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Symposium: "Newer Insights in Brain Research"

Dear colleagues, participants, and esteemed guests,
it is our privilege to present the proceedings of the 24th Brain Awareness Week in Croatia, which took place from March 10th to 16th, 2025. As part of the international Brain Awareness Week initiative, this event continues to serve as a vital platform for the dissemination of current scientific knowledge on brain structure, function, and pathology, as well as for fostering interdisciplinary dialogue within the neuroscience community.

The 2025 programme was organised by the Croatian Institute for Brain Research (CIBR), University of Zagreb School of Medicine, in collaboration with the Croatian Society for Neuroscience (HDN), the Federation of European Neuroscience Societies (FENS), and as partners of the European Dana Alliance for the Brain (EDAB). Our efforts were further supported by the University of Zagreb School of Medicine, the Student Section for Neuroscience, the Student Section for Neurobiology of the Biology Students Association (BIUS, PMF), and numerous academic and clinical partners throughout Croatia. We gratefully acknowledge the Croatian Academy of Sciences and Arts (HAZU) for their continued support.

This year's scientific focus addressed three pressing topics:

- Neurodevelopment in the context of the digital environment and its implications for the child's brain
- Emerging challenges in neuroethics, particularly as neuroscience interfaces with technology and society
- Neuroplasticity and adaptive mechanisms in the brains of deaf and blind individuals

The programme comprised a series of scientific workshops, public lectures, and the central symposium, "Recent Advances in Brain Research," held on March 13th, 2025, at the HAZU Library Hall in Zagreb. These activities were designed to encourage critical discussion, promote evidence-based understanding, and bridge the gap between neuroscience research and public health awareness.

We extend our sincere gratitude to all contributors, presenters, and attendees whose engagement and expertise have enriched this event. We trust that the abstracts and discussions presented herein will stimulate further research, collaboration, and innovation in the neurosciences.

With best regards,
Ivica Kostović

Prenatal development of synapses and modulatory systems in the cerebral cortex of the human fetal brain

Ivica Kostović[1], Janja Kopić[1]

[1] Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia

PRESENTING AUTHOR:
Ivica Kostović

ORCID:
0000-0002-4090-4114

E-MAIL:
Ivica.kostovic@hiim.hr;
ikostov@hiim.hr

Professor Emeritus, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia

Janja Kopić, PhD, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia

Abstract:
New data on structure, function and modulation of synapses are related to mechanisms of presynaptic vesicle fusion (Sudhof and Malenka, 2008), dynamic nature of synapse plasticity, trans-synaptic interactions, nanoscopic organization of synapse structure (Yang and Annaert, 2021), evolution of neuromodulation (Galvin et al. 2018) and specificity of cortical synapses in humans (Christopoulou and Charrier 2024) in terms of assembly of postsynaptic scaffold via post-translational regulation of postsynaptic proteins (several hundred!) and finally, prolonged synaptogenesis, with specific tempo and maturation during life.
In this presentation I am focused on development and distribution of synapses during prenatal development of the human cortex. The results confirm (Kostovic 2024) that typical chemical synapses emerge during early fetal life and show consistent laminar compartmental relationship with distribution of distal dendrites of pyramidal neurons and transient neurons of the subplate and marginal zone. Contrary to the prediction of experimental investigators, typical electrical gap junctions were not found during prenatal synaptogenesis. It was also found that early presynaptic elements belong to the evolutionary new, advanced cholinergic systems, which make synaptic input together with thalamic axons. Evolutionary conserved monoaminergic axons arrive in synaptic strata during the same period and grow in cerebral anlage even earlier, that is during embryonic period. Within the synaptic strata monoaminergic axons may release transmitters non-synaptically. These new results are in accordance wit the concept of protracted period and specific tempo of life-long cortical synaptogenesis in humans. We conclude that recently discovered specifically human genetic regulation of presynaptic and postsynaptic molecular structure is already present during the early human fetal life.

Keywords: synapse, prenatal cortical development, presynaptic elements, synaptic compartments

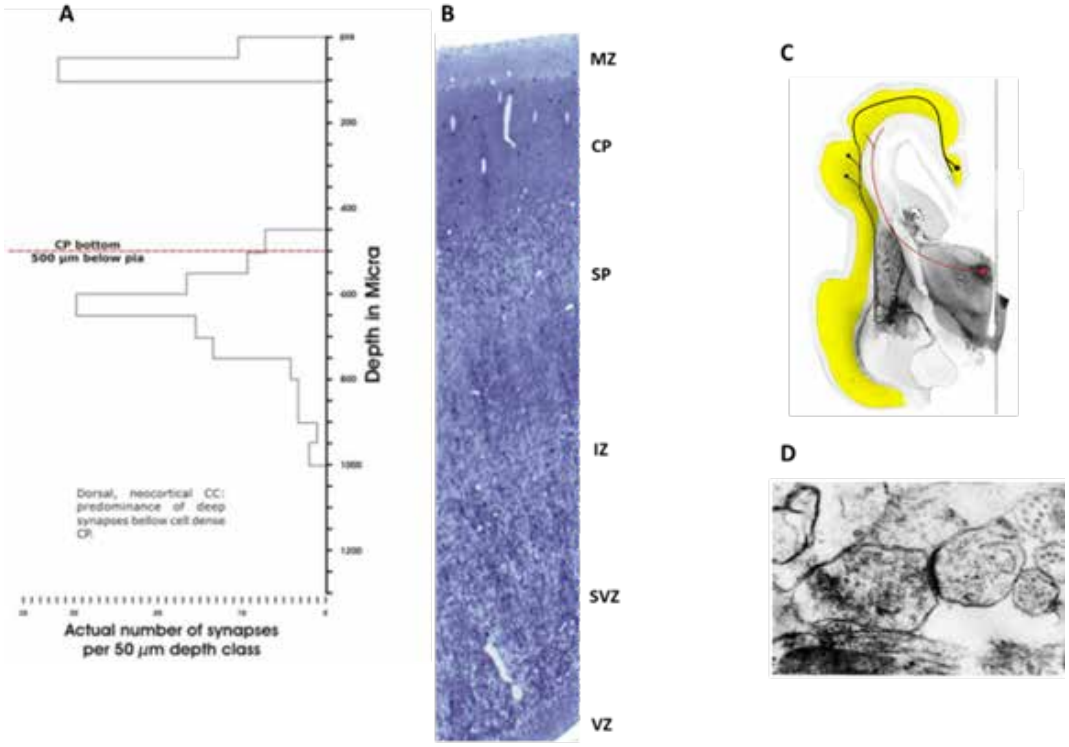

Figure 1. Distribution of synapses in neocortical part of gyrus cinguli in human fetal brain at 15 PCW. A- number of synapses per 50 µm depth class, B- Nissl stained 1 µm section adjacent to ultrathin section used for electron microscopy quanrification. Note that synapses are distributed in synaptic strata, C- potential afferent fibers to synaptic strata from cholinergic basal forebrain (Nucleus basalis Meynert), D- Typical asymmetric synapse at axonal terminal bouton and small dendritic profile in the SP.

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Contact information:

Ivica Kostovic, CIBR, School of Medicine, Šalata 12, 10000 Zagreb, e-mail: ikostov@hiim.hr

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Concept Neurons

Mario Vukšić[1], Fran Božić[1]

[1] Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia

PRESENTING AUTHORS:
Mario Vukšić
Fran Božić

ORCID:
0000-0003-2196-2244
0000-0003-3228-1161

E-MAIL:
mario.vuksic@hiim.hr
fran.bozic@mef.hr

CORRESPONDENCE:
Mario Vukšić
Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia,
Salata 12, 1000 Zagreb
e-mail: mario.vuksic@hiim.hr

Abstract:
Evidence from animal studies and the famous case of patient H.M. have convincingly demonstrated the crucial role played by the medial temporal lobe (MTL) in the formation, consolidation and retrieval of declarative memories. While many aspects of neural coding and retrieval of mnemonic information have been extensively studied, the specific neuronal mechanisms responsible for transforming our perceptions into long-lasting memories remain largely unknown. Recently, intracranial recordings in patients suffering from intractable epilepsy have shown that neurons in the MTL can be selectively activated when individuals are presented with stimuli corresponding to a specific concept, irrespective of the sensory modality through which the information is received.
For example, a single neuron recorded in the human hippocampus showed incredible specificity in its response to images of the actress Jennifer Aniston. This neuron was found to fire in response to different visual representations of the actress, as well as to a semantic representation of the same concept, such as the letter string "JENNIFER ANISTON". Furthermore, these neurons can activate when stimulus information is provided in other sensory modalities, such as when a specific name is spoken out loud. Due to this specificity, these neurons were initially named "*Jennifer Aniston neurons*". More recently, they have been referred to as "*concept cells*", reflecting their broader role in encoding abstract representations of semantic knowledge.
Given the well-established involvement of the MTL in the acquisition of declarative memory, one can conclude that concept cells serve as fundamental units for encoding the meaning of a stimulus for memory functions. Moreover, the way human memory tends to retain abstract concepts while discarding irrelevant details correlates well with the information encoded by the concept cells. The research has shown that these neurons sometimes fire to associated concepts, which are represented by different cell assemblies. When two concepts share a meaningful relationship, a subset of the neurons encoding one concept may also fire in response to another, giving a potential neural substrate for associative learning and offering a mechanistic explanation for how individuals transition from one concept to another in a fluid manner (Figure 1).
The described neural mechanism could serve as the neurobiological basis for episodic memory and even the stream of consciousness that characterizes human cognition. By linking perception with memory, concept cells generate an abstract and sparse representation of semantic knowledge that constitutes the building blocks for declarative memory functions, enabling humans to organize and recall information with remarkable efficiency (Quiroga 2012). Furthermore, recent studies have revealed that working memory representations are converted into long-term memory when concept cells become active.
Some researchers speculate that concept cells may represent a key component of human intelligence, potentially differentiating our cognitive abilities from those of other species. Future research on concept neurons could explore their precise role in the transition from perception to memory by investigating how their activity changes during different stages of learning and recall.

