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Contents

"Considering the country's longstanding history of neuroscience and clinical neurology, it was perfectly fitting for this year's annual EAN event to be held in the bustling Norwegian capital."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

EMJ is published quarterly and comprises review articles, case reports, practice guides, theoretical discussions, and original research.

EMJ also publishes 16 therapeutic area journals, which provide concise coverage of salient developments at the leading European congresses. These are published annually, approximately 6 weeks after the relevant congress. Further details can be found on our website: www.europeanmedical-journal.com

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- Peer review, which is conducted by EMJ's Peer Review Panel as well as other experts appointed due to their knowledge of a specific topic.
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Front cover and contents photograph: Oslo, Norway, home of the EAN 2019. © Leonid Andronov / 123rf.com

EMJ Neurol.

European Medical Journal 4.2 2019

By including articles from across the therapeutic spectrum, we hope to create fertile ground for the flowering of fresh ideas that will advance scientific knowledge and patient care.

Welcome

Esteemed readers, collaborators, and friends; I personally welcome you this year's edition of *EMJ Neurology*, accumulating what we believe are some the most exciting and innovative developments from across the entire neurological spectrum. Within these pages we present a brilliant selection of peer-reviewed articles, a collection of features written by our talented editorial team, and all the highlights from the 2019 5th European Academy of Neurology (EAN) Congress held in Oslo, Norway.

Considering the country's longstanding history of neuroscience and clinical neurology, it was perfectly fitting for this year's annual EAN event to be held in the bustling Norwegian capital. Since attaining its first full professorship in neurology back in 1895, we have seen Norway cement its place on the global neurology stage through steady contributions to the field. This year, over 6,000 neurologists were on hand to debate and discuss the most important, and indeed exciting, topics across 4 spirited days. Neuroinflammation was the academy's central theme this year, encapsulated within a simple message: that inflammation is involved in *all* neurological diseases, and better understanding its influence can identify new and previously unexplored therapeutic avenues.

Searching for new ways to solve old, and persistent, problems: whether it was established leaders in the field or aspiring young scientists boldly making their first waves on the international stage, it was clear during our attendance that outward thinking, innovation, and collaboration were on everyone's minds. As always, we include within a selection of abstract summaries that we believe deserve special acknowledgement, including a comparative analysis of high and low frequency transcutaneous electroneurostimulation for diabetic peripheral neuropathy treatment, and a characterisation of psychiatric abnormalities in paediatric migraine.

Alongside congress content, our selection of peer-reviewed articles is equally compelling. A variety of interesting topics are discussed, including landmarks of autonomic dysfunction in multiple sclerosis and seizures in the paediatric population. The breadth to which neurological disease can reach and impact on our lives highlights the importance of the work that our collaborators are doing towards finding actionable treatment regimens.

This is our 7th edition of *EMJ Neurology*, by which time we have already seen striking progress been made in this ever-evolving field. It is encouraging to see the international neurological community continually strive towards bigger and better things and not shy away from the unique challenges presented by these diseases that exact such a large toll on personal well-being. It is an exciting time for neurology, a sentiment we hope you will agree with reading through these pages. Enjoy.

Spencer Gore

Chief Executive Officer, European Medical Group

Foreword

Dear colleagues,

It is with great pleasure that I introduce to you this year's edition of *EMJ Neurology*.

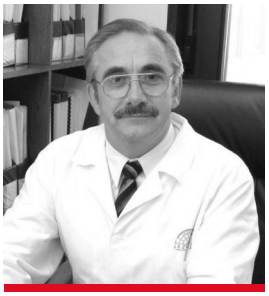
One of Europe's fasting-growing cities Oslo, Norway, played host to the 5th annual European Academy of Neurology (EAN) Congress this year, where >6,000 neurologists united to discuss developments in the exciting field that is neurology. Selected as the overarching theme, special attention was directed towards the involvement of inflammation in 'non-inflammatory' neurological disorders. With data suggesting that inflammation influences all neurological diseases, a better understanding of the mechanisms involved will result in new insights and hopefully a new era of treatment options. More information on this exciting aspect of neurology can be found in the congress feature "Neuroinflammation and its Influence on 'Non-Inflammatory' Neurological Diseases."

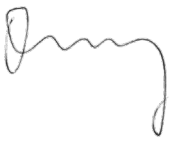
As always, on the following pages you can find the congress review for EAN; inside contains a collection of some of the best abstracts presented at the congress. In addition to the abstracts, you can find congress features, which summarise some of the key sessions that took place at the congress, including a social media trend called #brainlifegoals.

In addition, you will find a collection of great interviews with members of the EAN committee, in which they discuss their and EAN's current work and future challenges. One interviewee discusses the interesting differences in treatment across Europe, how this impacts patient care, and how the recently launched European Reference Networks (ERN) are trying to improve this, especially in regards to rare, complex disorders.

Similarly to the abstracts, this year's *EMJ Neurology* edition has an excellent collection of articles. Levy and Rubenstein provide an essential and detailed review of the aetiology, diagnosis, treatment, and future aspects of seizures in childhood. The authors not only discuss the positives and negatives of standard of care treatment options, but also genetically targeted drugs that are in current pharmaceutical pipelines and one of the most hotly debated therapeutics this decade: cannabidiol. Complementary to the congress review stories, Armstrong delineates a possible explanation for neurodegenerative disease, highlighting the importance the brain's natural ageing process has on such mechanisms. Improving our understanding of such neurodegenerative diseases is crucial if we are to improve the current treatment of them, a disease area in which disease progression altering treatment options are very limited.

This edition proves to be an interesting read in which I believe you all will find something of interest. Covering many hot topics, this edition of *EMJ Neurology* will spark discussion within the neurology community, achieving one of the main objectives of the journal as a whole.





Professor László Vécsei

University of Szeged, Szeged, Hungary

Congress Review

Review of the European Academy of Neurology (EAN) Congress 2019.

Location: Norges Varemesse, Oslo, Norway

Date: 29th June - 2nd July 2019

Citation: EMJ Neurol. 2019;7[1]:11-21.

Attracting over 6,000 neurologists from countries worldwide, the European Academy of Neurology (EAN) 2019 Congress was a hive for the sharing of knowledge, cementing itself as the largest general neurology congress in Europe. As host of the Nobel Peace Prize ceremony, the Norwegian capital of Oslo seemed to be the perfect city to host such an event, in which the coming together of neurologists from all over the world to combat the challenges that are currently facing the field is of utmost importance. A strong history of neurology is present in Norway, including great minds such as Fridtjof Nansen, who, amongst other outstanding achievements, defended the first Norwegian PhD for neuroscience in 1888, and Georg Herman Monrad-Krohn, a world-renowned neurologist best known for his masterpiece "The Clinical Examination of the Nervous System".

Following the screening of a 5-year anniversary video showcasing the growth and progress of the congress, Prof Erik Taubøll welcomed delegates from around the world to his city of birth, Oslo. Those in the audience listened in awe as Prof Günther Deuschl recounted the fascinating previous 5 years of EAN, instilling pride in all that EAN had already achieved and looking forward to the great possibilities the young congress has. Prof Edvard Moser, a recipient of a Nobel prize in Physiology or Medicine in 2014, delivered a world-class opening lecture regarding his latest work on the different types of positioning cells within the brain and how they work together with neural codes form the entorhinal cortex to facilitate episodic memories. He not only explained how these cells monitor direction, place, speed, and location, but also how when connections between these cells are disrupted, spatial-temporal orientation symptoms related to neurological diseases, such as Alzheimer's, can occur.

Presentation of ground-breaking research was evident in the abundance of abstracts at this year's congress. Covering a staggering 31 topics, including the

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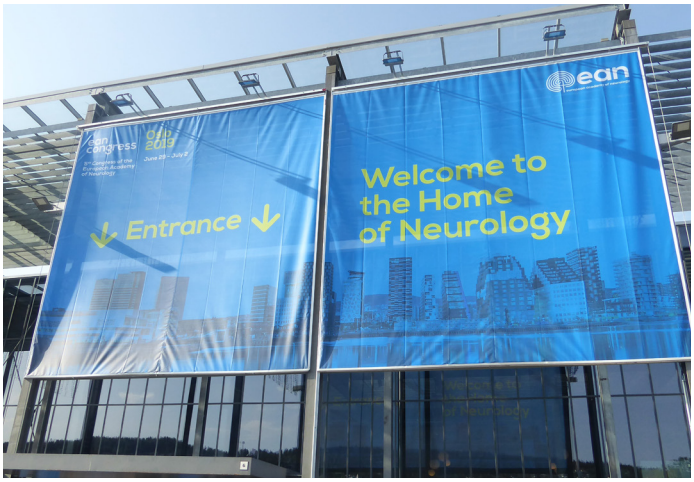
big 7 (epilepsy, stroke, headache, multiple sclerosis, dementia, movement disorders, and neuromuscular disorders), there was no lack of intriguing, novel approaches to the pathogenesis, diagnosis, and treatment of neurological disorders. As always, on the following pages we have compiled some of the abstracts we were most impressed by, two of which discuss Charcot-Marie-Tooth disease, an inherited disorder that involves the peripheral nerves, and therapeutic potential along with a new highly sensitive technology that can be used to monitor disease progression.

Highlighting the importance of the influence of inflammation on neurological disorders, the overarching theme of this this year's congress was 'Neuroinflammation – Science. Synergies. Solutions.'. Many sessions on offer at the congress touched on the potential involvement of neuroinflammation in diseases such as epilepsy, stroke, metabolic encephalopathy, Parkinson's disease, migraine, and more. This theme depicts thinking outside of the box by exploring pathways that were previously believed to not be involved, setting a precedent for the rest of the congress.

One of the most important aspects of congresses like EAN is the opportunity to discuss and debate controversial topics within the field. A perfect example of such communication was presented in the form of controversy sessions, in which the cases for and against the existence of chronic lyme disease, overuse of headache medication, pathogenesis of fibromyalgia, plus more, were disputed, all in an attempt to clarify discrepancies in each topic. In one's daily schedule, such valuable opportunities are few; therefore, by critically overviewing the current status and most recent advances, and allowing opinions to be formed, these EAN sessions further our understanding of science and clinical care immensely.

Complementary to these controversial sessions was a special session entitled 'Cannabinoids: between neuroinflammation and neurodegeneration'. Not only did this session educate audience members of the main characteristics of the endocannabinoid system, but also how the important mediators have fundamental functions within neuroinflammation and neurodegeneration. Special mention was given to the therapeutic potential cannabinoids



possess for the reduction of seizure frequency and intensity in epileptic patients. Balancing the apparently positive benefits surrounding the use of cannabidiol, the possibility of abuse was also discussed.

EAN 2019 proved, once again, to be a congress where thought-provoking discussions take place, so much so that we are already looking forward to EAN 2020, which will be held in the beautiful city of Paris, France. Until then, we hope you enjoy reading our review of this year's congress highlights.

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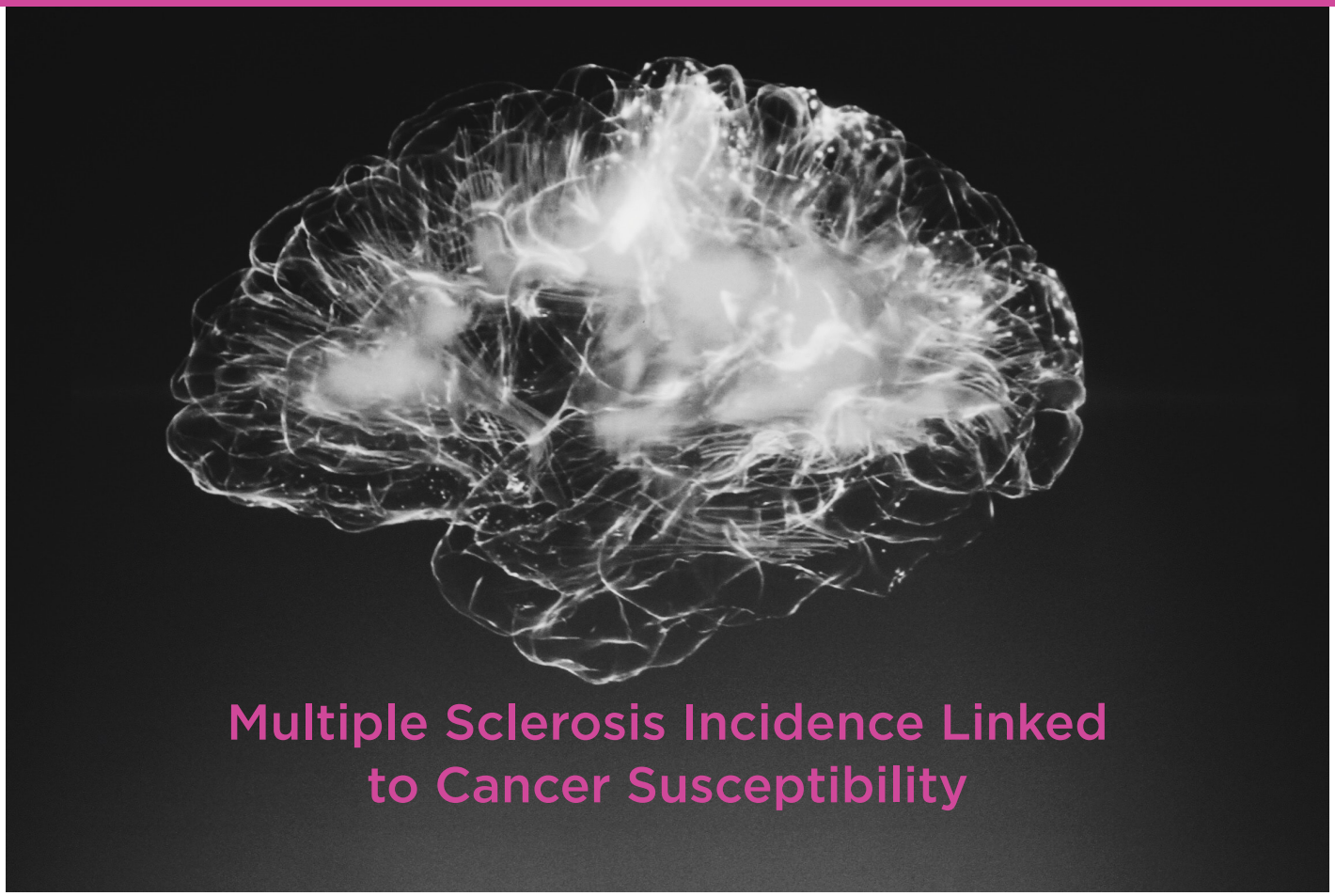
EAN 2019 REVIEWED →



Multiple Sclerosis Incidence Linked to Cancer Susceptibility

MULTIPLE sclerosis (MS) is a chronic condition that severely affects the brain, spinal cord, and optic nerves, and is one the most common neurological conditions to manifest in young adults; however, there has been little research conducted into determining cancer risk in this demographic. Emerging results from a 65-year follow-up analysis of approximately 7,000 Norwegian MS patients has shown that a higher cancer susceptibility exists in this patient population, especially malignancies of the central nervous system, respiratory system, and urinary tract. These findings were presented as part of a press release at this year's EAN Congress in Oslo, Norway.

