheim, cat# 165859) in HBSS (dissolved in HBSS and filtered with 0.45 µm filter).

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Poster Abstracts

Effects of oxytocin on mice depressive-like state induced by mother-pup separation

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Oxytocin is vital for initiation of maternal behaviour and recent studies have shown that it is also involved in maintaining aspects of maternal behaviour in the presence of the pup. Mothers separated from their pups display depression-like states. The aim of our study was to investigate whether oxytocin administration to mothers separated from their pups would reverse the depression-like state. We used 4 groups of nulliparous female NMRI mice. Two groups were separated from their pups for 5 hours per day, from day 2 to 14 after parturition, and were either intraperitoneally administrated oxytocin or placebo. The other two groups, which were not separated from their pups were injected subcutaneously with an oxytocin receptor antagonist (atosiban) or placebo. Before starting the administration of the drugs and after weaning, the female mice were subjected to behavioral tests (forced swimming test -FST, elevated plus maze -EPM, open field test -OFT - according to Current Protocols in Neuroscience, 2003); the tests were recorded using EthoVision XT Acquisition System (Noldus). Pup separation modified behavioral parameters. Pup separated placebo group showed decreased movement in OFT, a slight increase of immobility in FST and also spent less time in the opened arms in EPM ($p<0.05$). Overall they were more anxious, passive and showed a depressive-like behavior. In the atosiban treated group the duration of immobility in FST and the time spent in the central zone in OFT decresed, suggesting that the mice were rather anxious than depressed by atosiban administration. Administration of oxytocin in pup-separated mothers was correlated with an increase of immobility in FST; all the other parameters at the OFT and EPM did not changed significantly compared to the initial values for each subject. Compared to the pup-separated placebo group that expressed more anxiety than in the beginning, the pup-separated oxytocin group did not have a modified degree of anxiety in final recordings, indicating that oxytocin may reverse the anxiety observed in pup separation model.

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Interleukin-6 Immunoreactivity of Hippocampus Following Immediately After a Day After and Three Days After Exhausted Exercise in Non-Trained Rats

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Background and Purpose: Immune system is influenced by various types of psychological and physiological stressors including physical activity. Strenuous exercise induces an increase in the pro-inflammatory and anti-inflammatory cytokines, TNFα and IL-1β and IL-6. IL-6, more than any other cytokine, is produced in large amount in response to exercise in the skeletal muscle and is tightly related with both the duration and the intensity of the exercise. These cytokines are also key mediators of immune-to-brain communication. Acute and chronic stressors can impact pro-inflammatory cytokine production in some of the brain areas including hippocampus. We detected the differences between the amounts of IL-6 reactivity of the hippocampus immunehistochemically, following an exhausted running immediately after exhaustion and one day and three days after exhaustion in non-trained rats (6). **Methods.** Three groups of rats (n=6) were forced to run on a treadmill, in 20m/min speed, 10° inclination till to exhaustion. Immediately following exhaustion, one group was sacrificed and their brains were taken out to detect the amount of the IL-6 antibodies (this group constituted the immediate group). The second group of rats was sacrificed the day after the exhaustion and their brains were taken out for IL-6 antibody detection. The third group of rats was sacrificed 3 days after the exhaustion and their brains also were taken out for IL-6 antibody detection. The control group (n=6) didn't run, they were sedentary and their hippocampal IL-6 reactivity was detected to compare with other three experimental groups. **Results.** There was very little or negative IL-6 immunoreactivity in the hippocampal slices of controls. IL-6 immunoreactivity in the immediately sacrificed group's hippocampus was weak to mild. IL-6 immunoreactivity of the hippocampus in the group of rats which were sacrificed one day after exhaustion, was mild-to severe. However hippocampal slices of the third group which was sacrificed at the third day after exhaustion showed a weak-to-mild IL-6 immunoreactivity. **Conclusion.** Exhausting exercise (as a strong stress factor) promoted cytokine IL-6 immunoreactivity in hippocampus of all exhausted rats. But the most prominent effect of this exhausting exercise had been shown in hippocampus of the group which were examined a day after exhaustion.