Keywords: temporal lobe epilepsy, depth electrode, declarative memory, visual perception, working memory

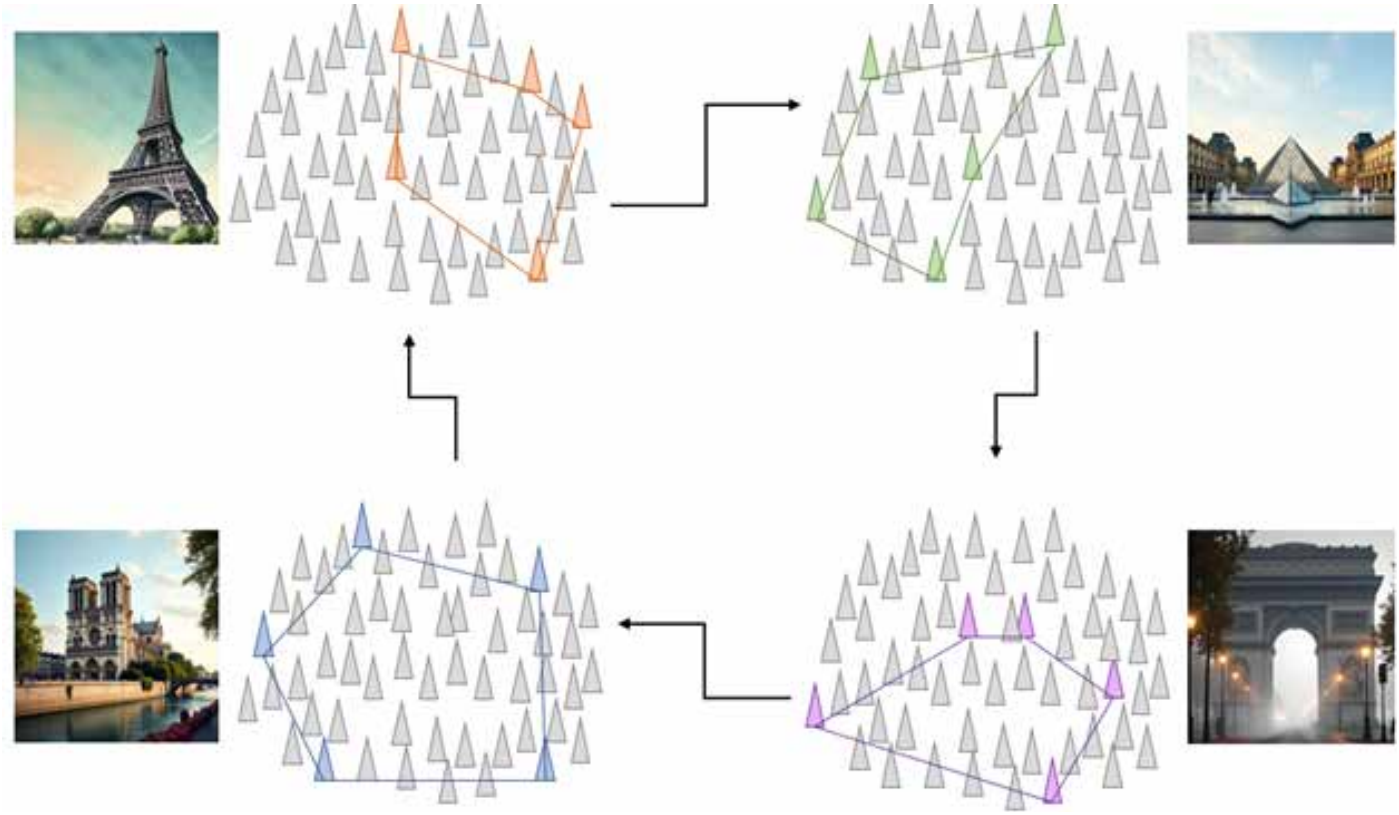

Figure 1. **Representation of the concept neurons in the medial temporal lobe.** *The encoded concepts of the representative Paris monuments are stored within a specific cell assembly. The top left (orange) assembly is activated upon recreating the concept of the Eiffel Tower, and at least one of these neurons is also included in the cell assembly that stores the concept of the Louvre museum shown in the top right (green), creating a potential mechanism of associative learning. The concepts of the Arc de Triomphe encoded by the cellular assembly in the bottom right (purple) and the Notre Dame of Paris cathedral stored by the assembly in the bottom left (blue) do not share a directly shared neuron but could be associated through the concept neurons encoding the concepts of the Eiffel Tower or the Louvre museum, existing within the same episodic memory (Quiroga 2012).*

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Extended developmental plasticity and vulnerability of human prefrontal microcircuits

Zdravko Petanjek[1], Ana Hladnik[1], Marina Čavka[1], Maura Zanze Beader[1]

[1] University of Zagreb School of Medicine, Department of Anatomy and Clinical Anatomy & Croatian Institute for Brain Research, Zagreb, Croatia

PRESENTING AUTHOR:
Zdravko Petanjek

ORCID:
0000-0001-5019-4198

E-MAIL:
zdravko.petanjek@unizg.mef.hr

CONTACT INFORMATION:
Zdravko Petanjek, zdravko.petanjek@unizg.mef.hr, University of Zagreb School of Medicine, Department of Anatomy and Clinical Anatomy, Šalata 11, Zagreb, Croatia

Abstract:

The transition from childhood to adulthood has become increasingly prolonged, with milestones such as completing education, marriage, and parenthood occurring later than ever before. This shift raises the question of whether adolescence should still be defined as the period between ages 12 and 18 or if its endpoint should be extended. Neurobiological research revealed that synaptic overproduction and developmental remodeling, including substantial elimination of synaptic spines, continue deeply into the third decade of life before stabilizing at adult level (Petanjek et al. 2023).

A recent study performing cross-species proteomic mapping of synapse development, tracked changes in over 1,000 postsynaptic density proteins from midgestation to young adulthood comparing human, macaque and mouse neocortex (Wang et al. 2023.). The results showed that human postsynaptic densities develop two to three times more slowly than those of other species, largely due to a higher abundance of RhoGEF proteins in the turquoise module. The turquoise module is linked to synaptic plasticity, human cognitive function and neuropsychiatric disorders. Overexpression of RhoGEFs was found to increase spine density and delay synapse maturation.

Several studies have previously demonstrated that gene expression changes during postnatal brain development in the human prefrontal cortex occur significantly later than in chimpanzees and rhesus macaques (Petanjek et al. 2023). The microcircuits responsible for processing the higher cognitive functions have the most extended period of synaptic overproduction, supporting high plasticity, which is crucial for acquiring complex cognitive abilities, including affective modulation of emotional cues, self-conceptualization, mentalization, cognitive flexibility, working memory and social skills. However, this prolonged development also extends the window of vulnerability, potentially increasing susceptibility to factors that may disrupt the formation of neural circuits involved in higher cognitive functions, which are impaired in neuropsychiatric disorders such as autism and schizophrenia (Petanjek et al. 2023).

A recent study performing single-nucleus RNA sequencing (Batiuk et al. 2022), found a reduction in GABAergic neurons and a concomitant increase in principal neurons within dorsolateral prefrontal cortex of patients with schizophrenia. The most pronounced changes were observed in the upper cortical layers, suggesting selective vulnerability and general cortico-cortical network impairment as a core substrate associated with schizophrenia symptomatology.

The dopamine system, whose disruption is considered to be one of the key features in schizophrenia, undergoes delayed maturation and changes throughout the whole stage of adolescence. Dopamine maturation interacts with changes in endocannabinoid signaling (Peters and Naneix 2022), characterized by transient increases in receptors expression and gradual rise in neurotransmitter levels. These changes enhance the recruitment of GABAergic interneurons and sustain the activity of pyramidal

neurons. Prefrontal dopamine signaling refines processing by improving the selection of specific inputs, decreasing the signal-to-noise ratio, most likely through regulation of glutamatergic synaptic spine pruning. Similar adolescent remodeling hasn't been observed in other neuromodulatory systems. Given their interactions with different neuronal populations and effects at different synaptic sites, dopamine and endocannabinoid signaling play a critical role in the late maturation of prefrontal circuits and their functioning.

Extensive reports showed that external factors, including drug use, nutritional habits, and stress, affect the functioning of the prefrontal cortex during adolescence. Research strongly indicates that these factors interfere with the development of dopamine and endocannabinoid pathways, ultimately impacting the reorganization of cortical microcircuits. A recent epidemiological study conducted on a large Danish cohort found an association between cannabis use disorders and the onset of schizophrenia (Hjorthøj et al. 2023). The study indicated that 15-30% of schizophrenia cases in males, and 5-10% in females could potentially be prevented if cannabis use disorders were avoided between ages 15 and 25. This provides a very explicit example of how the adolescent and post-adolescent brain remains extremely plastic and vulnerable to external factors.

Recognizing the extended developmental window in humans is crucial, as it entails both increased plasticity and vulnerability of prefrontal microcircuits. The influence of environmental factors on the shaping of these microcircuits is significant and may contribute to the development of late-onset neuropsychiatric disorders. Insights into prolonged neurodevelopment should be carefully considered when designing psychological, social, and educational strategies for adolescents and young adults, with a particular focus on legislation affecting those under 25.

This work was supported by the Croatian Science Foundation under the project number HRZZ-IP-10-2022-8943 (Uniqueness in development of interneurons in human prefrontal cortex during fetal life and first postnatal year – implications in pathogenesis of schizophrenia and autism)

Keywords: prefrontal cortex, dendritic spine, glutamate, dopamine, endocannabinoids

Contact information

Zdravko Petanjek, zdravko.petanjek@unizg.mef.hr, University of Zagreb School of Medicine, Department of Anatomy and Clinical Anatomy, Šalata 11, Zagreb, Croatia

Figure 1: High-power magnification images of rapid Golgi-impregnated layer IIIc pyramidal neurons in the dorsolateral prefrontal cortex showing synaptic spines on dendritic segments receiving thalamic (left panel) and cortical input (right panel) across various developmental stages: a 1-month-old infant, a 2.5-year-old child, as well as subjects aged 16, 28, and 49 years. The highest synaptic spine density is observed at the age of 2.5 years and dendritic segments receiving cortical input do not show significant decline at the age of 16 years, indicating that protracted reorganization of associative cortico-cortical circuits extends into the third decade of life.