"the risk assessment between these two groups is extremely interesting because they share the same genetics and environmental conditions."

Included within the study were 6,883 MS patients born between 1930 and 1979 who were registered with numerous Norwegian cancer and MS registries and prevalence studies. For control purposes, 8,918 siblings without MS were studied, as well as 37,919 non-MS individuals. In the MS cohort, increases in cancer prevalence were apparent: respiratory cancer (66% increase), central nervous system cancer (52% increase), urinary cancer (51% increase), and overall cancer (14% increase). Additionally, the unaffected sibling group displayed increased propensity for haematological cancers compared to their MS-afflicted siblings and the general population.

Dr Nina Grytten, lead researcher of the study from Haukeland University Hospital, Bergen, Norway, commented on the link between MS patients and their un-affected siblings saying that "the risk assessment between these two groups is extremely interesting because they share the same genetics and environmental conditions."

Whilst a significant association has been revealed in this study, the authors emphasise the need for further research to uncover the precise mechanisms by which this association presents. Developing a better understanding could lead to quicker cancer diagnoses for these patients and positively impact on clinical outcomes and survival.

One in Ten People Report Having had 'Near-Death' Experiences

FINDINGS have emerged from this year's EAN Congress attesting that mystical near-death experiences (NDE), in which people claim experiencing spiritual and physical symptoms such as out-of-body sensations and time distortion, affect an estimated 10% of the general population. These events occur to an equal degree in people who are not in imminent danger as to those who are, for instance in car crashes or combat situations.

In the study of 1,034 subjects from across 35 countries recruited through an online crowdsourcing platform, those who answered 'yes' to the question to whether having or not experienced an NDE proceeded to describe the perceptions they had using an assessment tool called the Greyson Near-Death Experience Scale. These included abnormal time perception (87%), enhanced speed of thought (65%), vivid senses (63%), and feeling separated from one's body (53%).

Of the 289 people who reported experiencing an NDE, 106 reached a threshold of 7 on the Greyson NDE Scale and thus confirmed having had a true NDE. Of the subjects who claimed experiencing an NDE, the majority (73%) perceived their experience as being unpleasant; however, when these figures are adjusted for Greyson threshold the degree of enjoyment actually increased (53% when a threshold of 7 was reached).

An additional association was made between NDE occurrence and rapid eye movement (REM) sleep intrusion into wakefulness. The latter, characterised by symptoms such as visual and auditory hallucinations and sleep paralysis, was more common in people with scores ≥7 (47%) compared to those with scores ≤6 (26%).

"Although association is not causality, identifying the physiological mechanisms behind REM sleep intrusion into wakefulness might advance our understanding of near-death experiences,"

"Although association is not causality, identifying the physiological mechanisms behind REM sleep intrusion into wakefulness might advance our understanding of near-death experiences," explained lead researcher Dr Daniel Kondziella form the University of Copenhagen, Denmark.

Finding the Time and Space to Combat Alzheimer's

SPACE and time mechanisms in the brain require greater levels of understanding to successfully combat Alzheimer's disease, according to Prof Edvard Moser, Kavli Institute for Systems Neuroscience, Trondheim, Norway, during EAN 2019. The Nobel Laureate explained how the inability to keep track of time, recall memories, and finding one's way serve as the first indications of Alzheimer's development.

"The neural networks that generate space and time are the very first cells that start to die, perhaps decades before we notice clear symptoms of Alzheimer's disease," commented Prof Moser. "The discoveries of how the brain encodes space, time, and memory is crucial to understanding how higher mental function is generated and of great importance to clinical neuroscience and the global efforts to fight brain disease."

Prof Moser was joint recipient of the Nobel prize for Physiology or Medicine in 2014 for the breakthrough discovery of a positioning system in the brain. At EAN 2019, he outlined the importance of establishing the existence of grid cells in 2005, which led to this award; these cells cover a person's spatial environment by generating regular hexagonal patterns, providing information on distances and directions.

Other research has revealed the presence of several types of navigation cells in the brain, each with their own distinct function, enabling an alternative way for people to find their way, for example in the hippocampus and medial entorhinal cortex regions. There are also so-called 'speedometer' cells, which gauge the speed of travel as well as head direction cells, alerting someone when they are close to the edge of something by setting off neural alarms.

Ultimately, it is building upon current insights into time-tracking and space-mapping neural systems that hold the key to tackling Alzheimer's disease, according to Prof Moser. He added: "The brain has specialised neural systems for encoding space, time, and memory that we are now starting to uncover. The next exciting steps are to understand how hundreds, or thousands of neurons interact, in order to create the sense of space or time."

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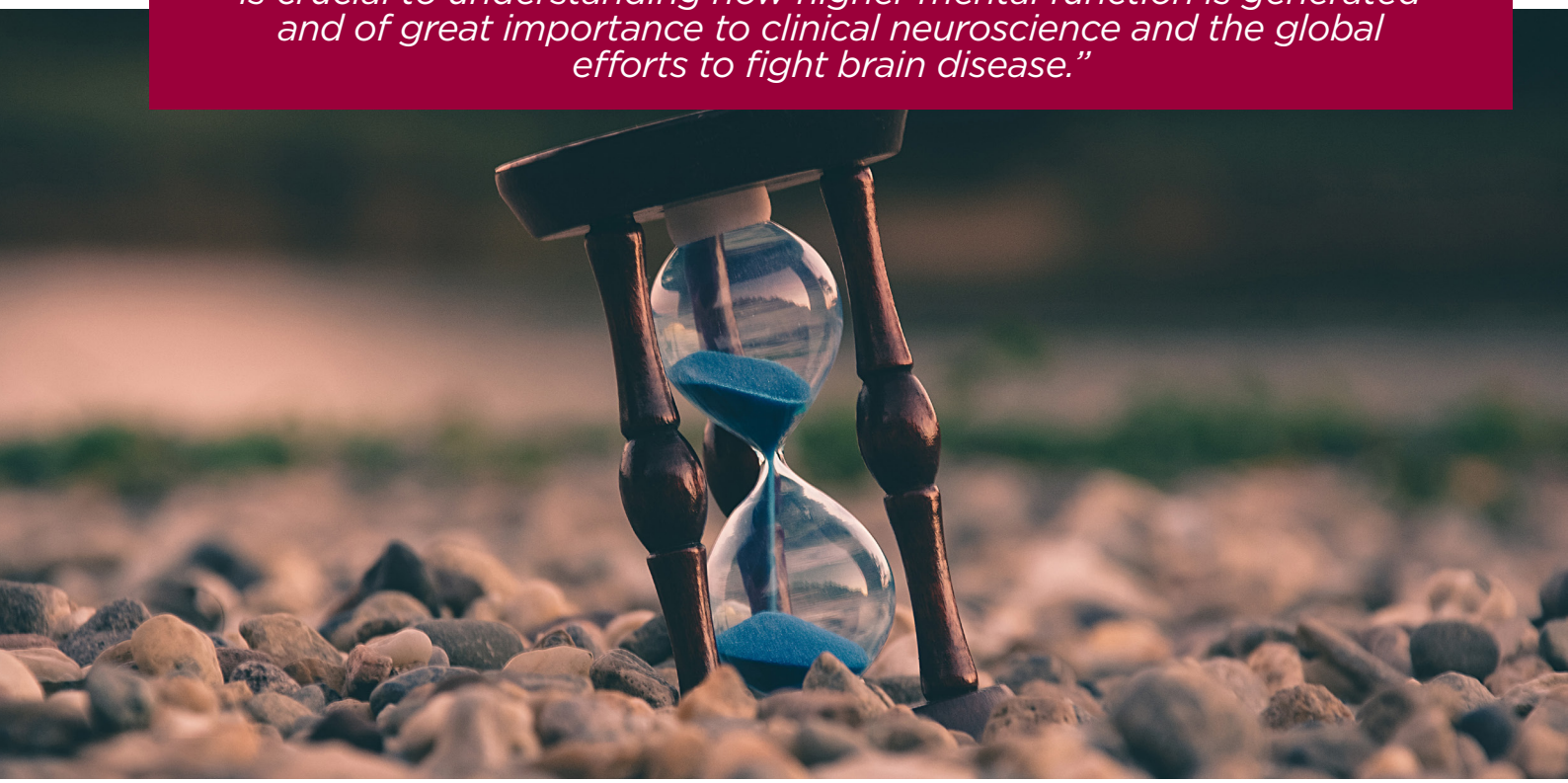

Study Reveals the Impact of the Holocaust on Brain Structure

HOLOCAUST survivors are afflicted with life-long effects on their brain function, with significantly lower volumes of grey matter in their brains compared with the general population, a study presented at EAN 2019 has shown. The authors displayed early evidence that the brain structures of the survivors' children and grandchildren are also negatively impacted. The findings are expected to spark greater efforts into learning how severe trauma impacts brain function in the long term, helping the creation of therapy strategies to support such people.

Prof Ivan Rektor, Masaryk University, Brno, Czech Republic, commented: "After more than 70 years, the impact of surviving the Holocaust on brain function is significant. We revealed substantial differences in the brain structures involved in the processing of emotion, memory, and social cognition, in higher levels of stress but also of post-traumatic growth between Holocaust survivors and controls. Early results show this is also the case in children of survivors too."

In the study, the volume of grey matter in areas of the brain responsible for stress response, memory, motivation, emotion, learning, and behaviour in 28 Holocaust survivors were revealed by MRI scanning to be significantly lower than that of 28 controls who do not have a personal or family history of the Holocaust. The average age of the participants was 79–80 years.

The team then compared the scan results of survivors above and below the age of 12 years in 1945. The younger survivors had significantly more expressed grey matter reduction, indicative of how a brain still developing in childhood is particularly vulnerable to a stressful environment. They also discovered there was a reduced volume of grey matter in parts of the brain associated with post-traumatic stress disorder (PTSD) in combat veterans and people who had experienced early life stress, aligning with earlier studies. However, grey matter reductions in other areas of the brain in the Holocaust survivors was far higher than observed in people with PTSD.

"Our hope is that these findings and our ongoing research will allow us to understand more about the effect of these experiences in order to focus therapy to support survivors' and their descendants' resilience and growth."

Prof Rektor added: "Our hope is that these findings and our ongoing research will allow us to understand more about the effect of these experiences in order to focus therapy to support survivors' and their descendants' resilience and growth. We may also reveal strategies that Holocaust survivors used to cope with trauma during their later lives and to pass on their experience to further generations."

Earlier Intervention is Pivotal for the Successful Treatment of Alzheimer's Disease

"Treating amyloid at a very early stage could protect against symptoms later on and we must target these processes if we want to make the most effective treatments,"

ILLUMINATING aspects of the mechanism of Alzheimer's disease, hereditary forms and results from failed clinical trials can progress our understanding of this perplexing disease. During the prestigious 'Brain Prize Lecture' at EAN 2019, Prof Bart De Strooper, Director of the UK Dementia Research Institute, London, UK, and Group Leader at the VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium, presented data from decades of Alzheimer's and genetic studies, outlining the need for early intervention to protect people against Alzheimer's symptoms later in life.

Prof De Strooper has contributed significantly to the characterisation of the pathogenesis of Alzheimer's disease. Notably, the discovery that abnormal amyloid plaques in the brains of Alzheimer's patients can be a result of mutations in presenilin: a section of the y-secretase enzyme that breaks down β-amyloid chains. The production of these amyloid-β plaques is thought to initiate a neurodegenerative process, thus understanding how these mutations drive Alzheimer's will help the development of new treatments.

Despite this knowledge, attempts to target the pathway with therapeutics have been unsuccessful. Acknowledging the possibility that current interventions are given at the latter stages of the disease, when symptoms are already present, Prof De Strooper commented: "scientists need to shift their focus to the earlier stage of the disease and think about the cellular environment in which the disease develops...". He added: "treating amyloid at a very early stage could protect against symptoms later on and we must target these processes if we want to make the most effective treatments."

Furthermore, Prof De Strooper argued that potential therapeutics have been tested in patients with a too advanced disease stage, therefore it is difficult to alter the disease progression. Looking to the future, intervening in the earlier stages of disease and altering the progression of disease seems to be a potential therapeutic option in treating Alzheimer's disease.

"However, the effect of statins on these two outcomes is not clear. The aim of this study was to analyse the association between the use of statins on the risk of death and stroke in patients diagnosed with dementia."

Reducing Mortality and Stroke Risk with Statins in Dementia Patients

THE IMPORTANCE of investigating stroke in dementia patients is highlighted by the fact that the risk of stroke is three times higher in patients with mild dementia and seven times more likely in patients with severe dementia. With an expectation that those affected by dementia in Europe to double to 20 million by 2030, research into mortality in dementia patients needs to be investigated. Results from a large cohort study into statins and the risk of stroke were presented at EAN 2019, Oslo, Norway, on the 1st July.

The study included 44,920 dementia patients from the Swedish Dementia Registry between 2008 and 2015. Results identified that patients who used statins had a 22% lower risk of all-cause death and a 23% lower risk of stroke. A protective effect on survival with statins was observed: a reduction in mortality rate was seen in those <75 years of age (27%) and in older patients (20%). Interestingly, differences in mortality with statin use were seen between men and women, with reductions observed in 26% and 17% of patients, respectively.

"Survival in patients in dementia is variable, and previous studies have identified many factors associated with survival and risk of stroke in these patients," commented first author Dr Bojana Petek from the University Medical Center Ljubljana, Ljubljana, Slovenia, and the Karolinska Institutet, Solna, Sweden. "However, the effect of statins on these two outcomes is not clear. The aim of this study was to analyse the association between the use of statins on the risk of death and stroke in patients diagnosed with dementia."

The authors explained that because the study is a cohort study and does not have a structure similar to clinical trials, only associations between statins can mortality could be identified, of which could not be definitively proved. Despite this, the results are encouraging and could provide the rationale for including statins in the treatment plan of dementia patients.

Norway Introduces First European Brain Health Strategy

BRAIN disease strategies for prevention and management were presented at EAN, on the 1st July 2019 in Oslo, Norway. The Norwegian Brain Health Strategy 2018–2024 was presented by Prof Anne Hege Aamodt, President of the Norwegian Neurological Association at the congress, outlining Norway's approach to brain diseases and their health strategy.

The initiative has four aims: 1) to provide good, lifelong brain health along with prevention and better quality of life; 2) to provide user-centred care and relative support; 3) to offer holistic care from a range of multi-disciplinary teams; and 4) to ensure adequate knowledge is available through more research and innovation.

Brain disease accounts for roughly 10% of the global disease burden, with one of the most common, dementia, affecting 50 million people across the world. This number is increasing; by 2030 the number of people living with dementia is expected to rise to 82 million, and by 2050, this could reach 152 million. Prof Aamodt said: "Brain diseases affect a wide range of people in all stages of life and, as people are living for longer, greater numbers now live with a range of brain diseases."