Key Words: Exhausted Exercise, Hippocampus, Chymokines, Interleukin-6

Comparative study between Phenytoin and Delphinium denudatum as Anti Epileptic Agents in cultured hippocampal neurons using Patch Clamp techniques

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Abstract

We examined the compound (N-22-4) extracted from ***Delphinium denudatum*** to see their effect on electrically induced epilepsy using sustained repetitive firing (SRF) protocol in whole cell configuration in current clamp mode for observing action potentials generated in response to depolarized pulses. When comparing with commercially available AED phenytoin, we found that the N-22-4 is more potent and almost completely blocked the SRF induced action potentials in mammalian cultured hippocampal neurons.

This compound was also tested in voltage clamp mode for measuring ionic currents generated in the presence of GABA, Acetylcholine and glutamate in the bath in micro molar concentrations. Unlike Phenytoin, N-22-4, not only decreases the deactivating phase of voltage gated sodium channels but also interact with the $GABA_A$, NMDA and nicotinic Acetylcholine receptors under different bath conditions.

We concluded that our natural compound is more effective than phenytoin for the treatment of temporal lobe epilepsy.

RAC-1 is necessary for the development of GABAergic interneurons in the cortex

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γ-amino butyric acid-producing (GABAergic) interneurons constitute ~20% of cortical neurons in rodents and provide the main source of inhibition to cortical microcircuits. Contrary to the principal projection neurons, which are born in the ventricular zone of the dorsal telencephalon, $GABA^+$ interneurons originate in the ventral forebrain (mainly in the medial ganglionic eminence-MGE) and reach the cortical plate by tangential migration during embryogenesis.

Rac1 is a member of the family of small Rho-GTPases which have been implicated in the re-organization of the actin cytoskeleton during cell migration and differentiation. Recent studies have specifically suggested that Rac-1 is involved in neuronal differentiation, axonogenesis and migration in the developing telencephalon. In our studies we use Cre-LoxP technology to delete *Rac1* from dividing progenitors or postmitotic precursors of GABAergic interneurons and examine the role of this G-protein on their differentiation and migration towards and within the developing cortex.

Mice carrying a floxed allele of *Rac1* (*Rac1^{fl}*) were crossed to *Nkx2.1-Cre* transgenic animals, which express Cre recombinase in the ventricular zone of the MGE, thus deleting *Rac1* from dividing interneuron progenitors. In addition, *Rac1^{fl}* mice were crossed with *Lhx6-Cre* animals which drive expression of Cre in MGE-derived postmitotic precursors of cortical interneurons. Here we present our phenotypic analysis of the two groups of mutant mice which suggests that *Rac1* activity is necessary for the tangential migration of cortical interneuron progenitors and their differentiation into cortical interneuron subtypes.

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Neuroethical Considerations in the Context of a Non-clinical Case of von Hippel-Lindau Syndrome which was suggested by Neuroimaging

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Novel pharmaceutical development - marketing and neuroimaging are two problematic avenues of neurotechnology “advancing” parallel to translational neuroscience. Neuroimaging, particularly functional brain imaging has recently been extensively promoted and exploited as a tool for precise, sometimes early, in some cases, even preventive diagnostic strategies in the context of “evidence-based medicine”. It is quite common that “neuroimaging diagnosed non-clinical subjects with incidental / variative findings” most often lead normal lives. We aim to 1) present a non-clinical case of VHL-S (an autosomal dominant multicancer syndrome diagnosed by clinical, neuroradiological and genetic findings) who as the sister of a clinical VHL-S case was diagnosed incidentally during screening of the family, and 2) propose for debate the consequent interventions conducted on the second patient with regards to prognosis and all other possible life events in terms of neuroethical issues / problems in the light of relevant literature. The pro’s and con’s of neuroimaging should be carefully questioned and discussed to avoid 1) any harm owing to the interventions directed by its results, 2) possible conflicts and disadvantages to emerge with regards to psycho-social development; school, profession, task and spouse choices, chances and opportunities as well as health insurance policies, and even law case practices in one’s life. Decision making for any treatment or intervention following neuroimaging-based diagnosis in such cases should be reckoned at least twice, and handled / conducted with multi-disciplinary / professional considerations for the welfare of the patient throughout his / her entire lifetime

Neurochemical and Behavioral Alterations in an Inflammatory Model of Depression: Sex Differences Exposed!