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Functional imaging of associative areas of the cerebral cortex

Jelena Božek[1], Marko Radoš[2], Milan Radoš[2,3]

[1] University of Zagreb Faculty of Electrical Engineering and Computing, Zagreb, Croatia
[2] Clinical Department of Diagnostic and Interventional Neuroradiology, Clinical Hospital Center Zagreb, Zagreb, Croatia
[3] Croatian Institute for Brain Research, Faculty of Medicine, University of Zagreb, Zagreb, Croatia

PRESENTING AUTHOR:
Jelena Božek

ORCID:
0000-0002-5986-5442

E-MAIL:
jelena.bozek@fer.unizg.hr

Abstract:

Brain imaging using functional magnetic resonance imaging (fMRI) enables the study of functional connectivity between different brain regions at rest, when the individual is not performing a specific task. Resting-state functional connectivity analysis has become a key tool in neuroscientific research of psychological and neurological disorders. The ability to observe how different brain regions communicate, as well as how these connections can be disrupted in various disorders, opens new horizons for understanding, diagnosing, and potentially treating these conditions.

In Alzheimer's disease, reduced connectivity in the Default Mode Network (DMN) affects memory processes, while in Parkinson's disease, reduced connectivity in dopaminergic networks impairs motor functions. In autism, decreased connectivity between temporal and frontal regions explains difficulties in social interaction, and in ADHD, reduced connectivity between prefrontal regions and other areas of the brain is linked to problems with attention and impulsivity. In depression, reduced connectivity in networks for emotional regulation, such as prefrontal and limbic areas, may help in understanding the disorder. For anxiety disorders and PTSD, changes in the connectivity of the amygdala with prefrontal regions explain hyperreactivity to stress.

In patients with schizophrenia, it has been shown that the functional connectivity of the thalamus and the primary motor and somatosensory cortex is increased, while the functional connectivity of the thalamus and the prefrontal cortex, striatum and cerebellum is reduced (Murray and Antičević 2017.). Recent research shows that the functional architecture of neural networks can be used as a neuroradiological biomarker for the recognition of mental disorders including schizophrenia (Spronk et al. 2021). Although at this moment the sensitivity and specificity of fMRI-based diagnostics are not satisfactory for clinical use, it is likely that with the development of MRI protocols and post-processing analysis the problem will be solved in the future.

Recent advancements in neuroimaging have also provided valuable insights into the auditory language comprehension and intrinsic network abnormalities associated with Autism Spectrum Disorder (ASD). Hua et al. (2024) conducted an ALE meta-analysis of fMRI studies to investigate auditory language processing among children and adolescents with ASD, revealing altered brain activity in key regions involved in speech and language. Similarly, Goodwill et al. (2023) employed meta-analytic connectivity modeling to examine functional connectivity patterns in ASD, uncovering disruptions in neural circuits that underlie social and cognitive functioning. In another study, Yoon et al. (2024) utilized independent component analysis to explore intrinsic network abnormalities in children with ASD, providing evidence of atypical connectivity within brain networks implicated in sensory processing and executive functions. These studies provide deeper insight into the neural mechanisms underlying

language and social cognition in individuals with ASD, offering potential targets for future therapeutic interventions.
Ongoing research in this field promises to enhance our understanding of neurological disorders and pave the way for the development of targeted therapies based on functional brain networks.

Keywords: Autism Spectrum Disorder; functional MRI; Schizophrenia

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The role of the extracellular matrix in neurodevelopmental brain disorders: a genetic perspective

Natasa Jovanov Milosevic[1], Mihaela Bobic Rasonja[1], Sara Trnski Levak[2], Ivona Kirchbaum[1]

[1] Department of Biology and Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia
[2] Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia

PRESENTING AUTHOR:
Nataša Jovanov Milošević

ORCID:
0000-0001-7897-6212

E-MAIL:
njovanov@hiim.hr

Abstract:

The fetal cerebral wall is rich in extracellular matrix molecules (ECM) that fill the intercellular space, estimated to comprise up to 60% of the volume of the early fetal brain. Four numerous families of ECM molecules (hyaluronan, proteoglycans, glycoproteins, and link proteins) are the main components of the biochemical niches that mediate the distribution of key signaling and trophic factors during prenatal development and build mechanical scaffolds to conduct actively morphogenetic processes such as cell proliferation, migration, axon pathfinding, and cortical folding. The transient fetal zones, such as the marginal zone, subplate, and early intermediate zone, are rich in hydrated extracellular matrix (ECM), and the last two are the bases of the cortical (hyperintensity) pattern on MRI scans, highlighting the importance of ECM in brain diagnostic and prognostic MRI follow-up in fetuses. In collaboration with their receptor, the ECM components also play a significant role in the differentiation of dendrites and the genesis of spines and synapses. Finally, some ECM molecules become integral to the quadripartite synapse and perineuronal nets, contributing to the formation of the functional connectome. Humans have more ECM in the cortical wall during fetal development compared to the evolutionary closest primate species. This supports the ECM as an evolutionary force, in conjunction with prolonged brain development, which facilitates the formation of more synapses and more elaborate, complex connections, while also being an additional substrate for vulnerability and plasticity in humans. Mutation of ECM coding genes or dysregulations of the ECM molecules' expression spatial and temporal patterns, can affect the processes of cell proliferation, migration, cortical folding, or differentiation, leading to a heterogeneous group of disorders- known as malformations of cortical development (MCD, such as microcephalia, megalocephalia, lissencephaly, polymicrogyria, periventricular nodular or subcortical band heterotopia), or neurodevelopmental disorders (NDD), such as epilepsy, autism spectrum (AS), schizophrenia, and/or intellectual disabilities (ID). With the latest advances in sequencing technologies, greater accessibility to whole-genome and whole-exome sequencing (WGS, WES) for diagnostic purposes has led to an increase in the number of mutated genes coding for ECM components identified in patients and linked to MCD or NDD. A few examples include mutations in the heparan sulfate proteoglycan 2 (*HSPG2*) and laminin subunit gamma 1 (*LAMC1*) genes, which are associated with disrupted proliferation in the ventricular and subventricular zones, leading to microcephaly, hemimegalencephaly, or focal cortical dysplasia. The mutations in genes coding glycoprotein reelin, ECM glycosylation enzymes, O-mannosyltransferase enzyme, and glycosyltransferase-like protein, *or the adhesion G—protein-coupled receptor (RELN, POMT1, LARGE, and GPR56, respectively), have been found* to hinder migration and cell adhesion, causing lissencephaly or cobblestone lissencephaly, bilateral frontoparietal polymicrogyria, or ID. Proteoglycans, including

versican, neurocan, lumican, hyaluronan, and proteoglycan link protein-1, as well as collagens (encoded by *VCAN, NCAN, LUM, HAPLN1*, and *COL4A1*, respectively), are expressed in the subplate and marginal zone. When mutated or dysregulated, these proteins alter synaptogenesis and gyrification, resulting in MCD or ID. The genes *PAX6, TBR1, SOX2, and MEF2C* coding for transcription factors, as well as members of the Wnt and Notch signaling pathways, enzymes involved in DNA methylation and histone modifications, and miRNAs, regulate the quantity and distribution of the ECM and their dysregulation can be part of the pathogenesis of MCD and NDD. For example, the *NDST1 and CHSY1 genes, which encode enzymes involved in ECM biosynthesis, are found to be* mutated in patients with AS and IP (for additional examples, see Table 1). The new research paradigm, which leverages the potential of generating induced pluripotent stem cells from patients with MCD or NDD to cultivate cerebral organoids, is an exceptional tool for elucidating the function of ECM and its receptor genes in the developing brain, both in health and disease. Therefore, knowledge of the genetic basis of brain-specific ECM and its MRI representation contributes to understanding the pathogenesis of cortical malformations and brain function abnormalities, and has proven essential in identifying potential new targets for diagnostic and therapeutic approaches to treating neurodevelopmental disorders.

Keywords: subplate, synaptogenesis, malformation of cortical development, autism spectrum disorders, perineuronal nets

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Contact information:
Nataša Jovanov Milošević, njovanov@hiim.hr, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Šalata 12, 10000 Zagreb, Croatia

Table 1 Overview of extracellural matrix (ECM) components and their genes associated with some malformations of cortical development (MCD) and neurodevelopmental disorders (NDD).

Table 1. Overview of extracellural matrix (ECM) components and their genes associated with some malformations of cortical development (MCD) and neurodevelopmental disorders (NDD).