"Prevention of brain diseases, the provision of equal treatment, follow-up, and rehabilitation, as well as increased research and expertise, is absolutely vital in providing patients with optimal outcomes. This strategy will help to facilitate this for a number of brain diseases, including dementia, multiple sclerosis, Parkinson's, and stroke-related conditions," continued Prof Aamodt.

The initiatives outlined are underway, including a €20 million investment from a National Clinical Research Centre to work towards a clinical treatment of brain diseases, including multiple sclerosis, dementia, and amyotrophic lateral sclerosis. The Norwegian Research Council are also set to receive €5 million for the improvement of research and innovation across neurological conditions.

Prof Aamodt concluded: "We believe that this national strategy should be replicated and implemented across Europe, tailored for each country. The continent will undergo major societal transformations, such as the ageing population, that will impact on brain diseases and health services must adapt to these changes."

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Infant Language Impairment Associated with Epilepsy Drugs in Pregnancy

EPILEPSY medication taken during pregnancy could increase the risk of language impairment in children aged 5 and 8 years. The research, presented at EAN on the 2nd July 2019 in Oslo, Norway, included an investigation into sodium valproate and carbamazepine. Results also showed that folic acid supplements taken during pregnancy had a protective nature against language impairment for children.

The study looked at two antiepileptic drugs (AED): sodium valproate, an AED that is used only in situations where there is no alternative for pregnant patients as there are known risks for unborn babies associated with the drug; and carbamazepine, which had been considered a safer alternative.

Children of mothers both with and without epilepsy, who had enrolled in the Norwegian Mother and Child cohort study from 1999–2008 were studied. Information was provided including epilepsy diagnosis, AED used in pregnancy, and the child's verbal ability at 5 and 8 years of age. Questionnaires were used to gather the information and validated using language screening tools. The concentration of AED in the blood was measured using blood samples taken at Weeks 17–19 of gestation and umbilical cord samples following birth.

AED-exposed children were found to have language impairment adjusted odds ratios of 1.6 at 5 years of age and 2.0 at 8 years of age when compared with children with non-epileptic mothers. Exposure to carbamazepine monotherapy showed a significantly increased risk of language delay in children when compared with the control group at 8 years. There was a correlation between higher maternal plasma valproate levels and language delay at 5 years. Mothers who took periconceptual folic acid as a supplement during pregnancy had children who were at lower risk of AED-associated impairment in both age groups.

Lead research, Dr Elisabeth Synnøve Nilsen Husebye, University of Bergen, Bergen, Norway, discussed the findings: "The main findings of the research are that fetal antiepileptic drug exposure is associated with an increased risk of language impairment in children of mothers with epilepsy, at age 5 and 8 years, especially after exposure to carbamazepine and valproate." She recognised the need for further research on new AED, such as lamotrigine, levetiracetam, and tompiramate.

Neuroinflammation and its Influence on 'Non-Inflammatory' Neurological Diseases

Layla Southcombe
Editorial Administrator

Citation: EMJ Neurol. 2019;7[1]:22-23.

Arguably, the development of treatments for neurological diseases is one the most challenging fields in life sciences. This is supported by the very apparent limited success of therapeutics for certain neurological conditions; for example, despite knowing that amyloid plaques are a hallmark of Alzheimer's disease, therapeutics targeted to amyloid clearance have not shown any success in clinical trials.[1] Recent evidence is now indicating that inflammation is involved in all neurological disorders, even those considered to be 'non-inflammatory', such as epilepsy, Alzheimer's disease, stroke, migraine, and many more.[2]

The brain is separated from the rest of the body by the blood-brain barrier, and even has its own immune cells: microglia. When activated, B and T lymphocytes situated in the body release inflammatory markers that pass through the blood-brain barrier and activate the microglia.[2] Because microglia play such a crucial role in the maintenance of a healthy brain, a change in their daily activities, such as alterations in received inflammatory markers or its own aberrant behavior, could result in neurological disorders.

Evidence has already identified such changes; it has been observed that a rapid increase in inflammatory markers occurs minutes after acute injury of the brain, for example from stroke or brain trauma.[2] Furthermore, increased microglia activity has been detected in chronic neurological disorders, including movement disorders, motor neuron disease, and migraines.[2] But one question remains to be answered: is this a curative or causative change, or perhaps even both?

Epilepsy

It is widely accepted that many factors can be attributed to epilepsy-epileptogenesis, for example genetic predisposition.[3] Evidence has identified a role for neuroinflammation in the cause and consequence of epileptic seizures, adding confusion to this complex disorder. IL-1β, TNF, IL-6, prostaglandin E2, and complement cascade are all upregulated in microglia and contribute to seizure generation.[2] Furthermore, their activities are not limited to this initial seizure; consequences of increased inflammatory signaling in the brain can alter synaptic excitability, eventually resulting in chronic epilepsy and the increased likelihood of pharmacoresistance to antiepileptic drugs.[2]

The identification of biomarkers in pre-symptomatic Alzheimer's disease will allow the initiation of interventions earlier to maximise the chance of halting or slowing the disease progression.

Alzheimer's Disease

Much evidence suggests that neuroinflammation is involved in the pathogenesis of Alzheimer's disease, causing both negative and positive consequences.[4] Generally, inflammatory mediators aid the eradication of pathogens in the brain. In Alzheimer's disease, the inflammatory mediators that reactive astrocytes secrete can actually break down the amyloid plaques and the astrocytes themselves can ingest amyloid β, leading to its degradation.[5] However, despite this seemingly positive behavior, there are other limitations; reactive astrocytes can secrete enormous quantities of amyloid β, and inflammatory mediators can compromise neuronal function and cause neuronal cell death, adding to the disease burden.[4,5]

Biomarkers

To develop better treatments and improve how disease progression is monitored, biomarkers need to be characterised on a patient level. In epilepsy, viable biomarkers could be used to help predict when a seizure is going to occur and the most likely cause, thus offering the chance to prevent seizure onset and to minimise their long-term effects, ultimately improving patient quality of life. The identification of biomarkers in pre-symptomatic Alzheimer's disease will allow the initiation of interventions earlier to maximise the chance of halting or slowing the disease progression. Not only would this open new avenues of therapeutic research to be explored, but current ineffective therapeutics could have new potential.

Conclusion

Does recent evidence conclude that neurological disorders are the result of unbalanced immune system mechanisms? If so, then current treatments and our understanding of the pathogenesis of neurological disorders need to be rethought. Future research into the relationship between inflammation and neurological disorders could illuminate answers to questions we have been asking for decades, and provide new rationale for potential therapeutics for diseases in which treatment success has not yet been achievable.

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#BrainLifeGoals

Kirstie Turner
Editorial Administrator

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INTRODUCTION

#BrainLifeGoals is a social media awareness campaign from the European Federation of Neurological Associations (EFNA), which was presented at EAN this year.[1] EFNA represent 18 neurology patient associations across Europe and further afield. Their slogan represents their mission: "Empowering neurology patient groups." They use four pillars to structure their work to ensure they make strides towards this goal.

EUROPEAN FEDERATION OF NEUROLOGICAL ASSOCIATIONS[1]

Advocacy

EFNA want to raise awareness of the different neurological diseases and be a voice for the patients that these associations represent. They want to generate more evidence to help shine a spotlight on the burden and impact of these diseases. Also, there is a drive towards improving the level of priority that neurology is given by the decision makers in healthcare.

Empowerment

This pillar ensures that the 18 patient associations feel both empowered and supported in order to take an active role in being an advocate for the neurological diseases they focus on. EFNA also work towards providing these organisations with the platforms and opportunities to be advocates for their patients.

Awareness

The third pillar focusses on the promotion of neurological conditions: making the public both more aware of the diseases that exist and aiding them in better understanding them. There is also a strong drive around destigmatising these conditions and removing any prejudice.

Engagement

Finally, EFNA want to fully engage their stakeholders, including working closely with their partners. This enables them to ensure that neurology patients' voices are heard and that their preferences and perspectives are represented. This creates a patient-led, multi-stakeholder approach.

THE CREATION OF #BRAINLIFEGOALS

Initial EFNA Survey

In 2018, EFNA, in working on their pillar of awareness, focussed their attentions on 18 to 35-year-olds to give this underrepresented audience amongst their organisation a voice. The issues that affect young people who are living with neurological disorders are not getting public or political attention, as they face additional burdens in education, employment, and socially.

EFNA produced a survey with 1,368 responders from 21 European countries,[1] made up of 80% females and 20% males. Participants in the survey had a range of neurological conditions, most commonly including multiple sclerosis (MS), migraine, chronic pain, and myalgic encephalomyelitis (ME). From these results, EFNA recognised the biggest issues that young people were facing:

1. Access to medication and treatment.
2. Isolation.
3. Stigma.

4. Depression and anxiety.
5. Access to employment.
6. Relationships and sexual health.
7. Medication and treatment costs.
8. Access to education.

They also looked at understanding of brain disorders and found that the participants ranked the public understanding of brain disorders as 2.5/10, family understanding as 5.7/10, and friend understanding as 4.3/10. When asked how comfortable they felt discussing their brain disorder with their employer, respondents gave the score 3.4/10.[1]

Highlighting the lack of public understanding and the frustrations that can coincide with neurological conditions, one survey participant said: "I am behind peers by years as far as education and employment goes, due to the inability to do many things most people take for granted and struggling just to deal with what little I do try to accomplish."

Another participant said: "I can't stand the stigma, no matter how hard I try to explain, it seems that they don't understand any better. Because I look 'healthy' I feel like it's hard to get the help I need."

#LifeGoals

EFNA recognised the severe need to improve understanding of these conditions. They came up with the idea to communicate something unknown using something familiar. The hashtag #LifeGoals is a common term on social media to portray people's ambitions, often in a tongue-in-cheek manner. While these often refer to superficial or luxury items, EFNA outlined how there are some people who have goals such as better treatment for their condition and employment opportunities. With 1 in 3 people living with a brain disorder, EFNA decided to put a spin on #LifeGoals, encouraging people to share their #BrainLifeGoals.

Aims of #BrainLifeGoals

- To increase awareness of the impact neurological conditions have on patients.
- To engage those living with brain disorders to advocate for better awareness.
- To showcase patient examples and show what is important to them and how this should steer decision-making and research and development.
- To identify and communicate unmet patient needs.

CAMPAIGN ENGAGEMENT AND PLANS FOR THE FUTURE OF #BRAINLIFEGOALS

EFNA are utilising the huge audience that social media offers and encouraging people living with brain disorders to get involved. Patients starting using the hashtag, sharing goals such as "Drive a car without pain #BrainLifeGoals" and "To live one day without thinking about my illness #BrainLifeGoals".[1] This method is helping to bring some public understanding to the neurological diseases these patients live with, as well as heightening awareness.

Some of EFNA's affiliated organisations also made use of the campaign, such as the International ME conference held in London, UK, in May 2019, which brought the campaign to attendees and encouraged them to share their #BrainLifeGoals.

The social media campaign is growing and on World Brain Day, EFNA will hold events in Brussels, Bucharest, Dublin, and Warsaw, where they will partner with patient organisation to raise awareness on the issues that are important to patients. They will also be working towards what can be done surrounding policy at both National and European Union (EU) levels to increase awareness of neurological disorders and get better results for patients.

EFNA are also offering grants to five projects to further the reach of this campaign, in which entrants must reflect the campaign themes, raise awareness of the neurological disorders, and improve understanding surrounding these in European countries.[2]

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Congress Interviews

Find out more about EAN from members of two of the important committees.

Featuring: Prof Antonio Federico and Prof Riccardo Soffietti

Prof Antonio Federico

Chairman of the EAN Task Force for Rare Neurologic Diseases

Past Chairman of the Scientific Committee of the European Academy of Neurology (EAN), Professor of Neurology, University of Siena, Siena, Italy, and Editor in Chief of Neurological Sciences.

Q1 What do you find the most inspiring aspect of working in neurology?

Neurology is the most important part of medicine because the nervous system co-ordinates all the other organ functions. Working in this field provides the opportunity to interact with clinics in addition to researchers in science and the humanities.

Q2 Throughout your time in the field, what is the biggest change you have seen in neurology?

The possibility to visually enter the brain through the use of new neuroimaging machines, which offers the possibility to learn about the basic mechanisms of nerve-nerve-other cell interactions. This is a huge change as they are the basis of many neurodegenerative diseases.

Q3 There are more than 5,000 rare diseases which the European Academy of Neurology (EAN) describe as a Pandora's box of diseases. How do you begin to tackle such a vast number of diseases?

There is a predicted minimum of 5,000 rare diseases, and of these around 50% have a neurological involvement. It is impossible to have a good knowledge of all of them; however, there are many websites that are very useful for the suggestion of diagnostic hypotheses, which then need laboratory confirmation.

Q4 What do you think will be the biggest challenge for the EAN Task Force in raising awareness and improving knowledge of rare neurological diseases?

Interest on this topic has been growing over the last few years. There have been two reasons for this. Firstly, for the interest of patients, who often are reliant on orphan drugs. Secondly, to enable doctors to take better care of such patients.

The Task Force will aim to further increase the interest of these diseases in the neurology community in Europe. Furthermore, they will

try to improve the care in many areas of Europe where the diagnosis and treatment of this group of diseases is not well-established or developed.

Q5 The EAN Task Force has many aims and objectives. Which of these has the Task Force already achieved and which are proving to be more challenging?

Amongst other successes, they have already been able to increase interest in rare neurological diseases in the younger generation from two perspectives: seeking to gain an understanding of the scientific mechanisms of the diseases, and developing new models of patient care.

The more challenging objective is to try to decrease the differences existing across the European countries. In fact, many differences are present between Western and Eastern European Countries, regarding the organisation of rare neurologic disease care; drug availability; facilities for diagnosis, because few molecular genetic laboratories exist; neonatal screening; plus more. I think that the recent organisation by European Union (EU) of the European Reference Networks (ERN) will be able to decrease the differences and establish several facilities for diagnosis and care. Some of the networks are mainly dedicated to rare neurological diseases: ERN rare neurological diseases, with special focus on neurodegenerative diseases, such as movement disorders, ataxia, choreas, atypical parkinsonism, leucodystrophies, dystonias, spastic paraparesis, frontotemporal dementias, and more; ERN neuromuscular diseases, focusing on rare forms of diseases of muscle and nerves, also including motor neuron diseases; and ERN rare forms of epilepsies.

Q6 You recently co-authored the paper 'Changes in grey matter volume and functional connectivity in cluster headache versus migraine.' What would you say is the take home message from this paper?

That the measuring of the grey matter volume and functional connectivity may be a useful tool for improving the diagnosis, and treatment, of many neurologic diseases.

Q7 How do you think the efforts against rare neurological diseases in Europe compare to those around the rest of the world?

Europe is on the same level as other Western countries, even better if we consider that over the last 2 years a big interest has been developed by the EU with the organisation of the ERN. This virtual network connects all the best centres in Europe and is an important means to improve general care organisation for rare diseases, in addition to diagnosis and research.