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It is firmly established that women experience major depression (MD) at roughly twice the rate of men and that dysregulation of the immune system is associated with the appearance and course of this condition. The aim of the present study was to identify whether LPS-induced sickness, a putative inflammatory model of depression, is characterized by sexual dimorphism focusing on both neurochemical and behavioural responses. Therefore, the serotonergic status of various brain regions implicated in the pathophysiology of affective disorders (hypothalamus, hippocampus, prefrontal cortex, amygdala and striatum) in response to a mild LPS challenge was investigated in rats of both sexes. According to our results, at 2 h post LPS administration (100 μg/kg, i.p.), the neurochemical substrate was altered primarily in female rats with the serotonergic function being markedly enhanced in all brain regions examined. The amygdala and striatum were the only regions in which serotonergic activity was increased in male rats, whereas in the hypothalamus serotonergic function was decreased. LPS-induced sickness was also found to affect behaviour to a different extent in male and female rats, as assessed in the forced swim test (FST) and hot-plate test (HPT). LPS treatment increased swimming duration in the FST only in female rats, possibly due to the serotonergic hyperactivity that was evidenced at the neurochemical level, but did not induce a "depressogenic effect" in either sex. The antinociceptive properties of LPS challenge were evaluated at 3 different time-points (30, 120 and 240 min after administration) and were evident in both sexes, though showing a sex-dependent motif. Analgesia was established sooner in females in contrast to their male counterparts (30 versus 120 min) but wore off at 240 min, while males still yielded higher thresholds compared to baseline values. These data are the first to demonstrate that the serotonergic system is affected to a greater extent in female rats upon LPS administration and further accelerates our understanding regarding sexual dimorphism upon sickness establishment.

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Transitional metals as markers for detecting gene polymorphism in schizophrenic patients of Pakistan

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Abstract:

The study of the interactions of double-stranded (ds) DNA by using base-specific Rhodium (II) acetate [Rh2 (O2CCH3)4] (Rh1) compound supports the presence of covalently linked DNA adducts. Rh1 has specific binding side N7 of the purine ring, Rh1 bound with Guanine (G) and Adenine (A). MunI digestion sequence has several A bases, If Rh1 bound with Guanine (G) and Adenine (A) MunI can not digested the DNA. A number of studies have indicated that neuregulin NRG1 gene located on 8p22–p12 is likely to harbor schizophrenia susceptibility loci. We performed the experiments using lower to higher concentrations of Rhodium and made adduct with human native DNA. We are targeting NRG1 gene and looking at its relationship in schizophrenic patients in Pakistani population using SNP1 primer to amplify.

We have also performed Metal- DNA reaction, digestion reaction by using MunI enzyme and Gel electrophoresis for the study of rhodium attachment to the DNA. Our results suggested that the NRG1 gene may play a significant role in conferring susceptibility to the disease and transitional metals could be used as markers for detecting gene polymorphism.

Modulation of Spinal Cord Sensory Entries by Opioids and Natural Toxins

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The axons of dorsal root ganglion neurons are known as afferents, which transmit sensory information from the peripheral nerve endings to spinal cord and, thus, form its main sensory entries. They are known to take part in pain modulation and regulation. The main role in such modulation is granted to P2X and opioid receptor families expressed in the DRG neurones.

We have shown that P2X3 and opioid receptors of DRG neurons are functionally coupled. Application of morphine leads to decrease in the amplitudes of ATP-activated currents (IC_{50} =8.5±1.3 nM). This indicates that the effect of morphin on P2X3 receptors is mediated by μ-opioid receptors. At saturating concentrations (10 μM) morphin inhibited ATP-induced current by 55% on the average.

P2X3 receptors are also found to be modulated by natural toxins, namely Central Asian Lycosa spider toxin (PT1) (IC50 =12±0.6 nM). PT1 is a small peptide of 35 aminoacid resides. It exerts an inhibiting effect on P2X3-mediated currents via prolongation of its desensitisation removal. The PT1 action is subtype-specific. It modulates only fast P2X3 responses, having no effect on other members of P2X family present in DRG (it also ineffective against number of voltage- and ligand-sensitive channels of DRG).

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