MCD or NDD group	MCD type or NDD	Gene	ECM component
Disorders of neuronal and glial proliferation or apoptosis	Microcephaly	*HSPG2*	Perlecan
		GPC1	Glypican-1
		LAMC1	Laminin subunit gamma 1
	Hemimegacephaly	*HSPG2*	Perlecan
		LAMC1	Laminin subunit gamma 1
	Hydrocephaly	*COL1A1*	Collagen I alpha 1
		COL1A2	Collagen I alpha 1
	Focal cortical dysplasia (type II)	*TNC*	Tenascin-C
		TNR	Tenascin-R
		VCAN	Versican
		BCAN	Brevican
		MMP9	Matrix metalloproteinase 9
		PLAT	Tissue-Type Plasminogen Activator
		PLAU	Urokinase-Type Plasminogen Activator
		HSPG2	Perlecan
		LAMC1	Laminin subunit gamma 1
	Tuberous sclerosis complex (TSC; cortical tuber)	*LAM*	Laminins
		COL	Collagens
		TNC	Tenascin-C
		ITGB4	Integrin beta 4
		PLAT	Tissue-Type Plasminogen Activator
		PLAU	Urokinase-Type Plasminogen Activator
Disorders of neuronal migration	Lissencephaly	*RELN*	Reelin
	Lissencephaly-pachygyria	*COL18A1*	Collagen XVIII alpha 1
	Cobblestone malformations	*LAMB1*	Laminin subunit beta 1
		LAMB2	Laminin subunit beta 2
		LAMC1	Laminin subunit gamma 1
		POMT1	Protein O-mannosyl-transferase 1
		LARGE	Glycosyltransferase-like protein LARGE1
		GPR56	Adhesion G protein-coupled receptor G1
		RELN	Reelin
Disorders of post migrational development	Polymicrogyria (PMG)/ bilateral persylvian PMG	*SRPX2*	Sushi repeat-containing protein SRPX2
		LAMC3	Laminin subunit gamma 3
		RELN	Reelin
		POMT1	Protein O-mannosyl-transferase 1
		LARGE	Glycosyltransferase-like protein LARGE1
		GPR56	Adhesion G protein-coupled receptor G1

NDDs	Schizophrenia	*RELN*	Reelin
		CPSG	Chondrotin suphate proteoglycan
		ADAMTSL3	*A Disintegrin-like And Metalloprotease domain with ThromboSpondin type I motifs-Like 3 gene*
		SEMA3A	Semaphorin 3A
	Autism spectrum disorders	*MMP9*	Matrix metaloproteinase-9
		RELN	Reelin
		VCAN	Versican V2
		NDST1	N-deacetylase and N-sulfotransferase 1
		CHSY1	Chondroitin sulfate synthase 1
	Intellectual disabilities	*VCAN*	Versican
		NCAN	Neurocan
		LUM	Lumican
		HAPLN1	Hyaluronan and proteoglycan link protein 1
		COL4A1	Collagen IV alpha 1
		RELN	Reelin
		POMT1	Protein O-mannosyl-transferase 1
		LARGE	Glycosyltransferase-like protein LARGE1
		GPR56	Adhesion G protein-coupled receptor G1

Contact information:

Nataša Jovanov Milošević, njovanov@hiim.hr, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Šalata 12, 10000 Zagreb, Croatia

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Human-specific genetics: new insights and research opportunities into the molecular basis of neurodevelopmental disorders

Martina Rinčić[1]

[1] School of Medicine University of Zagreb, Croatian Institute for Brain Research

PRESENTING AUTHOR:
Martina Rinčić

ORCID:
0000-0003-0390-5967

E-MAIL:
martina.rincic@hiim.hr

Abstract:

Neurodevelopmental disorders (NDDs) encompass conditions that impair the development of the nervous system, affecting cognitive, emotional, and behavioral functions. Their complex etiology involves a combination of genetic, environmental, and neurobiological factors. Advances in genomics have identified various genetic alterations, including chromosomal anomalies, copy number variations, single-nucleotide polymorphisms, and rare *de novo* mutations, all of which are linked to NDD pathogenesis. However, despite a strong genetic basis, the causes of many NDD cases remain unknown. Current research investigates how genetic modifications influence human neurodevelopmental evolution, shaping our distinct cognitive abilities. NDDs primarily affect functions distinguishing humans from other species, such as abstract thinking. Given DNA's role as a cellular blueprint, one might assume a "more is better" paradigm in human genome complexity. However, genome size alone does not define cognitive ability; for instance, the fork fern *Tmesipteris oblanceolate* has a much larger genome than humans measuring 160.45 Gbp/1C. Instead, evolutionary comparisons with our closest relatives, primates, offer better insights. Humans and chimpanzees share approximately 96% of their genomes. Yet, key genomic rearrangements differentiate them, falling into four categories: regions that rapidly accumulate changes through evolution, regions that have been lost, duplicated regions, and regions with variable copy numbers in humans.

One striking example of genomic evolution is the duplication of the *ARHGAP11B* gene, which is linked to brain expansion. Expressed in ape cerebral organoids, *ARHGAP11B* increases the number of basal progenitors to human levels (Fischer et al. 2022).

Earlier research focused on gene gains, assuming evolutionary advantages stemmed from additional genetic material. However, recent studies highlight the role of gene loss. Human-specific deletions of conserved elements (hCONDELs), primarily located in noncoding regions, can impact gene expression. A study identified 10,032 hCONDELs, affecting intergenic (59%) and intronic (35%) regions, with 800 showing species-specific regulatory activity. Many deletions lead to transcriptional repression rather than activation. For example, a single-base deletion in *LOXL2* removes a repressor binding site, resulting in increased gene expression in humans compared to chimpanzees. Genome editing confirmed this by reintroducing the ancestral base, restoring chimpanzee-like expression levels. Single-cell RNA sequencing further assessed the impact of this human-specific deletion. Edited cells containing the ancestral sequence showed significantly lower *LOXL2* transcription, mirroring chimpanzee profiles. This regulatory change triggered widespread alterations in gene expression, with 145 genes exhibiting differential expressions. Gene ontology analysis highlighted processes related to cell migration, development, myelination, intercellular transport, and synaptic function (Xue et al. 2023)032 human-specific conserved deletions (hCONDELs.

Another key feature of genome evolution are human-accelerated regions (HARs), which accumulate changes at an unusually high rate. A well-studied example, HARE5, regulates *FZD8*, a receptor in the WNT pathway. HARE5 is expressed earlier and more intensely during human brain development than in chimpanzees, leading to increased neural progenitor proliferation and larger brain size in transgenic mice (McLean et al. 2011)"properties":{"formattedCitation":"(McLean et al. 2011. Initial hypotheses suggested that human-specific structural variants (hsSVs) might alter genome folding, leading to enhancer hijacking—a phenomenon in which conserved enhancers regulate new target genes. However, recent studies mapping HARs and human-gained enhancers (HGEs) in neural stem cells indicate that most HARs still regulate the same genes in both species, challenging this hypothesis (Pal et al. 2025).

Genomic regions with variable copy numbers also contribute to human-specific traits. One such gene, *SRGAP2C*, arose from a partial duplication of *SRGAP2A*. *SRGAP2C* inhibits *SRGAP2A*, enhancing synaptic density in cortical pyramidal neurons, improving cortico-cortical connectivity, refining neuronal responses, and strengthening cognitive functions in transgenic mice (Schmidt and Polleux 2022).

What are the implications for NDDs? Genetic variations in human-specific regions are linked to NDDs. Patients with diverse clinical manifestations, such as microcephaly or reduced gyrification, often exhibit mutations or deletions in genes unique to humans. Traditional sequencing methods initially identified these changes, but research now incorporates single-cell epigenomic and transcriptomic comparisons. Brain development involves dynamic gene regulation across diverse cell types, rendering human brain evolution a complex and intricate process. The extended developmental period, increased synaptic complexity, and enhanced connectivity of the human brain stem from genomic and epigenomic modifications. These insights enhance our understanding of both human cognitive evolution and NDD genetics, informing future research on how our unique genome influences brain function.

Keywords: neurodevelopmental disorders (NDDs), genomic evolution, human-specific genetic variations, human-accelerated regions (HARs)

Contact information

School of Medicine University of Zagreb, Croatian Institute for Brain Research
Šalata 12, 10 000 Zagreb
martina.rincic@hiim.hr

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Imaging Mass Spectrometry – A Window into the Molecular Diversity of the Brain

Vedrana Ivić[1], Kristina Mlinac-Jerković[2,3], Željko Debeljak[4,5], Marija Heffer[1*]

[1] Department for Medical Biology and Genetics, Faculty of Medicine Osijek, J. J. Strossmayer University of Osijek, J. Huttlera 4, HR-31000 Osijek, Croatia;
[2] Laboratory for Molecular Neurobiology and Neurochemistry, Croatian Institute for Brain Research, Šalata 12, HR-10000 Zagreb, Croatia;
[3] Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb, Šalata 3, HR-10000 Zagreb, Croatia;
[4] Clinical Institute of Laboratory Diagnostics, Osijek University Hospital Centre, J. Huttlera 4, HR-31000 Osijek, Croatia;
[5] Department of Pharmacology, Faculty of Medicine, J. J. Strossmayer University of Osijek, J. Huttlera 4, HR-31000 Osijek, Croatia

PRESENTING AUTHOR:
Marija Heffer

ORCID:
0000-0001-6770-7359

E-MAIL:
mheffer@mefos.hr

Abstract:

The molecular complexity of brain composition underlies its anatomical complexity. The extraction of molecules from tissue homogenates facilitates molecular analysis but results in a loss of anatomical organization. By employing histological and multiplex immunohistochemical methods, the histology of the tissue is preserved, but only a limited number of molecules (1 – 100) can be visualized. Adding to the limitations of traditional research, histological epitopes are often selected based on hypotheses that may prove incorrect, leading to longer and more expensive investigations.

To address these challenges, the method of imaging mass spectrometry (IMS) was developed. IMS maintains the anatomical integrity of the tissue while measuring over a thousand molecular signals. Depending on tissue processing, the resulting signals can reflect protein composition (proteomics) or pertain to small metabolites (metabolomics) and lipids (lipidomics). When similar samples of normal tissue are available, additional statistical analysis helps identify molecules with statistically significant deviations. Further bioinformatics processing, using existing databases, allows these molecules to be placed within relevant metabolic processes or to determine transcription factors influencing their levels.

Unlike studies that begin with a hypothesis, IMS is often hypothesis non-driven or untargeted analysis. Such analysis aims to eliminate researcher bias and identify relevant pathophysiological mechanisms. IMS can also be targeted, focusing on just one or a very small set of molecules. This approach can reveal the distribution of a drug and its metabolites in brain tissue, assess viral neurotropism, or illustrate the distribution of a toxic metabolite.

In our previous works, we explored both approaches. Using untargeted IMS analysis of the brains of mice with a knockout gene for GD3 synthase and a deficiency of two (GD1b and GT1b) of the four main gangliosides, we demonstrated a disruption in the synthesis of ubiquinone, porphyrin, and long-chain fatty acids. In the study presenting a new method for isolating lipid rafts without the use of detergents, targeted IMS was employed to demonstrate the loss of all four main glycolipids in the brain (GM1, GD1a, GD1b, and GT1b) from cerebellar tissue sections after treatment with detergents Triton X100 and Brij O20.

Whether targeted or untargeted, IMS analysis generate large datasets, accelerate research, and enhance the utility of rare samples. Combined with other imaging or biochemical methods, IMS is a powerful tool in translational medicine.