Q8 Along with teaching at the University of Siena and chairing the EAN Task Force, you have authored over 400 journal articles. What motivates you to keep working in the field of neurology? What part of your job do you enjoy most?

I have been involved in many different jobs to promote neurology: at the national level, as President of the Italian Society of Neurology; at the European level, as chair of the EAN Scientific Committee; and at the global level, as a member of several research groups of the World Federation of Neurology (WFN). I will also be the president of the 2021 World Congress of Neurology (WCN), which will be held in Rome, Italy. I have also been very much in charge when involved in research, which was mainly in neurogenetic and neurometabolic diseases, whether that has been teaching neurology to students or caring for neurological patients. All these experiences gave me great enthusiasm and motivation, and the opportunity to learn more and more about the nervous system functions and dysfunctions and the possibility of treatments.

Q9 As someone who has worked extensively in the field of neurology, what advice would you give to someone starting out today?

To be curious, to recognise our limited knowledge, to be modest about but enthusiastic for the new possibilities given by new methodologies and technologies, and to think very critically.

Prof Dr Riccardo Soffietti

Chairman of the EAN Education Committee

Q1 How have your training and work experiences led you to focus your career on neuro-oncology?

At the beginning of my career, I was trained in the neuropathology of brain tumours at the University of Torino, where I grew up. Then, after the development of clinical neuro-oncology in the late 1970s, following an experience in the USA, I started my activity as a neuro-oncologist with an involvement in the medical treatments of gliomas and other primary brain tumours.

Q2 Alongside your clinical role, you are an advisor to the European Medicines Agency (EMA). Please could you outline your work with the EMA?

I have acted as a consultant for the EMA in the process of approval of new drugs, such as anti-vascular endothelial growth factors agents for glioblastoma multiforme and new drugs for brain metastases.

Q3 When interviewed in 2018 at the 4th EAN Annual Meeting, you discussed new classifications implemented by the World Health Organization (WHO) regarding brain tumours. What impact have these 2016 classifications had on work within the clinic?

The novel histologic-molecular classification of gliomas has changed, in a radical way, the management of Grade II and III gliomas (lower grade gliomas). It is now is based on the evaluation not only of clinical factors but also molecular factors such as isocitrate dehydrogenase (IDH) 1 and 2 mutations and 1p/19q codeletion.

Q4 The aforementioned classifications were published in 2016 and are now 3 years old. Do you think that another update is needed?

I agree. Now the process is to continuously update the 2016 Classification whenever new molecular data of prognostic significance are released. An example is the further subdivision of IDH 1/2 wild-type Grade II and III gliomas into low and high-risk categories.

Q5 Over the last year have there been any research developments or discoveries that suggest a new wave of successful therapeutics are on the horizon?

Regarding glioblastomas, a potentially active new treatment could be a new molecule (depatux) for epidermal growth factor receptor (EGFR)-amplified glioblastomas. This is a fusion molecule: a combination of a monoclonal antibody against EGFR and a toxin to microtubules. We are waiting on the final results of European Organisation for Research and Treatment of Cancer (EORTC) and Radiation Therapy Oncology Group (RTOG) studies. Regarding lower grade gliomas, the inhibition of IDH mutations by either small molecules or vaccination is promising.

Q6 Personalised medicine is of growing importance in healthcare. How translatable do you feel personalised approaches are to neuro-oncology? Do you think personalised protocols should be changed in any way to make them more advantageous for your patients?

Apart from O6-methylguanine-DNA methyltransferase methylation in glioblastoma of the elderly, thus far we have not personalised approaches in brain tumours. In the near future, some rare subgroups of primary brain tumours could be specifically targeted by BRAF inhibitors (such as gangliogliomas) or sonic hedgehog inhibitors (medulloblastomas).

Q7 You are the chair of the EAN Education Committee. What drove you to become involved with this aspect of the work of EAN? What is, in your opinion, the greatest achievement of the committee under your tenure?

I have been involved in EAN activities for several years, and the EAN Board decided last year to appoint me as chair of the education committee. Among the several activities that were already ongoing, two new projects will be launched: a revision of the European Core Curriculum for the Neurology residency and the development of an e-platform for both residency and continuing medical education.

"Rare brain tumours are of increasing interest, but neurological complications of systemic cancers (brain and leptomeningeal metastases, paraneoplastic neurological syndromes) are of utmost importance as well."

Q8 If you could direct the attention of the EAN to one neurological cancer, what would it be and why?

Rare brain tumours are of increasing interest, but neurological complications of systemic cancers (brain and leptomeningeal metastases, paraneoplastic neurological syndromes) are of utmost importance as well.

Q9 Lastly, if you could provide a new colleague with three pieces of advice for a successful career in neuro-oncology, what would they be?

1. To attend a residency in neurology at a site where neuro-oncology is part of the clinical training.
2. To get experience in neuropathology and translational research as well as in neuroimaging.
3. To obtain neuro-oncological experience in a centre of excellence in Europe or the USA to refine their skills for both daily practice and research.

The *EMJ Neurology* Editorial Board: A Roundtable Interview

Prof Nils Erik Gilhus

Professor in neurology at Department of Clinical Medicine, University of Bergen and senior consultant at Department of Neurology, Haukeland University Hospital, Bergen, Norway

Prof Emeritus Amos Korczyn

Professor of Neurology and Pharmacology
Tel Aviv University Medical School, Tel Aviv, Isreal

Q1 What fascinates you the most about neurology, and why did you decide to have a career in this field?

Prof Gilhus: The brain is a really fascinating organ: it is not simple at all and there are immense opportunities in trying to understand what is going on in the brain. Another part is that you really get to see the patients as part of your investigation and be in contact with them. The last thing I would say that drove me to have a career in this field is that, as part of my training that every doctor has to complete to get a licence, I worked in a very active neurology department; once I had just finished my studies at University, I went to another active department who actively participated in research and had a great clinic, this was also in neurology.

Prof Korczyn: The brain is the most fascinating organ in the body, the most complex one too. The only way to best learn about the function of the brain is to see what happens in people whose brain is ill and the dysfunction that has occurred. This will then tell us a lot about the normal function and the mechanisms and processes that are going on. I think if one wants to solve the problem of the brain, which maybe is not completely solvable at all (maybe the brain is too complicated for the brain to understand itself), then we need to study the dysfunction. This is what neurology is about, studying the brain and helping people.

Q2 This year, EAN is expected to host >6,000 neurologists, what are the benefits of congresses like EAN?

Prof Gilhus: I speak warmly towards the international aspect that meeting people from other countries brings. I think in Europe, and in the world, some countries are in conflict with other countries, some people are in political conflict with other people, and I think congresses like this are a really important contribution for neurologists to meet, to understand each other, to have sympathy for each other, and to unite.

Prof Korczyn: I believe that the main benefits of congresses are that people meet each other. So, it is not the formal lectures themselves, but the fact that you can communicate, debate, and discuss with other neurologists that makes meetings in general important and useful to me. Congresses are more of a teaching expedition as opposed to a research institution and therefore I do not think they would advance science but rather advance our understanding of science.

Q3 Neuroinflammation is this year's overarching theme at EAN, what developments have been made in this field since you started your career, and how will this understanding improve current and future therapies?

Prof Gilhus: I would say that there are some disorders that are defined as immunological disorders (autoimmune disorders) but in addition to these, you have all the other disorders that more or less have immunological aspects. I think this has been an important discussion point at this meeting, trying to find out to what degree Immunological mechanisms influence 'non-immunological disorders' such as neurodegenerative disorders, headaches, epilepsy, many more too. I think what is really interesting is the grouping of patients in diseases and the identification of specific biomarkers in subgroups of such patients. Additionally, that you can use these biomarkers to treat the patients by targeting them with monoclonal antibodies and now also with peptides. But you can find ways to interact with the biomarkers causing the disease in the whole patient group or often in subgroups of patients. Neuroinflammation in myasthenia gravis is a particular interest of mine, developments in this area have been made, for example the inhibition of complement is a new approach to treating the disorder and seems to be safe and very promising; it seems to influence a new part of the immune system.

Prof Korczyn: We have always considered inflammation as a protective process that occurs in the body. After all, during evolution inflammation developed to help the body recover from injuries. But, the study of inflammation has told us that it is very complex: sometimes it is protective but at other times it causes problems. We have also learned that quite a lot of the inflammation components, consisting of both the cellular part of it and the soluble mediators, can be lymphocytes, cytokines, or antibodies, each of which has a different function. This creates a complex picture that we need to understand.

Particularly over the past few years we learned a lot about inflammation in the brain and its involvement in different diseases. It turns out that inflammation occurs in many diseases of the brain and the question is, in which of them is the inflammation causative and in which is it trying to be curative or to suppress the disease. This is one of those difficult topics in which we try to find out what the function is. Maybe the function is not either protective or causing the damage but maybe a combination of both: maybe one arm does one thing and another arm does something else. All these things are being studied and we are learning more but we are far from a complete understanding.

"The brain is a really fascinating organ: it is not simple at all and there are immense opportunities in trying to understand what is going on in the brain. "

Q4 Are you working on any research projects at the moment; how does your current research differ to what/how you were investigating and experimenting 20 years ago?

Prof Gilhus: One of the differences now is that in the Nordic countries we have health registries and we have good patient cohorts. Recently, I have been focusing more of my efforts into examining and using data from National health registries. Additionally, my team and I have a joint Nordic project in which we use data from the Nordic Health Registries, combine it, and better define patient cohorts to produce them with very little selection bias. Furthermore, we are now combining the Nordic health registries with biomarker data and studies. For me, this has especially revolved around myasthenia gravis and epilepsy, and we are trying to identify good and viable biomarkers that could be linked to the health registries.

Prof Korczyn: We have a large prospective study called TABASCO, in which we monitor our patients who have had a transient ischaemic attack or stroke and try to see how they maintain their cognition; this might be related to inflammatory processes. In fact, we did find inflammatory markers that are associated with cognitive decline.

Q5 Biomarkers are increasingly being seen as the future of diagnosis and treatment monitoring; what potential do you believe they truly have?

Prof Gilhus: I think that biomarkers are very important and they seem to be very important when identifying groups of patients to try and identify a pattern of biomarkers that is not specific for a single patient but defining subgroups with more specific responses to therapy. This is also a challenge because if we say that the biomarker pattern is absolutely individual then you will have no study groups to look into. You must have groups with a certain number of patients included to be able to generalise the outcomes. However, I think that biomarkers lead to a higher specificity of treatment and better definition overall. For example, myasthenia gravis and multiple sclerosis are no longer one disorder: there are many subgroups and therapy has to be directed according to biomarker studies relating to subgroups. There are many ways of obtaining biomarkers, such as imaging from neurophysiology and of course from genetics, blood samples, cell markers, and more.

Prof Korczyn: Identifying a biomarker which reflects the disease activity is important because it may tell us about the cause of the disease. A good example of biomarkers is in migraines, in which we found a substance (CGRP) that is related to the appearance of a migraine attacks. Now we have developed antibodies against this substance which suppresses the migraine activity. The biomarker in this example was helpful in identifying a potential therapeutic. In another study, we identified biomarkers that are associated with neurodegeneration. For example, neurofilament light chains, which tell us that there are some degenerative processes occurring in the brain, can be used as a biomarker to monitor disease progression and more importantly to monitor the activity of drugs: whether an intervention is helping or not. In studies, we sometimes need to include numerous patients and monitor them for many years to see the clinical changes but if we can do this using a biomarker then this would make the process easier, cheaper, and faster.

Now, of course, it's not the whole story and we don't know when we have to prove in each case whether the biomarker can accurately tell us what is going on in the body, what is it marking?

Q6 How have recent advances in technology helped research and patient care? Do you have any examples that have been approved for clinical use?

Prof Gilhus: Although I am not particularly involved in technology, one aspect of my current research that is related to technology is big data. All the biomarker studies produce an immense quantity of data; new ways of recording and storing data is very important. To handle these data is technologically challenging, yet the way we handle it is very important: we want to ensure we are getting the most out of the data we produce. We work with a company who are applying the big data technology to the EEG data from our studies. They collect EEG data in a systematic way then they try to make the data meaningful for the clinicians for the diagnosis of epilepsy.

Prof Korczyn: I think that technological advances are a very important piece in the case of diseases of the brain because the brain is protected from the rest of the body by the blood-brain barrier. This means that when drugs are given to a patient

they do not necessarily arrive at the right time or in the right place where we have targeted them to go, in an effective concentration. There have been many attempts to overcome this by changing the way that the drug is given. Maybe it should not be administered orally, but transdermal to let the drugs get to the desired place of action more easily. Attempts to try to open the blood-brain barrier so that drugs can enter the brain and have an effect have been done and some successes have been reputed.

Q7 Currently, what are biggest challenges that are being faced in neurology?

Prof Gilhus: Basically, we need to find better treatments. For many disorders we have very good treatment options but there are very few disorders that we are able to cure. The ideal situation would be to be able to cure the diseases, or even try to prevent them: that is always a challenge. Usually, improvements in treatment come in steps; we have to take one step at the time. Again, myasthenia gravis and epilepsy are the diseases I am most interested in and we have very good treatment for them. We have a very good understanding of the diseases but when it comes to the question that all patients ask: 'what causes the disease?' We don't know. But I think what is true for nearly all diseases is that treatment is not 100% effective and many improvements on the understanding and treatment of all diseases can, and needs to, be made.

"I believe that the most important challenge is to understand 'how does the brain form the mind'?"

Prof Korczyn: I believe that the most important challenge is to understand 'how does the brain form the mind'? We believe that the mind is an activity of the brain but it is different from the brain itself. The brain acts according to principles that we understand in neurons, axons, and synapses, and all of these are what can be described and understood in terms of physical and chemical processes. But when we talk about the mind, this does not apply anymore; somehow, the physical chemical processes change into a new entity that we call the mind. How this happens and what the underlying processes are is something that we do not understand at all. This is the most important secret nature has kept from us.

Q8 What advice would you give to young neurologists just starting their career, and what opportunities should they try and make the most of?

Prof Gilhus: They should work hard and have fun. I think for me they are the two most important things. I believe it is important to always try to make the most of the possibilities where you are. When you are starting a department, hospital, or office you should take full advantage of those opportunities that are there. Find and network with those who are the best at their role and go to the best activities on offer. In addition, try to be an Internationalist. Travel and try to cooperate and meet people at congresses such as EAN. Seek out young people and try to make scientific contact to improve treatment but also for fun and for widening your views on right and wrong, be open-minded.

Prof Korczyn: I think the most important thing is that now when technology takes so much forefront, they should not forget the basis. We have this acronym of VOMIT: Victim Of Modern Imaging Technology. We apply this frequently to patients in whom by chance we find something that was not relevant to their disease and its discovery is not helpful to the patient; now that it has been observed, this is probably a matter of concern to the patient but it does not always improve their care and prognosis. VOMIT in general also applies to the physician because many of the physicians pay too much attention to the pictures that they see on their screens and forget about the patient. We believe that this is something that should be resisted and that we should understand that the important thing, namely the patient; we cannot overcome this by looking at imaging pictures. We always have to start with the patient and end up with the patient, and all the technologies and laboratory techniques can be helpful along the way but should not affect us from looking at the human being who came to us to get help.