Keywords: imaging mass spectrometry, multiplex methods, gangliosides

Contact information:
Marija Heffer, Department for Medical Biology and Genetics, Faculty of Medicine Osijek, J. J. Strossmayer University of Osijek, J. Huttlera 4, HR-31000 Osijek, Croatia; e-mail: mheffer@mefos.hr

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Abnormal orbits of genetic mechanisms in the formation of brain tumors

Nives Pećina-Šlaus[1,2], Anja Kafka[1,2], Petar Brlek[1,3], Niko Njirić[1,4]

[1] Laboratory of Neurooncology, Croatian Institute for Brain Research, Šalata 12,10000 Zagreb, Croatia
[2] Department of Biology, School of Medicine, University of Zagreb, Šalata 3, 10000 Zagreb, Croatia
[3] St. Catherine Specialty Hospital, Branimirova 71E, 10000 Zagreb, Croatia.
[4] Department of Neurosurgery, University Hospital Center "Zagreb", School of Medicine, University of Zagreb, Kišpatićeva 12, 10000 Zagreb, Croatia.

PRESENTING AUTHOR:
Nives Pećina-Šlaus

ORCID:
0000-0002-3334-7671

E-MAIL:
nina@mef.hr

Abstract:

In spite of recent progress, molecular mechanisms responsible for brain tumor formation are still inadequately explained. These are very complex mechanisms that include changes in genes and proteins involved in numerous vital cellular processes - proliferation, apoptosis, DNA repair, mobility, angiogenesis, immune surveillance, genomic instability and cellular metabolism. The famous hallmarks of cancer proposed by Hanahan and Weinberg in 2000. have been constantly upgraded and now number 14 characteristics. Latest additions include unlocking phenotypic plasticity, nonmutational epigenetic reprogramming, polymorphic microbiomes, and cellular senescence. However, signal transduction pathways are fundamental in the development and progression of cancer. The imbalances in cellular signaling networks are also causative of brain tumors. The recent failure of a number of targeted therapies, particularly for glioblastoma, shows that CNS tumors do not just respond to a single pathway-driven targeted therapy. On the contrary, targeting multiple pathways simultaneously could be an alternative way to overcome tumorigenesis. Our group is studying brain tumor genetics with particular focus on Wnt signaling. We believe that genetic and expression changes are associated to phenotypic characteristics and behavior of tumor cells. The results of in silico analysis by cBioPortal for Cancer Genomics (Figure 1) database following Array Comparative Genomic Hybridization (aCGH) and Genomic Identification of Significant Targets in Cancer (GISTIC) identified significantly deleted regions: 9p21.3; 17p13.2; 10q24.2; 14q21.3; 1p36.11 and 13q12.11, but also amplified ones: 3q28; 12q13.3 and 21q22.3 in higher malignancy grades of gliomas. We identified copy number aberration (CNA), pathways and genes that are biologically and functionally significant by the use of DAVID, an enrichment analysis tool designed to estimate the biological relevance of a given collection of genes, and data repository KEGG (Kyoto Encyclopedia of Genes and Genomes). It should be emphasized that other cellular mechanisms are also crucial for the formation of brain tumors, for example oncogenic roles of long non-coding RNAs in signaling regulation. Novel research focuses on the tumor microenvironment and glioma stem cells, the appearance of neoantigens and cellular senescence that creates a specific SASP phenotype (senescence-associated secretory phenotype) responsible for therapy resistance. All this contributes to a better understanding of the brain tumor genetic profile. The highlighted molecular changes can offer prognostic markers and directions for the development of improved treatment.

Keywords: genetic profiles of brain tumors, cBioPortal, aCGH, GISTIC

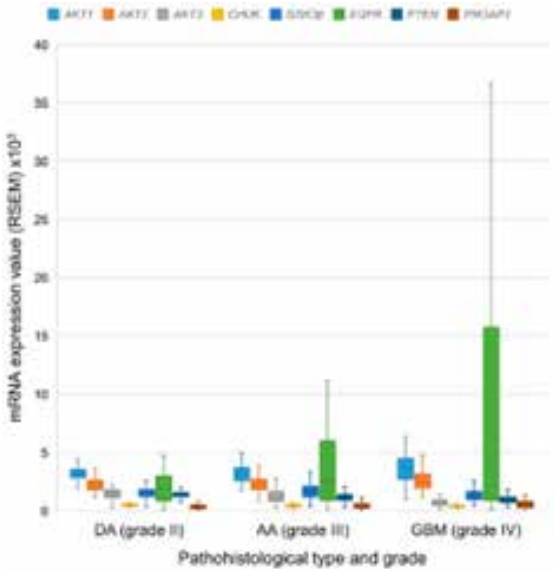

Figure 1. Distribution of mRNA expression of the examined genes according to the pathohistological type and grade of diffuse gliomas obtained by the RSEM method. DA—diffuse astrocytoma; AA—anaplastic astrocytoma; GBM—glioblastoma multiforme (Brlek et al, 2021).

Contact informations:

Prof. dr. sc. Nives Pećina-Šlaus
Email: nina@mef.hr
Phone: +385 1 46 21 140
Fax:+385 1 45 90199; +385 1 49 20 050
Orcid: 0000-0002-3334-7671.

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Cerebral Organoids enable studying live surrogates of brain tissue, patient/donor specific, in long term experiments *in vitro*

Ante Plećaš[1], Dinko Mitrečić[2], Ivan Alić[1,2,3], Dean Nižetić[3*]

[1] Dept. of Anatomy and Embriology, Faculty of Veterinary Medicine, University of Zagreb, Croatia
[2] Stem Cell Lab, Croatian Institute for Brain Research, Faculty of Medicine, University of Zagreb, Croatia
[3] The Blizard Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, U.K.
[*] presenting author

PRESENTING AUTHOR:
Dean Nižetić

ORCID:
0000-0001-5486-5761

E-MAIL:
d.nizetic@qmul.ac.uk

Abstract:

Cerebral organoids (COs) are millimeter-sized, self-organizing 3-dimensional live tissue spheres derived from human induced pluripotent stem cells (iPSCs) differentiated into neural tissue. Standard COs represent mixed-regions of cortical tissue, but directed organoids represent specific cellular subtypes specific for some neuro-anatomical regions, such as dorsal forebrain cortex, hippocampus, striatum, medial gangliomic eminence, midbrain and cerebellum. Functional connectivity between different parts of the brain can be studied by deriving each regional organoid separately and then fusing the parts into assembloids. Organoids were initially thought as useful models of neurodevelopmental (such as microcephaly)[1] and related disorders (such as ZIKA-virus infections causing microcephaly), but not for conditions related to old-age neurodegeneration, due to embryonic nature of the starting cells, and due to erasing of epigenetic ageing marks by the iPSC re-programming. Recent studies however, including studies by Nizetic group in collaboration with Croatian Institute for Brain Research (CIBR), uncovered that cerebral organoids, most probably due to the lack of clearing mechanisms (such as microglia, blood-derived macrophages, glymphatics linked to circulation) surprisingly can accumulate aggregates causative of neurogenerative conditions[2,3]. We and others have uncovered that organoids can model the true causative sequence of events representing Alzheimer's disease (AD) pathology, and allow studies of the effects of gene dose and drugs on AD prevention, progression and spreading[2]. Studying patients born with an extra-copy of the gene for the Amyloid Precursor Protein (APP) that causes early onset Alzheimer's disease (EOAD), including both patients with the segmental duplication of this gene (Dup*APP*) and people with Down Syndrome ((DS), born with an extra copy of human chromosome 21, harbouring the *APP* gene), we uncovered that their cerebral organoids develop in just over 3 months in culture typical hallmarks of Alzheimer's disease (AD) pathology: secretion of toxic soluble oligomeric aggregates of β-amyloid peptide, phosphorylated Tau and inflammasome ASC-specks[3], extracellular amyloid insoluble fibrillary deposits, intracellular pathologically conformed Tau protein, and progressive neuronal loss[2]. We also uncovered that the profile of β-amyloid peptides secreted by the organoids to the culture media, closely recapitulates the profile visible in cerebrospinal fluid of people with DS[2]. Importantly, all 3 pathological processes can be reversed by the inhibition of the β-amyloid peptide, therefore recapitulating the true sequence of events in human AD: β-amyloid-peptide-driven Tau-opathy. This is also providing the proof-of-principle that the organoid technology can be used for drug-screening approaches to uncover compounds with potential to prevent or slow-down AD pathogenesis[2].

In other recent studies, we have shown that not only AD, but also cellular and neuronal ageing can be modelled using COs[4]. We revealed that premature ageing in DS is underpinned by cellular senescence that can be modelled in induced pluripotent stem cells (iPSCs) and cerebral organoids. We

also showed that DNA damage-associated progeria, with a decrease in LaminB1 levels, is a significant component of DS, that trisomy of the chromosome 21-encoded gene *DYRK1A* causes this, and that it can be pharmacologically alleviated in iPSCs and COs[4]. This study included a collaboration with 6 institutions in Zagreb and was partly funded by the Croatian Science Foundation. Through this study, a transfer of technology was achieved enabling the group at the Croatian Institute for Brain Research (CIBR) to adopt the iPSC and COs technologies. This is now further advanced, in collaboration with Nizetic lab in London, by producing assembloids between striatal and cortical organoids, for the mechanistic study of neuronal connectivity defects in DS.

Our studies demonstrate that COs, despite lacking many cell types and precise 3D tissue organization of the human brain, can model processes that mimic those in the human brain, including ageing, neuro-inflammation and neurodegeneration. Furthermore, lack of certain brain types in organoids is also an advantage, as it allows sequential addition of the missing cell types (such as astrocytes, microglia or endothelial cells derived from same, or genetically altered iPSCs), and thereby the precise dissection of the roles of each cell type in the disease being studied.

Organoid technology appears promising for the uncovering of new insights about the mechanisms, biomarkers of disease risk and progression of prognostic significance, as well as detecting new chemical compounds and antibodies for the developments of hitherto unknown therapeutic approaches[5].

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Changes in gene regulation in brain aging

Goran Šimić, Ena Španić Popovački, Lea Langer Horvat

PRESENTING AUTHOR:
Goran Šimić

ORCID:
0000-0002-6339-9261

E-MAIL:
gsimic@hiim.hr

Abstract:

The greater the mortality caused by environmental factors, the less natural selection will favor the maintenance of somatic cells, giving preference to the vitality of germ cells. Since the pressures of natural selection act more strongly towards positive effects in earlier life stages (gene variants that lead to dysfunction before puberty will quickly be outcompeted by alleles that favor the production of healthy offspring), the aging process is a consequence of the absence of these pressures after the reproductive period. Cognitive abilities peak during the reproductive phase and need only remain stable until offspring become independent. Events after reproduction are less significant, so the heritability impact on longevity is generally low: approximately 0.23 for men and 0.26 for women (**Herskind et al., 1996**). Genetic effects on lifespan shorter than 60 years are minimal, and their impact then increases proportionally with age. After reaching sexual maturity, the priorities shift from adaptation and cognitive development to finding a mate, procreating, and caring for offspring, leading to a reduction in synaptic density and plasticity in the cerebral cortex (**Huttenlocher and Dabholkar, 1997**). The duration of reproduction and neurodegeneration in mammals is proportional regardless of differences in lifespan, suggesting that the aging process is similar across mammalian species, differing only in pace. This is likely due to a unique epigenetic clock that "ticks" at different rates, as evidenced by Horvath's algorithm, which determines a person's biological age with greater accuracy than chronological age based on a specific DNA methylation pattern at 353 selected CpG sites in the genome (**Horvath, 2013**). Unlike the epigenetic clock, which broadly reflects the general aging process, epigenetic drift is a unique collection of all acquired changes related to the environment in which an individual or cell culture ages. Some parts of this mechanism are common across all tissues, but it is known that each tissue can have its specific mechanisms (Hannum's epigenetic clock) and that during aging, the clock ticks faster for longer genes compared to shorter ones. Since pluripotent stem cells avoid age-related changes in DNA methylation, it is believed that reprogramming aged cells could lead to regeneration (epigenetic rejuvenation), but it is still unclear how to achieve rejuvenation without the risk of dedifferentiation.

The process of brain aging initiates at the cellular level, with distinct types of neurons aging at varying rates due to differential susceptibility to cellular aging mechanisms. Key alterations in gene regulation involve those encoding proteins responsible for synaptic function, responses to oxidative stress, and neuroinflammation (a state of low-grade inflammation due to heightened expression and activation of inflammasomes **[Figure 1]** and the secretion of pro-inflammatory cytokines). Alongside epigenetic modifications, the most significant changes in neuronal gene regulation include genomic instability (characterized by the accumulation of DNA damage and reduced efficiency of repair mechanisms, which are hallmarks of aged neurons leading to somatic mutations), alterations in mtDNA (mutations occur 10-20 times more frequently than in nuclear DNA due to the generation of reactive oxygen species during oxidative phosphorylation), and changes in the expression of genes associated with proteostasis, particularly those regulating autophagy and the ubiquitin-proteasome system for tagging and degrading damaged and misfolded proteins. The endothelial cells of brain capillaries maintain the integrity of the blood-brain barrier, which protects the brain from pathogens and other harmful factors. During aging, these cells are among the first to undergo transcriptional changes, likely due to

circulating signals. Indeed, heterochronic parabiosis experiments demonstrate that the plasma of aged mice accelerates the aging of brain capillary endothelial cells, while the plasma of young mice exerts a rejuvenating effect (**Chen et al., 2020**). The most significant rejuvenating interventions currently under investigation include metabolic interventions (metformin, mTOR antagonists, GLP-1R agonists), the removal of senescent cells, and epigenetic rejuvenation using Yamanaka transcription factors. A comparative analysis of gene expression in the brains of 19,300 individuals revealed that the primary "drivers" of brain aging are neuronal insulin resistance in the 40s (notably increased expression of the insulin-dependent glucose transporter gene *GLUT4* and decreased expression of the monocarboxylate transporter gene *MCT2*), vascular changes (reduced expression of *VEGFR1*), and heightened activation of innate immunity signaling pathways (increased expression of *APOE* and *IL1B* genes) (**Antal et al., 2025**). Therefore, future interventions targeting genes that are over or underexpressed during aging could represent a promising strategy for achieving longevity. A metabolic intervention study involving 101 participants demonstrated that ketones have a potent effect on re-stabilizing cortical network activity, with the maximum effect occurring between the ages of 40-60, suggesting that in midlife, carbohydrate intake should be reduced, while protein and healthy fat consumption should be increased, as has been indicated by the results of caloric restriction experiments for years.

Keywords: aging; brain; epigenetic drift; epigenetic clock; regulation of gene expression.

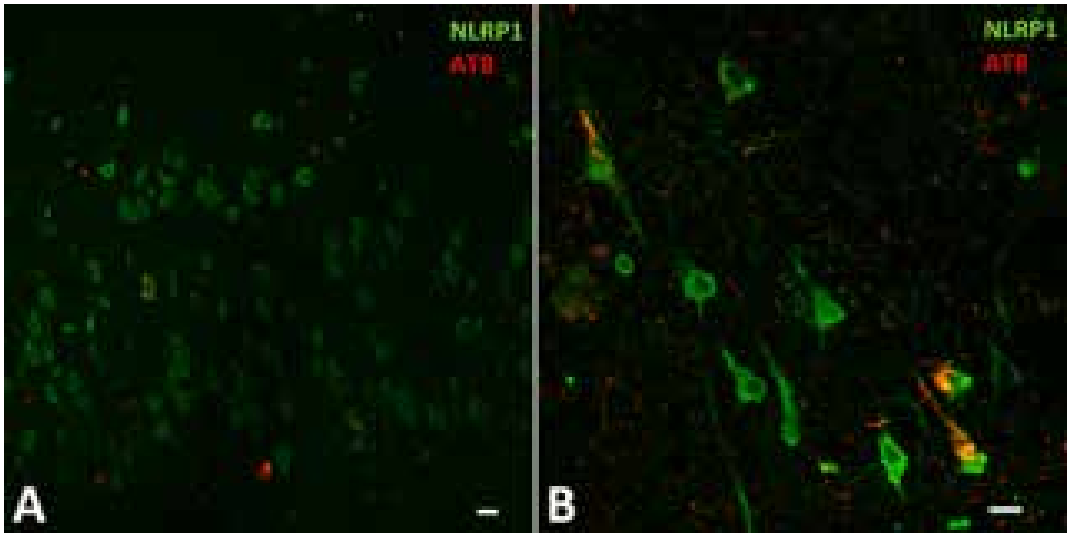

Figure 1. Expression of neuronal (NLRP1) inflammasome in the CA1 field of the hippocampus of a cognitively healthy 77-year-old woman (A) and a person with clinically and neuropathologically confirmed Alzheimer's disease aged 84 (B), whose disease lasted 3.5 years from diagnosis to death. Phosphorylated tau protein is visualized using AT8 antibody (binds to phosphorylated epitopes Ser199, Ser202, and Thr205). Scale bars = 20 μm.

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New possible treatments for dementia

Nenad Bogdanovic

Karolinska University Hospital

PRESENTING AUTHOR:
Nenad Bogdanovic
Senior consultant, Professor
Karolinska University Hospital
Theme Aging and Inflammation R94
Cognitive Clinics
14186 Stockholm, Sweden

ORCID:
0000-0001-6092-2515

E-MAIL:
nenad.bogdanovic@ki.se

Abstract

Dementias are complex neurodegenerative and cerebrovascular disorders that necessitate distinct pharmacological strategies due to their diverse pathological mechanisms. Among the most prevalent forms are Alzheimer's disease, dementia with Lewy bodies, frontotemporal dementia, and vascular dementia. While no curative treatment currently exists to halt disease progression, recent advancements in Alzheimer's research have introduced therapeutic approaches aimed at slowing neurodegeneration.

A dominant framework in Alzheimer's disease pathophysiology is the amyloid cascade hypothesis, which posits that the accumulation of β-amyloid (Aβ) in the brain initiates a sequence of neurodegenerative events, ultimately leading to widespread neuronal degeneration and cognitive impairment. Aβ is a ubiquitous protein with several essential functions, including synaptic regulation, injury recovery, microangiogenesis, cell growth inhibition, antimicrobial activity, and maintenance of the blood-brain barrier. In individuals with Alzheimer's disease, Aβ accumulation begins 15 to 20 years before the onset of clinical symptoms. The etiology of this accumulation differs between early-onset and late-onset Alzheimer's; in early-onset cases, overproduction of Aβ drives its pathological aggregation, whereas in late-onset cases, impaired clearance mechanisms are primarily responsible. Current symptomatic therapies do not facilitate Aβ clearance; however, experimental and clinical studies involving monoclonal antibodies have demonstrated promising efficacy in reducing amyloid burden and slowing disease progression.

Recent FDA-approved monoclonal antibodies, such as lecanemab and donanemab, have garnered significant attention for their ability to target Aβ fibers, remove Aβ plaques, and mitigate cognitive decline. Clinical trials indicate that these therapies can slow cognitive decline by approximately 30 percent over 18 months. Lecanemab preferentially binds to Aβ protofibrils, preventing the formation of mature plaques, whereas donanemab binds to existing amyloid plaques, facilitating their rapid clearance via immune-mediated mechanisms. Both therapies, however, are associated with amyloid-related imaging abnormalities (ARIA), including cerebral oedema and microhemorrhages. Notably, the incidence of ARIA is lower with lecanemab compared to donanemab.

To address the limitations of first-generation monoclonal antibodies, trontinemab, a novel therapeutic currently in late-stage development, employs a brain shuttle mechanism that enhances blood-brain barrier penetration. Unlike lecanemab and donanemab, trontinemab incorporates a transferrin receptor binding domain, allowing superior brain penetration at lower doses, thereby improving efficacy while reducing systemic exposure and side effects.