Optimism and Opportunities with Anti-CGRP Biologics in Migraine: Where Are We Today?

This symposium took place on 30th June 2019 as part of the Teva-sponsored satellite symposium at the 5th European Academy of Neuroscience (EAN) Congress in Oslo, Norway

Chairpeople: Messoud Ashina[1]

Speakers: Anthony Dickenson,[2] Zaza Katsarava,[3] Patricia Pozo-Rosich[4]

1. Department of Neurology, Danish Headache Center, Rigshospitalet Glostrup, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
2. Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK
3. Evangelical Hospital Unna, Unna, Germany; Department of Neurology, University of Duisburg-Essen, Essen, Germany; EVEX Medical Corporation, Tbilisi, Republic of Georgia; IM Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russian Federation
4. Headache Unit, Neurology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain

Disclosure: Prof Ashina has been a principal investigator for trials sponsored by Alder Biopharmaceuticals, Amgen, electroCore Medical, and Novartis and has received consultancy fees from Alder Biopharmaceuticals, Allergan, Amgen, Eli Lilly, Novartis, and Teva Pharmaceuticals. Prof Dickenson has served as a speaker for Teva Pharmaceuticals, Grunenthal, and Allergan; as a consultant to Janssen; and on the advisory boards of Regeneron, Sandoz, and Teva Pharmaceuticals. Prof Katsarava has received honoraria from Allergan, Novartis, Eli Lilly, Merck, and Teva Pharmaceuticals. Dr Pozo-Rosich has received honoraria as a consultant and speaker for Allergan, Almirall, Chiesi, Eli Lilly, Novartis, and Teva. Her research group has received research grants from Allergan and has received funding for clinical trials from Alder, Electrocore, Eli Lilly, Janssen Cilag, Novartis, and Teva.

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Meeting Summary

In his opening remarks, Prof Ashina explained the theme behind the Teva-sponsored satellite symposium: to inform the audience about the science behind the emergence of calcitonin gene-related peptide (CGRP) as a target for migraine prevention, the clinical evaluation of anti-CGRP monoclonal antibodies (mAb), including the latest clinical data on fremanezumab leading to its licensure, and the importance of considering the patient experience when initiating anti-CGRP treatment. Prof Ashina also highlighted the greatest unmet needs with respect to current migraine management, ranging from underdiagnosis and underutilisation of preventive therapies,

suboptimal efficacy and tolerability of existing medications, poor adherence, comorbidities, and migraine-related disability. Prof Dickenson introduced the audience to the identification of CGRP as one of the key mediators of migraine pathophysiology and nociception. He then delineated central and peripheral pathways in which CGRP plays a role in the neurovascular processes associated with migraine to show why anti-CGRP interventions hold the promise for better preventive therapies against migraine. Prof Katsarava stated the shortcomings of current preventive therapies and highlighted low adherence to current chronic treatment. He then showcased the clinical data from the HALO and FOCUS trials, which found fremanezumab to be a good candidate for migraine preventive therapy. Finally, Dr Pozo-Rosich discussed the importance of a patient-oriented approach when deciding which is the right treatment for the right patient, noting that this is a component of both personalised and precision medicine. She also added that before evaluating the benefits of or commencing preventive migraine treatment, both the patient perspective and the experience of the physician should be taken into consideration.

Anti-CGRP Biologics: A New Era for Migraine Prevention

Professor Anthony Dickenson

Migraine is a complex neurological disorder with an approximate global prevalence of 15%.[1] It is characterised by a moderate-to-severe unilateral headache that is aggravated by routine physical activity and is also generally accompanied by photo and phono-sensitivity, nausea, and vomiting. The impact of migraine on physical, social, and occupational functioning is reflected in it being the leading cause of neurological disability, as well as one of the top five causes of chronic disability.[1,2] While the pathophysiology of migraine is still not fully understood, one of the key mediators involved has been shown to be CGRP, a 37-amino acid peptide primarily localised to the C and A∂ sensory fibres that are widely present in the body and have nociceptive as well as effector functions.[3]

Prof Dickenson began his presentation with a brief timeline of the discovery of CGRP[4,5] and its identification as a potential target for migraine therapy, before proceeding to brief the audience on the neurophysiological basis of migraine and what has been discovered so far. Migraine is thought to be a disorder of sensory processing in the brain, involving both the central and peripheral systems, and characterised by generalised neuronal hyperexcitability.[6] The symptomatology of migraine is complex, suggesting abnormal functioning in multiple neuronal systems, including those in the brain stem and diencephalic regions, which results in premonitory symptoms. The subsequent involvement of the dural trigeminovascular system is manifested as the pain phase of migraine. The central sensitisation hypothesis proposes that altered processing of sensory inputs in the trigeminal nucleus caudalis could account for numerous temporal and symptomatic characteristics of migraine.[7] In addition, peripheral sensitisation is thought to contribute to migraine, especially in conditions in which the threshold for stimulation of the peripheral sensory neurons is reduced,[7] through irritation of peripheral trigeminal fibres by inflammatory mediators,[7,8] or the release of CGRP in the periphery, which can amplify and sustain inflammatory responses. Such conditions are known to also sensitise peripheral nociceptive neurons.[9]

While CGRP is proposed to have various functions in normal physiology and pathology, its role as a vasodilator in the cardiovascular system is well known and there has been a substantial amount of research to explore its somatosensory function in modulation of neuronal sensitisation and pain.[10] Evidence indicates that CGRP is involved in the development of peripheral sensitisation and the enhanced pain associated with it. CGRP has also been found to be upregulated in inflammatory processes and neuropathic pain, hinting at a role in neurogenic inflammation. CGRP is thought to contribute to the development and maintenance of a sensitised neuronal condition at the primary afferent sensory neurons, as well as secondary pain transmission neurons in the central nervous systems, thereby contributing to central sensitisation as well. Such a hypersensitised neuronal state is a key factor underlying migraine pathophysiology.

Prof Dickenson further explained the models postulating the role of CGRP in peripheral and central sensitisation as stated in Iyengar et al.[10] and showed that CGRP is a key signalling molecule at both ends of pain fibres.[11,12] Furthermore, Prof Dickenson shared results published by McCoy et al.[13] which have shown that CGRPα-expressing sensory neurons terminate in the dorsal spinal/trigeminal cord and respond to noxious stimuli that evoke pain and itch sensation.

Given the role played by CGRP in both the central and peripheral processes associated with migraine, Prof Dickenson concluded that mAb targeting CGRP at sites on the dura, the peripheral neurons, or the trigeminal ganglia offer the potential for more effective migraine prevention.

Changing Pathways, Changing Lives: Taking Control of Migraine Development

Professor Zaza Katsarava

Depending on their frequency, migraines are classified as episodic or chronic.[14] In episodic migraine, patients experience headache on <15 days per month, while chronic migraine is characterised by ≥15 headache days per month for at least 3 months; with headaches bearing migrainous features for at least 8 of those days in both cases. The pharmacologic treatment of migraine depends on the frequency of headaches and may be either acute (abortive) or preventive (prophylactic).[15] Preventive therapy has been shown to possess numerous benefits. Besides reduction in frequency, duration, and severity of attacks,[15] preventive treatments may also enhance response to acute treatments, reduce patient disability,[16] and even result in reduction of healthcare costs associated with migraine.[17]

Despite established guidelines, a large proportion of patients do not receive preventive therapy, even in developed countries, an issue Prof Katsarava illustrated with the findings of the Eurolight study.[18] In addition, he emphasised the poor adherence to oral preventive treatments in migraine patients.[19] These factors, compounded further by inadequate efficacy and tolerability of current oral preventive therapy for migraine, have driven the search for new treatments. The most promising of which are the CGRP-targeting mAb, which specifically target key pathways in migraine and have an acceptable safety and efficacy profile. Following a series of clinical evaluation programmes, currently three anti-CGRP mAb (erenumab, fremanezumab, and galcanezumab) have been approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), while eptinezumab is currently awaiting licensure.[20,21] Prof Katsarava summarised the landmark clinical trials for these mAb, as well as their salient target and pharmacokinetic properties, before proceeding to discuss the results of the HALO trial programme for evaluation of fremanezumab.

HALO EM was a multinational, randomised controlled trial (RCT) conducted across 123 sites in 9 countries between March 2016 and April 2017.[22] The objective of this study was to assess the efficacy of subcutaneous fremanezumab, administered monthly over 12 weeks or as one single higher dose (intended to support quarterly dosing), in preventing episodic migraine attacks in patients in whom multiple migraine medication classes had not previously failed. The study recruited a total of 875 patients (fremanezumab monthly, n=290; fremanezumab quarterly, n=291; placebo, n=294) and the primary endpoint was the mean change from baseline in the monthly average number of migraine days during the 12-week intervention period after the first dose. Results from participants who completed the HALO EM study showed that the least-squares mean reduction in the average number of monthly headache days was 3.9 days with fremanezumab single higher dose (n=288), 4.0 days with fremanezumab monthly (n=287), and 2.6 with placebo (n=290) ($p<0.001$ for both comparisons with placebo). Moreover, in comparison to those receiving placebo, patients in the fremanezumab groups had a decrease in the monthly average number of migraine days, with both monthly dosing (difference: -1.5 days; 95% confidence interval [CI]: -2.01 to -0.93 days; $p<0.001$) and the single dose (difference: -1.3 days; 95% CI: -1.79 to -0.72 days; $p<0.001$) regimens. In addition, the proportion of patients achieving a reduction of ≥50% in the monthly number migraine days over 12 weeks with fremanezumab monthly, fremanezumab single higher dose, and placebo was 47.7%, 44.4%, and 27.9%, respectively ($p<0.001$ for both fremanezumab doses versus placebo).

HALO CM was a multinational RCT conducted across 132 sites in 9 countries between March 2016 and January 2017.[23] Similarly to HALO EM, the objective of this study was to assess the efficacy of subcutaneous fremanezumab, administered quarterly or monthly over 12 weeks, in preventing chronic migraine. The study recruited 1,130 patients (fremanezumab quarterly, n=376; fremanezumab monthly, n=379; placebo, n=375) and the primary endpoint was the mean change from baseline in the average number of monthly headache days of at least moderate severity during the 12-week intervention period. In those who completed the trial, the least-squares mean (±standard error) reduction in the average number of monthly headache days was 4.3 (±0.3) with fremanezumab quarterly (n=375), 4.6 (±0.3) with fremanezumab monthly (n=375), and 2.5 (±0.3) with placebo (n=371) ($p<0.001$ for both comparisons with placebo). In addition, the proportion of patients achieving ≥50% reduction in the monthly number of headache days of at least moderate severity over 12 weeks for fremanezumab quarterly, fremanezumab monthly, and placebo was 38%, 41%, and 18%, respectively ($p<0.001$ for both fremanezumab doses versus placebo).

Having detailed the findings of the HALO trials, Prof Katsarava emphasised the need for long-term efficacy and safety data to inform decision-making before introducing data from the HALO Long-Term Study, which had recently been presented at the 13th European Headache Federation (EHF) Congress 2019 in Athens, Greece. These data showed sustained reductions in the average number of monthly migraine days over 12 months in patients with both episodic and chronic migraine.[24] In addition, the average number of patients with episodic and chronic migraine who achieved a reduction of ≥50% in the average number of monthly migraine days was sustained.[25] Prof Katsarava then proceeded to share unpublished data from the FOCUS study, an RCT that assessed the efficacy of monthly or quarterly fremanezumab over 24 weeks in difficult-to-treat patients, defined as those with episodic and chronic migraine who had previously failed to respond to 2–4 classes of preventive treatment. Results from the FOCUS study not only reiterate previous findings by showing a significant reduction in the average number of monthly migraine days in fremanezumab-treated patients compared with placebo,[26] but also showed a rapid change in the average number of weekly migraine days[27] and a decrease in the use of acute headache medication in the fremanezumab groups.[26] In addition, fremanezumab demonstrated a favourable long-term safety and tolerability profile with low treatment discontinuation rate due to adverse events.

Prof Katsarava concluded his presentation by summarising the acceptable safety and efficacy of anti-CGRP therapies and their advantages for use as preventive treatment for migraine.

The Right Treatment for the Right Patients

Doctor Patricia Pozo-Rosich

Multiple evidence-based guidelines recommend preventive therapy as part of the overall approach to migraine management. Preventive treatment is especially recommended for patients with frequent attacks (starting at ≥4 monthly headache days); patients in whom migraine substantially interferes with daily activities despite acute treatment; or those in whom acute treatments are contraindicated, ineffective, or lead to overuse.[15,28-30] Because the currently available oral preventive treatments were not designed for treating migraine, they offer a suboptimal efficacy and tolerability profile, and their use is limited by contraindications and drug interactions.[30] These factors may explain why the proportion of migraine patients who use preventive therapies is low, despite many being candidates for a preventive approach.[31,32] To optimise use of preventive therapy, it is necessary to individualise treatment based on severity and frequency of attacks, presence of other comorbidities and associated symptoms, type and severity of disabilities, contraindications and concomitant medications, and just as importantly, patient preference.

Dr Pozo-Rosich's presentation focussed on the importance of taking into account the patient perspective when considering preventive treatments for migraine. The common goal of migraine treatment, from the physician's and patient's perspectives, is to improve patient

quality of life and minimise disease burden. Hence, the choice of the intervention needs to be viewed from both these perspectives for it to be successful. Dr Pozo-Rosich stated that while personalised medicine allows for this to some extent, this process needs to be fine-tuned by improving patient–physician communication and practicing precision medicine. Patient factors, such as demographics, age, sex, and diagnosis are usually taken into account by the treating physician when recommending particular preventive treatments in migraine; however, other factors, such as lifestyle and work, the presence of comorbidities, and response to previous treatments, also need to be taken into account in selecting an appropriate treatment.

Dr Pozo-Rosich further stated that this approach of acknowledging the patient perspective needs to be implemented at the clinical evaluation phase to identify patient factors that could have a bearing on the appropriateness of preventive treatments. Moreover, besides objective diagnosis based on clinical evaluations, physicians also need to take into consideration how patients feel about prescribed treatments despite this being a subjective issue. To illustrate this, Dr Pozo-Rosich first presented data from a study by Mitsikostas et al.,[33] which evaluated patient preferences in the acute and preventive treatment of headaches. When study patients were asked whether safety, efficacy, or route of administration were important to them with respect to symptomatic treatment, >80% of the patients with migraine listed efficacy as the factor most important to them. Gathering such feedback from patients is essential in understanding the factors that they regard as important in their treatment. While direct face-to-face communication is helpful in understanding these, this process could be improved by the use of standardised algorithms to gather patient-reported outcomes (PRO). Though PRO are useful in gauging patient perception, numerous factors such as the appropriateness of an outcome being reported need to be examined when designing the PRO questionnaire. To demonstrate this, Dr Pozo-Rosich listed the various instruments used to collect PRO during the clinical evaluation of CGRP mAb,[34] as well as data from the fremanezumab trial programme, which could be evaluated according to patient factors such as age, sex,[35] diagnosis,[36] and even comorbidities, such as depression.[37] Dr Pozo-Rosich added that although these data give us some insights into the influence of some patient factors, there remains much to be learned. One key approach would be to find better ways of evaluating what the term 'efficacy' means for each patient in order to first choose a preventive treatment, and then evaluate whether the treatment is effective or not before deciding about continuing or stopping treatment.

Dr Pozo-Rosich concluded her presentation by reiterating the importance of harmonising the patient–physician perspectives and stating that, in the future, prognostic factors will need to be defined in order to ensure that "the right treatment is offered to the right patient at the right time."

Conclusion

CGRP plays a key role in the peripheral and central pain mechanisms involved in migraine and its chronification. Hence, CGRP is a rational target for designing biological or chemical agents for migraine prevention. The discovery of mAb that bind either CGRP or its receptor, and subsequently prevent the activation of downstream pathways that are intrinsic to migraine, offer improved outcomes for those with both episodic and chronic migraine. Phase III trials with anti-CGRP mAb have shown promising results with a reduction in the number of migraine days and use of acute migraine medications, a rapid onset of action for some patients, and an acceptable safety profile. The successful integration of anti-CGRP biologics in preventive treatment of migraine will rely on adopting a precision medicine approach in the informed clinical decision-making process where patient preferences will also be taken into account.

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Abstract Reviews

Here, you will find some a collection of hand-selected abstracts that were presented at EAN this year, covering diseases such as Charcot-Marie-Tooth, amyotrophic lateral sclerosis, migraine, plus more.

Nuclear Factor Erythroid 2-Related Factor 2 as a Gene Modulator of Response to Oxidative Stress in Amyotrophic Lateral Sclerosis

Authors: Annalisa Lo Gerfo,[1] Costanza Simoncini,[1] Lucia Chico,[1] Vincenzo Montano,[1] Cecilia Carlesi,[1] Filippo Baldacci,[1] Simona Daniele,[2] Maria Rosaria Maluccio,[3] Giulia Ricci,[1] Michelangelo Mancuso,[1] and Gabriele Siciliano[1]

1. Department of Clinical and Experimental Medicine, University of Pisa, Italy
2. Department of Pharmacy, University of Pisa, Italy
3. Clinic of Neurology, Department of Clinical Medicine, ATNO, Italy

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Keywords: Amyotrophic lateral sclerosis (ALS), Nrf2–ARE pathway, oxidative stress (OS), therapeutic target.

Citation: EMJ Neurol. 2019;7[1]:40-41 Abstract Review No. AR1.

BACKGROUND AND AIM

Among the pathogenic mechanisms on motor neuron degeneration leading to amyotrophic lateral sclerosis (ALS), the oxidative stress (OS) theory has been put forward.[1] Although it remains hard to understand whether or not OS is the cause or effect of the disease, the association between oxidative damage and the disease makes therapeutic targeting of the antioxidant systems an attractive option.[2,3] Nuclear factor Erythroid 2-related factor 2 (Nrf2)–anti-oxidant response elements (ARE) pathway is a primary sensor and a master regulator of OS via its ability to modulate the expression of hundreds of antioxidant genes.[4,5] Considering the pivotal defensive role exerted by the Nrf2–ARE pathway (demonstrated in animal models of many neurodegenerative disorders), it is evident that the dysregulation of Nrf2-regulated genes offers a possible explanation for the direct and indirect association between OS and ALS.[6-8] This work was aimed to evaluate a possible association between -653 A>G, -651 G>A, and -617 C>A functional polymorphisms in the *NRF2* promoter gene with the NRF2 mRNA and OS biomarkers in ALS.

RESULTS

Analysis of 150 ALS patient's data showed that the allelic -653G variant is associated with increased risk of disease (odds ratio: 1.71; 95% confidence interval: 1.18–2.48); in relation to the polymorphisms -651 G>A and -617 C>A, no significant differences have been found in either the genotypic distribution or in the allelic frequencies of patients with ALS compared to the controls. The evaluation of peripheral OS biomarkers showed a significant increase in advanced protein oxidation products (AOPP) levels ($p<0.001$) and a significant decrease in thiol groups (-SH) levels ($p<0.01$) in ALS patients; the authors did not find any imbalance in the Iron-reducing capacity of plasma (FRAP) level of ALS patients compared to controls. mRNA expression in ALS lymphocytes carrying -653 AG or -653 GG genotype was significantly decreased ($p>0.05$) compared to wildtype (AA) carriers at this position. Finally, the data obtained showed a correlation between the -653G variant, mRNA expression level, and OS biomarkers in ALS patients.

CONCLUSIONS

The data obtained suggest that the -653G variant in Nrf2 promoter gene can be a risk factor for ALS. This variant is associated to decreased level of Nrf2 mRNA as evaluated in peripheral lymphocytes. All together these data reinforce the statement that the Nrf2–ARE pathway can be one of the pathogenic molecular mechanisms to be considered in motoneuron neurodegeneration in ALS. Conclusive remarks can be assumed in terms of relevance of OS events as integral part of the pathogenic complex of this disease.

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Paediatric Migraine: A Multidimensional Disorder

Authors: Yasser A. Elheneedy,[1] *Wafik S. Bahnasy,[1] Shereen A. ELahwal,[1] Reham A. Amer,[2] Shereen D. Abohammar,[2] Heba A. Salem[3]

1. Department of Neurology, Tanta University, Tanta, Egypt
2. Department of Psychiatry, Tanta University, Tanta, Egypt
3. Department of Chest Diseases, Tanta University, Tanta, Egypt

*Correspondence to wafiq.elbahnasi@med.tanta.edu.eg

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Keywords: Child behaviour checklist, paediatric migraine, PedMIDAS, polysomnogram.

Citation: EMJ Neurol. 2019;7[1]:41-42 Abstract Review No. AR2.

BACKGROUND

Paediatric migraine is a common medical problem with consecutive impairment in psychosocial development.[1-3] Concurrently, school-age children with migraine (SCM) commonly

experience sleep disturbances in the form of insufficient sleep, bedwetting, parasomnias, and excessive daytime sleepiness which negatively influence their school performance.[4,5]

OBJECTIVES

This study aimed to investigate the existence and types of sleep and psychiatric abnormalities in SCM.

METHODS

The study included 40 SCM and 20 age and sex-matched healthy control subjects submitted to headache induced disability assessment used the Pediatric Migraine Disability Assessment questionnaire (PedMIDAS). Psychiatric assessments were done using the Arabic-translated and validated versions of the Child Behavior Checklist (CBCL), and sleep assessment used the Epworth Sleepiness Scale for Children and Adolescents (ESS-CHAD) as well as one-night polysomnography.

RESULTS

SCM showed higher incidence of psychiatric disorders than healthy control subjects. The most common psychiatric disturbances included anxious depressed symptoms, withdrawal depressed symptoms, social problem, somatic complaints, and attention problems. At the same time, SCM experienced excessive daytime sleepiness as well as polysomnography abnormalities in the form of decreased total sleep time and sleep efficiency, in addition to increased sleep latency, wake after sleep onset, arousal index, and rapid eye movement sleep without atonia index. The headache-induced disability measured by PedMIDAS was positively correlated with each of wake after sleep onset, sleep stage transition index, arousal index, as well as periodic limb movement index, and negatively correlated with total sleep time and sleep efficiency.

CONCLUSION

Sleep and psychiatric abnormalities are common paediatric migraine comorbidities greatly reducing headache control and school performance in a very important period of psychosocial development.

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Spanish Normative Data for TMA-93 (Binding by Images): Descriptive Analysis

Authors: *Emilio Franco-Macías, Silvia Rodrigo-Herrero, Pablo Baena, Eugenia Graciani-Cantisán, María Bernal Sánchez-Arjona, Andrea Luque-Tirado, Ana Baquero-Ramírez, María Dolores Jiménez-Hernández

Department of Neurology, Hospital Virgen del Rocío, Seville, Spain
*Correspondence to efranco17@gmail.com

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Keywords: Binding memory, normative study, Spanish, TMA-93.

Citation: EMJ Neurol. 2019;7[1]:42-43 Abstract Review No. AR3.

Table 1: Normative data stratified by age and education for TMA-93 total scores.

Age (years)	Educational attainment	n	P_5	P_{10}	P_{25}	P_{50}	P_{75}	Range
<65	<First grade	82	21	24	28	30	30	20–30
≥65	<First grade	210	19	21	25	28	30	17–30
<65	First grade	168	24	25	28	29	30	18–30
≥65	First grade	188	20	23	26	29	30	18–30
<65	>First grade	251	26	27	29	30	30	19–30
≥65	>First grade	192	22	25	28	29	30	16–30

BACKGROUND AND AIM

TMA-93 is a French memory test that examines binding by images.[1] Drawings of familiar objects of everyday life are displayed in semantically related pairs during the encoding phase; participants must recall the missing drawing when the associated one is provided. This retrieval phase is repeated up to three times to assess participants' learning abilities.[1] The maximum total score is 30 out of 30.[1] The aim of this study was to report the preliminary results of an ongoing normative study from Spanish population for the test.

MATERIALS AND METHOD

Partners of patients who attended the Memory Unit, Hospital Virgen del Rocío, Seville, Spain, from July 2018 to April 2019 were invited to participate. These patients were aged ≥50 years old; had no memory complaints; scored ≥10th percentile on Phototest, a short cognitive test with normative data in Spain; and gave informed consent. Age (<65, ≥65), educational attainment (<first grade, first grade, or >first grade), and gender were considered as sociodemographic variables. A stratified analysis by combinations of significant socio-demographic variables was performed.

RESULTS

In total, 1,091 subjects were included from July 2018 to April 2019. Total scores for TMA-93 showed a non-normal, left asymmetric, and leptokurtic distribution (P_5=22, P_{10}=24, P_{25}=27, P_{50}=29, P_{75}=30). There were significant differences between groups according to age and educational attainment, so a stratified analysis by these sociodemographic variables was undertaken (Table 1).

CONCLUSIONS

Total scores for TMA-93 show a non-normal, left asymmetric and leptokurtic distribution in Spanish people aged ≥50 years old. The test works with a ceiling effect that is mitigated by older age and lower educational attainment.

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Benefit of Coenzyme Q10, Riboflavin, Petasites, and Relaxation in Migraine Prophylaxis: A Randomised Controlled trial

Authors: *Anastasia Bougea, Nikolaos Spantideas, Ioanna Spanou, Paraskevi-Zacharoula Katsika, Panagiota Voskou, Dimos D Mitsikostas, Evangellia Kararizou

1st Department of Neurology, Eginition Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece
*Correspondence to annita139@yahoo.gr

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Keywords: Complementary and alternative medicine (CAM), migraine, nutraceutical.

Citation: EMJ Neurol. 2019;7[1]:44-45 Abstract Review No. AR4.

Migraine headache is one of the most common and debilitating neurological disorders. According to the European Federation of Neurological Societies (EFNS) guideline, prophylactic treatment should be considered ''when the quality of life is severely impaired, when two or more attacks occur per month, when migraine attacks do not respond to acute therapy, or in case of frequent, very long, or uncomfortable auras.''[1] However, patients are often dissatisfied due to ineffectiveness, cost, and side effects of conventional drugs. Therefore, there is a high demand of non-pharmacologic alternatives for migraine prophylaxis.

Complementary and Alternative Medicine (CAM) is a group of diverse medical and healthcare systems, practices, and products that are not presently considered to be part of conventional medicine.[2] There is growing evidence of CAM in the prevention of migraine, including the use of nutraceuticals. Non-pharmacological therapies include behavioural treatments, physical therapies, and acupuncture. Nutraceuticals include vitamins, supplements (magnesium, riboflavin, coenzyme Q10, and alpha lipoic acid), and herbal preparations (feverfew and butterbur), and its usage is frequently determined by dissatisfaction with conventional medical therapies. Behavioural techniques comprise a series of strategies, including relaxation, thermal and electromyographic biofeedback, and cognitive behavioural therapy, which have been used in migraine therapy mostly with the aim of teaching patients to better cope with symptoms and identifying potential triggers for headache. Relaxation techniques include progressive muscle relaxation, autogenic training, and meditation.

Currently, there is increasing evidence for the efficacy and tolerability of these CAM approaches in the migraine prophylaxis.[3-6] Although these strategies may be used instead of traditional medications, using them in conjunction with conventional pharmacological therapies as part of a multidisciplinary treatment plan is more likely to result in an optimum response. The authors aim is to evaluate the prophylactic effect of the combination of nutraceuticals and relaxation methods in migraine prophylaxis.

The randomised-controlled trial was conducted at the outpatient department of the Headache Clinic of Eginition Hospital, Athens, Greece, between 2016 and 2017. Ninety patients meeting criteria for chronic migraine according to International Classification of Headache Disorders (ICHD)- 3 beta criteria were enrolled.[7] The study used a parallel-group design, with participants randomly assigned to the nutraceuticals group (n=20) (coenzyme Q10, riboflavin, Petasites hybridus, 400 mg each), relaxation group (n=20), combined group (n=20), and control group (standard care n=20) for 2 months. Inclusion criteria were: 1) male/female 18–75 years old; 2) diagnosis of migraine <50 years (ICHD-3beta); 3) 15–28 headache days/month, of which >8 met criteria for migraine. Exclusion criteria were: 1) other types of primary headache; 2) medication overuse; 3) failure of previous prophylaxis; 4) the use of migraine prevention for the past 2 months; 5) pregnancy. Patients were instructed to complete the diary at the end of each day, noting whether they had a headache and to record the mean intensity of pain on a 0–10 scale. There were no differences in headache-related and demographic characteristics between the groups at baseline.

At the 12-month follow-up assessment, both nutraceuticals and relaxation had significantly reduced headache frequency and intensity, and effects were maintained ($p<0.05$ for all). The combined intervention was significantly superior to either therapy alone ($p<0.05$).

Combination of coenzyme Q10, riboflavin, petasites, and relaxation could be beneficial and safe for the prevention of migraine. Large clinical trials are especially needed in this field and will hopefully allow a better understanding of headache disorders, as well as a more individual-based approach to treatment in the future.

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Action Observation Training Increases Dynamic Functional Connectivity in Patients with Multiple Sclerosis

Authors: Claudio Cordani,[1] Paola Valsasina,[1] Luca Gavazzeni,[1] Alessandro Meani,[1] Paolo Preziosa,[1] Federica Esposito,[2] Massimo Filippi,[1,2] *Maria A. Rocca[1,2]

1. Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy
2. Departement of Neurology, IRCCS San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy

*Correspondence to rocca.mara@hsr.it

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Keywords: Action observation training (AOT), hand motor impairment, multiple sclerosis (MS), time-varying functional connectivity.

Citation: EMJ Neurol. 2019;7[1]:45-47 Abstract Review No. AR5.