Despite the therapeutic promise of monoclonal antibodies, their clinical application necessitates stringent patient selection. These treatments are indicated exclusively for early-stage Alzheimer's disease, specifically in individuals with mild cognitive impairment or mild dementia, representing only 10 to 20 percent of patients. They do not provide significant benefit in moderate or severe Alzheimer's disease, where extensive neurodegeneration has already occurred.

Prior to initiating treatment, confirmation of Aβ pathology via PET imaging or cerebrospinal fluid biomarkers is mandatory. Additionally, APOE genotype testing is strongly recommended due to its

significant influence on treatment safety. Carriers of the APOE ε4 allele, particularly homozygous ε4/ε4 individuals, exhibit the highest ARIA risk, with approximately 30 to 40 percent developing complications. Heterozygous ε3/ε4 carriers face a moderate risk, while non-carriers (ε3/ε3 or ε2/ε3) are at lower risk. Given these risks, APOE ε4 carriers require enhanced MRI surveillance throughout the course of treatment.

The efficacy and safety of monoclonal antibody therapy are also influenced by patient comorbidities. Individuals with a history of stroke, cerebral microbleeds, or severe cardiovascular disease are at an increased risk of ARIA and may not be suitable candidates. Furthermore, patients on anticoagulant therapy, such as warfarin or direct oral anticoagulants, face an elevated risk of intracranial hemorrhage. Those with severe hepatic or renal impairment may also have altered drug metabolism and clearance, necessitating careful assessment prior to treatment initiation.

Beyond clinical considerations, logistical challenges further limit widespread implementation. The administration of monoclonal antibodies requires specialized infusion centers, highly trained medical personnel, and frequent MRI monitoring, significantly increasing healthcare burdens. MRI scans are required at baseline and at regular intervals, such as at one, three, and six months' post-initiation, to monitor ARIA. Additionally, the high cost of treatment remains a major barrier, restricting access to only a subset of eligible patients.

In conclusion, monoclonal antibody therapy represents a significant milestone in the treatment of Alzheimer's disease, offering moderate yet meaningful cognitive benefits in select patient populations. However, stringent patient selection criteria, the risk of ARIA, the necessity for continuous MRI surveillance, and cost constraints pose substantial challenges to widespread clinical adoption. While these therapies mark a critical advancement in Alzheimer's disease management, their long-term clinical impact and cost-effectiveness remain areas of ongoing investigation.

Keywords: Alzheimer, amyloid, antibodies, lecanemab, donanemab

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News in the prevention and treatment of stroke

Vida Demarin[1], Hrvoje Budinčević[2,*], Barbara Čaić[2], Hrvoje Jurlina[3]

[1] International Institute for Brain Health, Zagreb
[2] Sveti Duh University Hospital, Department of Neurology, Zagreb
[3] Health Center of Zagreb County, Velika Gorica

PRESENTING AUTHOR:
Hrvoje Budinčević
Sveti Duh University Hospital, Department of Neurology, Sveti Duh 64, 10000 Zagreb, Hrvatska

ORCID:
0000-0002-0184-8949

E-MAIL:
hbudincevic@gmail.com

Abstract

The leading cause of disability and mortality in Croatia and the world is stroke. Up to 90% of strokes can be prevented if ten modifiable risk factors are controlled. Stroke treatment has made significant progress in the last twenty years, primarily with mechanical thrombectomy, systemic thrombolysis, and other supportive measures in stroke units. Treatment in specialized stroke units increases the probability of a good outcome by 14%, the use of systemic thrombolysis by 30%, and mechanical thrombectomy by more than 50%.

This lecture aims to present news in research on stroke prevention and treatment, including the following research funded by the European Union: Validate, Umbrella, TRUSTroke, RES-Q+, POC4Triage, and EAST Stroke. These projects use artificial intelligence and/or advanced technology to improve stroke care from different angles.

Validate project uses artificial intelligence to revolutionize stroke treatment by analyzing patient data and helping physicians make faster, more informed decisions. To make artificial intelligence a reliable medical tool, VALIDATE focuses on 1) training artificial intelligence with diverse data to reduce bias, 2) collaborating with physicians, artificial intelligence experts, and patients, 3) following EU safety and ethical regulations, and 4) continuously testing for accuracy and reliability. The UMBRELLA project will use advanced technology, artificial intelligence, and real patient data to help physicians make better treatment decisions, improve recovery, and prevent future strokes. The UMBRELLA aims to improve stroke care by 1) faster diagnosis & treatment, 2) personalized care, 3) better access to care, and 4) understanding unknown strokes. The RES-Q+ project aims to improve the quality of stroke care by combining artificial intelligence with stroke data. RES-Q+ project will use technology to 1) automatically collect hospital data, 2) AI-powered virtual assistant tools will help physicians assess risks and improve treatment plans, 3) support recovery by tracking progress, and 4) provide information and ensure access to post-stroke care and rehabilitation. TRUSTroke project uses artificial intelligence to improve stroke recovery by helping predict future health risks. TRUSTroke project aims to develop an AI-driven system (easy-to-use application) to personalize treatment and reduce risks such as serious post-stroke health issues, mobility challenges, and recurring strokes.

The EAST-STROKE trial ('Early treatment of Atrial fibrillation for Stroke prevention Trial in acute STROKE') tests a new treatment strategy (rhythm control) for patients with acute ischemic stroke and atrial fibrillation. The EAST-STROKE trial aims to change that by integrating this treatment early after a stroke alongside standard care. The POC4Triage project will use advanced technology and artificial intelligence to improve the speed and accuracy of diagnosis in stroke and other cardiorespiratory conditions. POC4Triage is developing four innovative, portable medical devices designed to provide information for ambulance professionals and physicians to make faster treatment decisions in patients with acute stroke. These portable medical devices are 1) multi-diagnostic monitoring patch, 2) electroencephalography patch, 3) functional near-infrared spectroscopy (fNIRS) device for stroke monitoring, and 4) handheld blood test device (for biomarkers). The four devices will connect to a device hospital connectivity platform.

Keywords: stroke, stroke prevention, stroke treatment, trials

ORCID numbers and e-mails:
Vida Demarin : https://orcid.org/0000-0003-4942-259X; vida.demarin@gmail.com
Hrvoje Budinčević: https://orcid.org/0000-0002-0184-8949; hbudincevic@gmail.com
Barbara Čaić: https://orcid.org/0009-0000-0909-6107; barbaracaic@gmail.com
Hrvoje Jurlina: https://orcid.org/0000-0003-1953-2974; hrvjurlina@gmail.com

Early habilitation in cerebral palsy and the role of robotic-assisted therapy

Ivana Kern[1], Ana Katušić[2]

[1] Special Hospital for Children with Neurodevelopmental and Motor Disorders, Zagreb, Croatia, https://orcid.org/0000-0003-0411-9394

[2] Early Motor Behaviour Research Lab, Faculty of Education and Rehabilitation Sciences University of Zagreb, Croatia, https://orcid.org/0000-0002-7648-131X, ana.katusic@erf.unizg.hr,

PRESENTING AUTHOR:
Ana Katušić

ORCID:
0000-0002-7648-131X

E-MAIL:
ana.katusic@erf.unizg.hr

Abstract:

Cerebral palsy (CP) is the most common motor disorder in childhood. In the last decade, major breakthroughs have been made in the early diagnosis, prevention, and intervention of CP, changing its incidence and prognosis, as well as the response to intervention. Severe motor impairments leading to immobility are becoming less common, meaning that more children than ever before can walk. The reduction in the incidence and severity of the clinical picture of CP is likely a result of a combination of extensive interventions during childbirth and neonatal intensive care, as well as early intervention programs during the period of greatest brain plasticity.

One of the key components of early habilitation is effective early diagnosis, which facilitates timely access to therapeutic interventions. Identifying children who may be at risk for CP involves standardized assessments and clinical criteria that effectively categorize their risks (McNamara et al., 2021). Delays in diagnosis often lead to missed opportunities for intervention, contributing to poorer long-term functional outcomes. In recent years, the body of evidence on early intervention in CP has continued to expand rapidly, providing professionals and families with the possibility of newer, safer, and more effective interventions. For instance, interventions initiated in infancy have been shown to leverage the peak neuroplasticity period, enabling children to achieve better motor and cognitive function (Li et al., 2023; Kwong et al., 2018).

Research supports the effectiveness of therapies that focus on promoting proper movement patterns and improving muscle control, such as robotic-assisted therapy. Robotic-assisted therapy has emerged as a promising intervention for children with CP, focusing primarily on enhancing gait and upper limb function. These therapies leverage advancements in robotics to provide structured and measurable rehabilitation that can be precisely tailored to individual patient needs. Particularly, robotic-assisted gait training has demonstrated beneficial effects by allowing children with CP to engage in repetitive movement patterns that improve gross motor function. Unlike traditional therapies, robotic therapy allows for training in much higher doses (performing a larger number of required movements) and higher intensity (number of movements per unit of time). This dosage per unit of time is considered a key factor in habilitation. This method can also provide objective measures for assessing treatment outcomes. A meta-analysis has confirmed that robotic gait training leads to significant improvements in mobility, indicating the effectiveness of these interventions in promoting locomotor skills (Conner et al., 2022). Further evidence suggests that robotic systems can help overcome specific deficits associated with CP, such as balance and endurance challenges, by enabling task-specific practice in a controlled and motivating environment. Additionally, interventions such as Constraint-Induced Movement Therapy (CIMT) are increasingly integrated with robotic systems. CIMT encourages intensive use of the affected limb by constraining the unaffected one, fostering functional improvements. With robotic systems adopting CIMT principles, children's engagement in task-specific training can enhance their manual abilities in daily life (Faccioli et al., 2023).

In conclusion, robotic-assisted therapy presents a valuable addition to the treatment landscape for children with CP, offering prospects for enhanced mobility, engagement, and measurable outcomes. Continued integration of these technologies into habilitation practices could shape the future of pediatric therapy for individuals affected by CP.