BACKGROUND

Motor disability has a high prevalence and dramatic effects on daily life activity of multiple sclerosis (MS) patients. Several rehabilitative strategies are currently available to treat these patients but often their efficacy is suboptimal and their benefits are generally not long-lasting. In patients

with neurological diseases, action observation therapy (AOT) has been proposed as an effective rehabilitative intervention for regaining motor function.[1,2] AOT is based on visual stimulation and it is thought to act through the function of the mirror neuron system, an observation-execution matching system.[3-6] In MS, functional MRI (fMRI) techniques provide valuable measures to monitor disease evolution and treatment effects and might increase our understanding of the mechanisms responsible for a favourable clinical outcome following rehabilitation. Recently, a novel fMRI analysis method has been introduced. Such a method allows a time-varying (dynamic) assessment of resting state (RS) functional connectivity (FC), thus capturing reoccurring patterns of interaction among intrinsic functional networks at rest.[7] In this present study, AOT was applied in right (R)-handed MS patients with motor impairment of their R hand to assess: 1) whether this strategy may lead to a clinical improvement of motor deficits; 2) changes over time of dynamic RS FC following AOT; 3) the correlations between RS FC changes and improvement at functional motor scales.

METHODS

Eighty-seven R-handed subjects were randomised into two experimental-groups (healthy control (HC)-AOT=23; MS-AOT=20) and 2 control (C) groups (HC-C=23; MS-C=21). The groups underwent 2-weeks training of ten 45-minutes sessions. AOT-groups watched three motor-task videos alternated by their execution; C-groups performed the same tasks watching landscapes-videos. At baseline (t0) and after 2 weeks, RS fMRI and clinical evaluations were performed, including Nine-hole peg test, finger tapping (FT), Jamar and Pinch dynamometers, the Expanded Disability Status Scale (EDSS) test, Paced Auditory Serial Addition Test, and Functional Independence Measure. Independent-component analysis identified 41 FC-networks assigned to the sensorimotor, default mode (DMN), attention, executive, visual, auditory, basal ganglia, and cerebellar networks (Figure 1). Between-group differences and dynamic RS FC changes over-time were evaluated using a dynamic approach, assessing FC on small temporal segments using sliding-windows, and grouping FC correlation matrices into recurrent FC-states. Correlations between clinical and MRI-measures were also assessed.

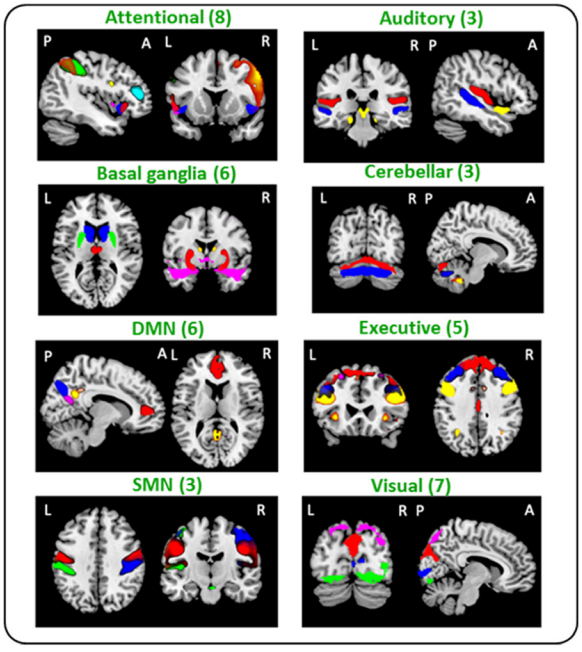

Figure 1: Composite map of the 41 identified independent components (IC) in all study subjects.
IC were sorted into eight subcategories (sensorimotor, default-mode, attentional, executive, visual, auditory, basal ganglia, and cerebellar networks).
A: anterior; DMN: default mode; L: left; P: posterior; R: right; SMN: sensorimotor.

RESULTS

At t0, no significant differences were found in the comparison between the AOT and the respective control groups in both HC and MS. Decreased dynamic RS FC was detected in MS patients versus HC, mainly between the basal ganglia and cerebellar networks/DMN (p range: <0.001–0.01). Increased dynamic RS FC among visual, executive, and attention networks was also detected (p range: <0.001–0.008).

Dynamic RS FC significantly increased after two weeks of training in both MS groups, with more evident effects in MS-AOT than in MS-C, involving sensorimotor, visual, basal ganglia, DMN, and attention networks (p range: 0.001–0.009). Conversely, both HC groups showed a decrease of dynamic RS FC at Week 2 versus t0, mainly for the sensorimotor, basal ganglia, cerebellar, and attention networks in HC-AOT, and DMN and attention network in HC-C (p range: <0.001–0.01) .

Both in MS-AOT and MS-C, significant correlations were found between dynamic RS FC modifications of sensorimotor, DMN, and attentional networks, and improvement of motor performance at FT and Pinch (r range: 0.49–0.77, p<0.05).

CONCLUSIONS

A 10-day manual dexterity training with AOT modulated dynamic RS FC of several functional networks, with stronger effects in the MS-AOT than in the MS-C group. Dynamic RS FC modifications correlated with concomitant motor performance improvements, suggesting an adaptive role of the detected RS FC increase. These findings might improve the understanding of the functional substrates underlying motor deficit recovery in MS patients and contribute to developing individualised treatment strategies.

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Baclofen, Naltrexone, and Sorbitol All Contribute to the Efficacy of PXT3003 in CMT1A Rats

Authors: *Rodolphe Hajj,[1] Thomas Prukop,[2] Stephanie Wernick,[2] David Ewers,[2] Anthony Brureau,[3] Nathalie Cholet,[3] Julien Laffaire,[3] Klaus-Armin Nave,[2] Daniel Cohen,[3] Michael Sereda[2]

1. Pharnext SA, Issy-les-Moulineaux, France
2. Max-Planck Institute for Experimental Medicine, Department of Neurogenetics, Göttingen, Germany
3. Pharnext SA

*Correspondence to rhajj@pharnext.com

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Keywords: : Charcot-Marie-Tooth type 1A, combination therapy, peripheral neuropathy, pleotherapy, repurposing.

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The most common type of Charcot-Marie-Tooth disease (CMT1A) is associated with a duplication of *PMP22*, leading to dysmyelination, axonal loss, progressive weakness and wasting of the muscles, chronic pain, and muscle cramps.[1] Although the overexpression of *PMP22* is a hallmark feature of the disease, the molecular mechanisms that lead to CMT1A phenotype remain unknown.

Currently, no approved treatment for CMT1A patients is available. Thus, in order to understand the mechanisms underlying CMT1A and develop a therapeutic strategy to treat the disease, a rat model overexpressing the *Pmp22* mouse gene was generated.[2] The pathology in CMT1A rats mimicked closely the pathophysiological features of the human disease, such as loss of muscle strength and nerve sensitivity.

The novel polytherapeutic drug combination PXT3003 (a low dose of baclofen, naltrexone, and sorbitol) was previously administered to CMT1A rats and demonstrated a positive efficacy to slow down disease progression after 3 months of treatment. Moreover, in a Schwannoma cell model PXT3003 decreased *Pmp22* expression. Furthermore, the treatment improved myelination in a dorsal root ganglion co-culture model derived from CMT1A transgenic rats.[3] Interestingly, it has also been demonstrated that an early short-term treatment of 2 weeks with PXT3003, starting at postnatal Day 6 in CMT1A rats, was enough to alleviate motor and molecular deficits 3 months later. This data suggested that PXT3003 is a potential promising therapy for children and young adolescent CMT1A patients.[4] Finally, a clinical Phase III study recently revealed positive efficacy in CMT1A adult patients.[5]

Here, the authors performed a long-term treatment study in adult CMT1A rats with PXT3003 to test the superior effect of PXT3003 compared to its three binary combinations of baclofen/naltrexone, baclofen/sorbitol, and naltrexone/sorbitol, and to demonstrate the contribution of each compound in the global efficacy of PXT3003 on CMT1A symptoms. CMT1A and wildtype rats were included as placebo controls. The treatment was performed in adult rats starting at postnatal Week 6, after the manifestation of symptoms commonly observed in CMT1A rats, and was continued until 18 weeks of age. Behavioural analyses were performed at the age of 6, 10, 14, and 18 weeks (grip strength, bar test, and inclined plane test). Furthermore, electrophysiological measurements and hot plate test were carried out at the end of the study. Post-mortem histological analyses were also performed to study the effect of PXT3003 on neuromuscular junctions and muscle function alterations.

PXT3003 treatment for 3 months starting in clinically affected adult rats improved the motor and sensory functions of CMT1A animals. The data suggest a synergistic effect of the three drug components contained in PXT3003: no significant effect on motor endpoints was actually observed with any of the binary combinations of baclofen/naltrexone, baclofen/sorbitol, or naltrexone/sorbitol, while in contrast, PXT3003 was significantly efficacious and proved its superior efficacy to the binary combinations on the behavioral level in CMT1A rats. Electrophysiological recordings revealed an improvement in distal motor latencies after PXT3003 treatment. This data also demonstrated that PXT3003 treatment restored functional neuromuscular innervation, which may be responsible for the observed correction in muscle fiber composition and subsequent increased muscle strength.

In conclusion, baclofen, naltrexone, and sorbitol all contribute to the effect of PXT3003 in CMT1A disease. The authors hypothesise that PXT3003 improves the function of neuromuscular junctions and consecutive muscle innervation, which may contribute to the improved motor function observed in CMT1A rats.

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Characteristics and Evolution of the Charcot-Marie-Tooth Hand: An Observational Study

Authors: *Valeria Prada,[1] Giulia Mennella,[1] Mehrnaz Hamedani,[1] Chiara Gemelli,[1] Laura Mori,[1] Marina Grandis,[1] Angelo Schenone[1,2]

1. Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Sciences (DINOGMI), University of Genova, Genova, Italy
2. Policlinico IRCCS San Martino, Genova, Italy

*Correspondence to valeria.prada@gmail.com

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Keywords: Charcot-Marie-Tooth disease, hand, muscles weakness, neuropathy, outcome measures, rehabilitation, upper limbs.

Citation: EMJ Neurol. 2019;7[1]:49-50 Abstract Review No. AR7.

BACKGROUND AND AIMS

Charcot-Marie-Tooth (CMT) disease is the most common hereditary neuropathy.[1] It is caused by multiple genetic mutations, but the typical symptomatology mainly involves the feet in the lower limbs and the hands in the upper limbs.[2] The fact that this condition is rare implies that there is not an abundance of rehabilitative indications in the literature, especially regarding the functionality of the upper limbs and the progression of impairment over time. Moreover, there are few validated measurement tools, and they often have limitations. The aim of this work was to analyse the characteristics of the CMT hand, monitoring the evolution in time and demonstrate the responsiveness of an innovative tool to evaluate the hand function: the hand test system (HTS; Figure 1).[3]

METHODS

The authors analysed the hands of 105 patients with a diagnosis of CMT. Assessment took place at the authors integrated outpatient clinic for hereditary neuropathy (Policlinico San Martino IRCCS, Genova, Italy). Evaluation took place between 0–6 months, 7–13 months, and 14–20 months. The outcome measures, in addition to the HTS, were: thumb opposition test,[4] Sollerman hand function test,[5] tripod pinch, and hand grip strength.[6] HTS is an engineered glove that measures the dexterity of the hand.[3] Briefly, finger opposition movements were evaluated using motor sequences in a quantitative spatial-temporal way. The patients, with their eyes closed, were instructed to execute a finger tapping sequence (opposition of thumb to index) and an index-medium-ring-little sequence (opposition of thumb to index, medium ring, and little fingers) at maximum velocity. Each sequence lasted 30 seconds alternating the hands. Data were processed with customised software measuring the following parameters: touch duration (contact time between thumb and another finger [ms]), inter-tapping interval (time between the end of the contact of the thumb and another finger and the beginning of successive contact [ms]), and movement rate (or frequency of complete motor task [Hz]).

Figure 1: Hand Test System: an engineered glove that can be used to assess hand dexterity.

RESULTS

At baseline, the study results confirmed that all parameters are significantly altered compared to healthy subjects, as previously shown.[7] The long-term analyses demonstrated that in a range time of 20 months thumb opposition test and hand grip did not show a significant worsening (n=25). Tripod pinch showed a slightly, but not significant, higher impairment of intrinsic muscles strength. Performances at the Sollerman hand function test constantly diminished with time (75.8±4.6, 74.8±6.3, 73.4±5.0), although not significantly. Interestingly, the HTS showed significant worsening in the performances of the hand with the index-medium-ring-little task (non-dominant hand: 5.0±1.5, 3.3±0.9, 2.7±1.0 Hz, $p<0.0001$; dominant hand: 3.5±1.2, 3.3±1.3, 2.9±1.2 Hz; $p<0.001$).

CONCLUSION

This study confirms that impairment of the hands is common in CMT patients. The authors speculate that intrinsic muscles strength is the first to be impaired whereas flexors muscles, as expected, are less affected. Importantly, the authors' longitudinal evaluation suggests that HTS is a responsive tool to evaluate the hand function, of which is probably more sensitive than other outcome measures. Because hand dysfunction is an important problem for the quality of life of CMT patients, innovative tools for its evaluation are strongly needed. Finally, a detailed exam of the hands is important to address specific rehabilitative or occupational solutions for the patients. For all these reasons, the authors aim, in increasing the number of cases and time of the long-term follow-up, to have better and more complete results.

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Comparative Analysis of High Frequency and Low Frequency Transcutaneous Electroneurostimulation in Treatment of Diabetic Peripheral Neuropathy

Authors: Mustafa Al Zamil, Natalia Gennadevna Kulikova

Department of Physiotherapy, Peoples' Friendship University of Russia, Moscow, Russian Federation
*Correspondence to alzamil@mail.ru

Keywords: Diabetic polyneuropathy, transcutaneous electroneurostimulation (TENS).

Citation: EMJ Neurol. 2019;7[1]:51 Abstract Review No. AR8.

BACKGROUND AND AIMS

Transcutaneous electroneurostimulation (TENS) has been used in the treatment of neuropathic pain for many years;[1] however, the effectiveness of various modality of TENS for the treatment of diabetic peripheral neuropathy (DPN) has not been sufficiently studied.[2]

To study the dynamics of neuropathic pain and neurologic deficiency in management of DPN by using high frequency-low amplitude (HL) and low frequency-high amplitude (LH) TENS.

METHODOLOGY

A cohort of 122 patients with DPN of the lower limbs were examined. All patients were diagnosed with Type 2 diabetes mellitus and aged between 40 and 60 years. Forty one patients underwent pharmacotherapy only. In addition to pharmacotherapy, 41 patients were managed with HL TENS (100 Hz, 100 µs, 7mA) and 40 patients were treated with LH TENS (1 Hz, 200 µs 15 mA) of peroneal and tibial nerves. The cathode was fixed on the proximal end of nerve. The stimulating anode was moved from the proximal end of nerve distally. The course lasted for 15 days with 1 procedure per day.[3] Neurologic examination before and after treatment included the following tests:

- Negative sensory symptoms (NS): Intensity of vibration, temperature, tactile, and pain hypoesthesia were measured using 5-point scales.
- Positive sensory symptoms (PS): Patients described subjective sensory disorders such as tingling, numbness, burning pain, and shooting feeling using 10-point scales.
- Neuropathic pain was studied using visual analogue scales (10-scores).
- Electromyography of peroneal and tibial nerves.