Keywords: early intervention, cerebral palsy, robotic-assisted therapy

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Deep Brain Stimulation – Clinical Experience, Indications, and Future Perspectives

Darko Chudy[1,2], Vladimira Vuletić[3,4], Marina Raguž[1,5]

[1] Department of Neurosurgery, University Hospital Dubrava, 10 000, Zagreb, Croatia.
[2] School of Medicine, University of Zagreb, 10 000, Zagreb, Croatia.
[3] Department of Neurology, Faculty of Medicine, University of Rijeka, 51000 Rijeka, Croatia.
[4] Department of Neurology, Clinical Hospital Center Rijeka, 51000 Rijeka, Croatia.
[5] School of Medicine, Catholic University of Croatia, 10 000, Zagreb, Croatia

PRESENTING AUTHOR:

ORCID:

E-MAIL:

Abstract:

Deep brain stimulation (DBS) represents one of the most significant advancements in functional neurosurgery. Since its stereotactic origins in the mid-20th century, DBS has evolved into a standard neurosurgical procedure for various movement disorders, most notably Parkinson's disease, dystonia, and essential tremor. With over 210,000 devices implanted globally and more than 12,000 new procedures annually, the scope of DBS continues to expand.

At University Hospital Dubrava, more than 400 DBS procedures have been successfully performed to date. Depending on the indication, surgical targets include the subthalamic nucleus (STN), internal globus pallidus (GPi), ventral intermediate nucleus (VIM), posterior subthalamic area/caudal zona incerta (PSA/cZI), and the centromedian-parafascicular complex (CM-pf), as well as various thalamic nuclei in the treatment of chronic pain. In addition to movement disorders, DBS has demonstrated efficacy in treating Gilles de la Tourette syndrome and is emerging as a promising option in the management of disorders of consciousness (DOC).

Our multidisciplinary team has recently focused on the application of DBS in patients with DOC, including those in vegetative and minimally conscious states. Preliminary findings indicate that structural preservation of the thalamus, basal ganglia, and brainstem, along with a favorable gray matter proportion, may serve as predictive markers of clinical responsiveness to CM-pf stimulation. Although early results are encouraging, this indication remains under active investigation, requiring further studies to determine optimal patient selection and therapeutic mechanisms.

Despite its generally favorable safety profile, DBS is not without risks. Potential complications include intracerebral hemorrhage, infection, hardware malfunction, and neuropsychiatric side effects. Therefore, careful patient selection, thorough preoperative imaging, and multidisciplinary evaluation are essential to achieving optimal outcomes.

This abstract provides an overview of the historical evolution, current indications, and institutional experience with DBS, with particular emphasis on its expanding role in neuromodulation for patients with disorders of consciousness. The future of DBS lies in broadening its clinical applications, refining targeting strategies, and identifying reliable predictors of treatment response.

Keywords: Deep brain stimulation, movement disorders, disorders of consciousness, stereotactic neurosurgery, subthalamic nucleus

Neurotechnology in Psychiatry

Neven Henigsberg

Medical School University of Zagreb, Croatian Institute for Brain Research and Vrapce Psychiatric Hospital

PRESENTING AUTHOR:
Neven Henigsberg

ORCID:
0000-0002-5303-1834

E-MAIL:
neven.henigsberg@zg.ht.hr

Neurotechnology is a rapidly evolving field at the intersection of neuroscience, engineering, and medicine, offering interconnected approaches to understanding and treating psychiatric disorders. Traditionally, key categories include neuroimaging, neuromodulation, and brain-computer interfaces (BCIs). Together, these technologies offer complementary insights: neuroimaging to observe brain patterns and activation of brain patterns in psychiatric disorders, neuromodulation to intervene in brain activity, and BCIs to interface with neural signals either in a one-way, or bi-directionally.
MRI-based techniques like fMRI, DTI and MR spectroscopy, or others like fNIRS, are employed to non-invasively visualize brain structure or function to identify neural correlates of mental disorders. Neuromodulation involves technologies that directly alter brain activity – for example, transcranial magnetic stimulation (TMS), vagal stimulation, deep brain stimulation (DBS), tDCS and tACS (transcranial direct/alternate current stimulation). These techniques use magnetic or electrical stimuli to influence brain areas or circuits linked to mood, cognition, or behavior. BCIs provide a direct communication link between the brain and external devices and were initially developed for neurological conditions (e.g. paralysis) but are increasingly relevant to cognitive enhancement and other psychiatric applications.
Clinical Applications
Neurotechnology is transforming both the understanding and treatment of psychiatric disorders. In depression, repetitive TMS applied to the dorsolateral prefrontal cortex is an established, FDA-approved treatment for patients who do not respond to medications. It is considered safe and effective, with about 50–70% of treatment-resistant cases achieving significant symptom relief, and considerably higher remission rates are reported when MRI-guided neuronavigation is used to personalize stimulation targets. Brain stimulation, an invasive neuromodulation mainly used in movement disorders, has been experimented in severe depression and obsessive-compulsive disorder. Notably, a recent closed-loop DBS approach – in which an implanted device delivers stimulation only when a neural signature of depression is detected – produced rapid and sustained remission in a patient with depression. This individualized, on-demand neuromodulation highlights the promise of precision neurotechnologies for psychiatry and the closer integration between the neuroradiology, the psychiatry and the neurosurgery. MRI-based neuroimaging genomics has revealed subtle but widespread brain alterations, as in schizophrenia and autism, reflecting the disorders' complex polygenic risk architecture. Such insights may guide biomarker development and early diagnosis in the future. In PTSD, neuroimaging consistently finds hyperactivity in fear circuits (e.g. amygdala) and hypoactivity in prefrontal regulatory regions, informing neuromodulation trials targeting those networks. Early studies with TMS in PTSD and anxiety suggest potential benefits, though results are mixed and further trials are ongoing.
Emerging therapies include neurofeedback and neurobiofeedback, a BCI-related technique where patients learn to modulate their own brain activity (via real-time EEG, preferably fNIRS or, theoretically, fMRI feedback), is being explored to alleviate PTSD, ADHD, and anxiety, capitalizing on neuroplasticity. Meanwhile, other BCIs are mostly in research phases for neurological impairment, but their rapid progress indicates their potential use in psychiatric use-cases. A recent breakthrough BCI enabled a person with paralysis to generate fluent speech by decoding cortical activity into words, demonstrating that high-bandwidth decoding of complex mental content is feasible. In principle, similar

interfaces might one day assist patients with psychiatric conditions. Evidence Base and Limitations: Some neurotechnologies are already backed by robust clinical trials (e.g. TMS for depression), whereas others remain experimental. Neuromodulation trials in disorders like schizophrenia and PTSD have had variable outcomes, underscoring that what works for one condition (or individual) may not readily translate to others. Also, many neuroimaging findings in psychiatry have not yet yielded actionable clinical tools, partly due to small effect sizes and heterogeneity. It is increasingly recognized that psychiatric illnesses involve distributed brain network dysfunctions rather than single lesions, which means interventions may need to be personalized and circuit-specific.
The trajectory of neurotechnology in psychiatry is clearly toward more personalized, objective, and biologically grounded care, complementing traditional pharmacotherapy with novel brain-based interventions.

Keywords: neurotechnology, neuroimaging, neuromodulation

How to Increase Treatment Effectivenes and Efficiency in Psychiatry?

Miro Jakovljević

University of Zagreb, School of medicine Croatian Institute for Education and Research of Mental Health

PRESENTING AUTHOR:
Miro Jakovljević
Professor emeritus,
University of Zagreb, School of medicine
Croatian Institute for Education and Research of Mental Health

ORCID:
0000-0003-0420-357X

E-MAIL:
jakovljevic.miro@yahoo.com

Abstract:
As approximately one third of psychiatric patients with serious mental disorders are treatment resistant and the other third shows only partial therapeutic response the question how to increase treatment effectiveness and efficiency is crucial one. Current psychiatry in our turbulent VUCA (*volatile*, *uncertain*, *complex*, *ambigous*) world is overwhelmed with the most varied disciplines, ideas, values, treatment techniques and methods, but short with comprehensive scientific theories and unfortunately not efficient enough. Different fields in psychiatry are oriented to different perspectives of mental disorders and their treatment: the disease/illness perspective, the perspective of the person, the cognitive perspective, the behavioral perspective, the narrative perspective, the spiritual perspective and systems perspective. Psychiatry should move from a pluralistic coexistence of the many separated brainless and mindless, not rarely even confronting disciplines to a coherent transdisciplinary and comprehensive mental health science and practice. Our genome operates within the context of the cell, the cell within the context of the body, the body within the context of the self, the self within the context of the society, and the society within the context of the universe. Human brain is where biological, psychological, social and spiritual processes and mechanisms meet and interact so psychiatric treatment should stimulate patients' brain cybernetics or psychocybernetics to work friendly and creatively for them. Transdisciplinary integrative psychiatry is the theory and practice of mental health care, research, treatment and prevention of mental disorders that 1.promotes the emphatizing therapeutic relationship between psychiatrists and patients and their families using shared decision making and a person-centered approach; 2.focuses on the whole person and total health, considering body/brain-mind-spirit and its systems inter-related with biological, psychological, social, cultural, ecological and spiritual aspects; 3.involves evidence-based practice and practice-based evidence and uses combination of different appropriate therapeutic methods and mental health disciplines; 4.eliminating illness and stopping pathogenesis as well as promoting salutogenesis and increasing wellness. Transdisciplinary integrative psychiatry with a „person life-story centered integrative diagnosis" approach is promising in search of appropriate answers to very relevant inter-individual variability in order to 1.close the gap between evidence-based medicine, value-based medicine, and narrative-based medicine with regards to effective care and valid clinical trials; 2.improve the course of mental disorders with earlier diagnosis and prevention measures; 3.improve adequate monitoring of vulnerability, resilience and psychological growth factors. As each patient is a unique, responsive and responsible subject creative, person-centered narrative psychopharmacotherapy with emphatizing cognitive-emotional-behavioral interaction with the patient evoking and expanding the patient's empathy, well-being and creativity may significantly increase treatment effectiveness. Arteficial intelligence offers many benefits to clinical psychiatry, treatment effectiveness and efficiency and professional and scientific development in psychiatry.

Keywords: treatment effectiveness, treatment resistance, transdisciplinary integrative psychiatry, emphatizing cognitive-emotional-behavioral psychotherapy

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