RESULTS

The analgesic effect of HL TENS proved to be more effective than LH TENS by 34.7%. Improvement of sensory impairments was observed in patients who underwent LH TENS more than HL TENS by 77.0%. On the second month of the follow-up period, NS continued to decline significantly after LH TENS by 19.0%. PS significantly regressed after application of HL TENS in comparison with LH TENS by 24.0%. Motor deficit regressed only after the use of LH TENS. Strength of ankle in dorsal flexion increased to 16.7% and in dorsal extension to 21.0%. Electromyography values of M-response and velocity of peroneal and tibial nerves were not significantly changed; however, in some cases there was an improvement in the M-response rate in electromyography after LH TENS.

CONCLUSION

HL TENS is a more effective treatment of neuropathic pain and PS. LH TENS is more effective in treatment of NS and motor deficit. In many cases, it also leads to a decrease in EMG disorders. TENS has a prolonged effect that lasts for 6 months of the follow-up period. The maximum therapeutic effect is observed in the second month of the follow-up period.

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Classification of Pontocerebellar Hypoplasia: Where does it End?

EDITOR'S PICK

Our Editor's Pick for this year's edition of *EMJ Neurology* is the review paper by Appelhof et al. Providing a detailed explanation of the differences between the 12 types of pontocerebellar hypoplasia, this article is essential if improvements in the diagnosis and knowledge of the disease are to be made. The ability to distinguish which disease type the patient is afflicted by and the cause behind it will allow appropriate, type-specific treatments to be developed. Pontocerebellar hypoplasia is a prime example of the positive influence next-generation sequencing is having on such rare, complex diseases.

We hope you enjoy reading this review as much as we did.

Authors: Bart Appelhof,[1,2] Peter G. Barth,[3] *Frank Baas[2]

1. Department of Clinical Genetics, Amsterdam University Medical Centre, Amsterdam, the Netherlands
2. Department of Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands
3. Department of Pediatric Neurology, Amsterdam University Medical Centre, Amsterdam, the Netherlands

*Correspondence to f.baas@lumc.nl

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Keywords: Classifications, next-generation sequencing, pontocerebellar hypoplasia (PCH), rare neurodegenerative disease.

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Abstract

Pontocerebellar hypoplasia (PCH) represents a heterogeneous group of congenital neurodegenerative diseases. Patients are afflicted by severe motor and mental impairments and most patients die at a young age. The hallmark of PCH is hypoplasia of the cerebellum and the pons, often in combination with supratentorial involvement. PCH is caused by autosomal recessive mutations in genes, most of which play a role in RNA metabolism. Twelve types (PCH1-12) are described, mainly based on clinical features. Identification of the responsible genes showed that the clinical classification did not match with the genetic classification leading to definition of subtypes. The authors expect that the increasing use of next-generation sequencing will lead to the identification of even more new PCH genes, widening the genetic and phenotypical spectrum. This will expand the classification and make it more complex. Therefore, the authors suggest that a new adjusted classification should be formulated to save the functionality of the PCH group.

INTRODUCTION

Pontocerebellar hypoplasia (PCH) describes a heterogeneous group of rare neurodegenerative diseases. PCH is characterised by hypoplasia of the cerebellum and pons with variable atrophy of supratentorial structures. The first in-depth neuropathological report of a PCH case was published in 1928.[1] PCH was first described as a distinct disease group in 1993 by Barth with the classification of PCH1 and PCH2.[2] The PCH spectrum was broadened to six PCH types over the following years based on phenotypes. *RARS2* was the first gene to be associated with PCH and was reported in 2007.[3] In the following years, the gene defects for the other five subtypes were reported.[4,5] It also became clear that mutations in different genes could give a similar clinical PCH phenotype. The number of PCH (sub)types increased rapidly due to the introduction of next-generation sequencing, with a (sub)type classification for each new PCH related gene. Currently, the PCH spectrum describes twelve clinical PCH types with eleven genetic subtypes (MIM PS607596) (Table 1).[5-27] All forms show an autosomal recessive inheritance pattern.

Table 1: Categorisation of the PCH group.

Subtype	MIM number	Gene	Distinctive features	Ref.
PCH1A	#607596	*VRK1**	SMA-like, mild motor impairments, normal/very mildly affected cognition, variable microcephaly.	5
PCH1B	#614678	*EXOSC3*	SMA-like, combined cerebral and spinal motor involvement, microcephaly.	6,7
PCH1C	#616081	*EXOSC8*	Identical to PCH1B.	8
PCH1D	#618065	*EXOSC9*	Identical to PCH1B.	9
PCH1E		*SL-C25A46**	SMA-like, combined cerebral and spinal motor involvement, optic atrophy.	10,11
PCH2A	#277470	*TSEN54*	Neonatal onset, clonus, dystonia/chorea, spasticity, swallowing disorder, progressive microcephaly, 'dragonfly' pattern on MRI.	4,12
PCH2B	#612389	*TSEN2*	Identical to PCH2A.	4,12
PCH2C	#612390	*TSEN34*	Identical to PCH2A.	4,12
PCH2F	#617026	*TSEN15*	Milder than PCH2A.	13
PCH2D	#613811	*SEPSECS*	Cerebral atrophy, spasticity, ataxia, variable pons atrophy.	14,15
PCH2E	#615851	*VPS53*	Cerebral atrophy, spasticity.	16
PCH4 (PCH5)	#225753 and #610204	*TSEN54*	Severe form of PCH2A, polyhydramnios, primary hypoventilation, severe generalised clonus neonatal death.	4,12
PCH3	#608027	*PCLO*	Optic atrophy, dysmorphic facial features.	17
PCH6	#611523	*RARS2**	Variable hyperlactataemia, seizures, hypotonia, respiratory chain disfunction.	3,18
PCH7	#614969	*TOE1*	DSD, enlarged ventricles, thin corpus callosum, diminished white matter.	19
PCH8	#614961	*CHMP1A*	Non-progressive, non-degenerative, thin corpus callosum.	20
PCH9	#615809	*AMPD2**	Abnormal muscle tone, dysmorphic facial features, 'figure 8' shape of upper brainstem.	21,22
PCH10	#615803	*CLP1*	Abnormal muscle tone, seizures, mild cerebellar atrophy, motor neuron degeneration.	23,24
PCH11	#617695	*TBC1D23*	Non-progressive, non-degenerative, dysmorphic facial features.	25,26
PCH12	#618266	*COASY**	Severe prenatal symptoms, micrognathia, arthrogryposis.	27

DSD: disorders of sexual development; PCH: pontocerebellar hypoplasia; SMA: spinal muscular atrophy.
*PCH (sub)types in which mutations in the involved gene also can result in another disease.

There is much clinical variability between different types but in general PCH presents as a severe disease with a prenatal onset or an onset of symptoms in the first months of life. Frequently, early symptoms are difficulties swallowing, apnoea, and respiratory difficulties indicating brainstem dysfunction. Prominent other symptoms are severe intellectual disability, epilepsy, and central motor impairment.[4,12,28] Most PCH subtypes follow a progressive disease course and most patients die at a young age.[12,29]

Both hypoplasia and atrophy contribute to the reduction of cerebellar volume in PCH. Hypoplasia usually refers to a static state, while atrophy implies a progressive condition. However, it is often difficult to differentiate between the two mechanisms, as follow-up MRI scans are required to determine progression of cerebellar volume loss. PCH patients typically show severe hypoplasia of the cerebellar hemispheres with relative sparing of the vermis resulting in a typical 'dragonfly' pattern on the coronal MRI, as seen in PCH2A patients (Figure 1e and Figure 1h). Another pattern is the 'butterfly'; this reflects a milder form with less atrophy of the hemispheres (Figure 1b).[12] Furthermore, patients display (progressive) supratentorial atrophy which underlies the microcephaly. In contrast to the gross infratentorial abnormalities, typical cerebellar symptoms are absent. This can be explained by the supratentorial involvement which hampers intentional motor skills. Below, this review will discuss the most common disease pathways in PCH, followed by a detailed description of the twelve types of PCH.

MECHANISM OF PONTOCEREBELLAR HYPOPLASIA

Although the pathology of different PCH subtypes is similar, there is no common pathomechanism for all of the PCH types; however, many genes are linked to different aspects of RNA metabolism (Table 1). This review will only focus on the most common pathway: RNA processing. *EXOSC3*, *8*, and *9* (PCH1B-C) encode for components of the exosome complex; this complex is responsible for degrading different types of RNA, such as messenger RNA (mRNA) and transfer RNA (tRNA).[6,8,9] *TOE1* (PCH7) modifies specific RNA types called small nuclear RNA.[19] The four components of the tRNA splicing endonuclease (TSEN) complex (PCH2A-C,F) together with CLP1 (PCH10) are involved in intron removal from tRNA.[4,13,23] Mutations in these genes possibly trigger stress pathways caused by accumulation of aberrantRNA fragments in the cells. Activation of stress pathways can suppress protein synthesis leading to neurodegeneration.

Another possible mechanism is shortage of properly processed tRNA, which are responsible for connecting the correct amino acid to the corresponding trinucleotide code on the mRNA in the ribosomes during translation.[23] There are 417 human tRNA genes while there are only 20 different amino acids. Despite the vast redundancy in tRNA genes, the lack of one specific tRNA gene can already cause progressive cerebellar atrophy in mice.[30] Almost 7% of the tRNA genes contains an intron;[31] thus, it is conceivable that tissue-specific expression of intron containing tRNA metabolism genes can result in dependence of the cerebellum on the tRNA splicing machinery. Other tRNA-related PCH genes, such as *RARS2* (PCH6) and *SEPSECS* (PCH2D), are responsible for the production of tRNA coupled to their corresponding amino acids.[3,14] However, it remains unclear why these aberrations in tRNA metabolism cause a brain specific defect.

The remaining PCH-related genes are thus far not functionally groupable and vary widely in mechanistic functioning.

PONTOCEREBELLAR HYPOPLASIA 1

PCH1 is hallmarked by anterior horn cell degeneration (as seen in spinal muscular atrophy) in addition to PCH. The severity of the disease course of PCH1 in patients is varied. PCH1 is often neonatal lethal because of respiratory insufficiency and severe hypotonia. Congenital contractures and polyhydramnios can also be present at the severe end of the PCH1 spectrum. In milder cases however, the pons is spared, and patients survive into adolescence. So far, five subtypes have been described (PCH1A-E).[6] PCH1B (MIM #614678), caused by *EXOSC3* mutations, is the most prevalent subtype of PCH1.[32] A PCH1B patients' disease course can be mild or severe, depending on the genotype.[7] A homozygous p.(Asp132Ala) change in *EXOSC3* is associated with sparing of the pons and a mild disease course (Figure 1a–b): patients display some

cognitive development and limited speech. Other *EXOSC3* mutations are associated with a more severe disease course, which is characterised by arthrogryposis and respiratory insufficiency which is often lethal before the first year of life (Figure 1c–d).[6] *EXOSC8* and its paralogue *EXOSC9*, also components of the exosome complex, underlie PCH1C (MIM #616081) and PCH1D (MIM #618065), respectively. The phenotypes of patients with *EXOSC8* or *EXOSC9* mutations are similar to those of *EXOSC3* patients.[8,9]In 2016,

Wan et al.[33] reported mutations in *SLC25A46* in patients with PCH1E. Later, the authors of this present review showed that the PCH1 patients, described by Barth (1993), were compound heterozygous for mutations in *SLC25A46*.[2,10] The clinical spectrum of patients with *SLC25A46* mutations is very broad, ranging from death within the first postnatal month, to survival into adulthood and with patients becoming symptomatic later in life, presenting with mild cerebellar atrophy and axonal peripheral neuropathy.[10,11,33] Notably, all patients have optic atrophy in this subtype.[34] Mitochondrial disorganisation and enlargement was detected in cells of PCH1E patients, which is in line with the function of *SLC25A46* in mitochondrial fission.[10]

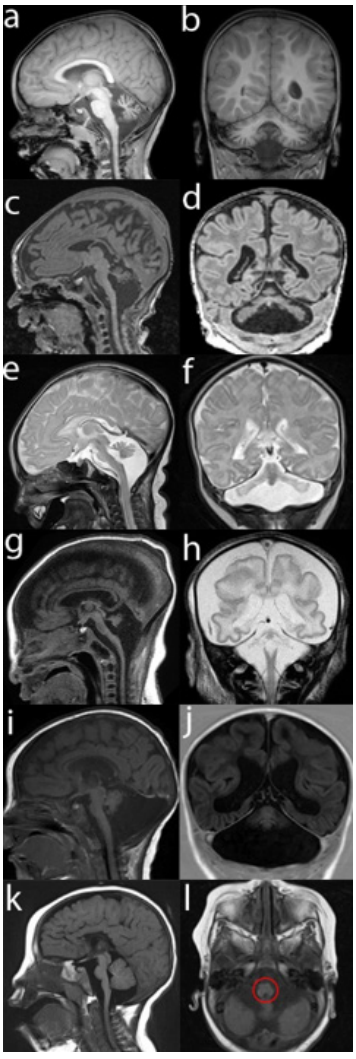

Figure 1: MRI images of PCH patients in sagittal (a, c, e, g, l, k), coronal (b, d, f, h, j), and axial (l) cross sections. MRI 'a' and 'b' display a PCH1B patient with the mild phenotype correlating with the p.(Asp132Ala) change in the *EXOSC3* gene. Other mutations in *EXOSC3* are associated with more severe atrophy of pons and cerebellum ('c', 'd'). PCH2A ('e-f') and PCH4 ('f-h'), both caused by *TSEN54* mutations, display the classical 'dragonfly' pattern seen on the coronal MRI ('f', 'h'); however, in PCH4 more cerebral involvement is seen. *TOE1* mutations cause PCH7 with severely dilated ventricles ('i', 'j'). *AMPD2* mutations cause PCH9 ('k', 'l'), which show a typical 'figure 8' pattern of the midbrain on the axial MRI ('l', red circle).

Lastly, PCH1A (MIM #607596) is caused by mutations in the *VRK1* gene and is sometimes called spinal muscular atrophy with PCH. It is a rare and atypical subtype often reported in patients with an Ashkenazi Jewish origin. Patients seem to present no mental impairments but do display a delayed motor development or only slow, progressive loss of motor functions later in life.[35]

PONTOCEREBELLAR HYPOPLASIA 2

Of the six PCH2 subtypes (PCH2A-F) that are currently described, PCH2A (MIM #277470) is the most common. Typically, neonates show jitteriness, clonus, poor sucking, and impaired swallowing. Later extrapyramidal movement disorders such as dystonia and chorea, spasticity, and the lack of intentional motor skills become apparent.[36] Patients often are afflicted by generalised seizures, sleeping disorders, and chorea which often necessitates institutional care. Respiratory insufficiency and infections are a common cause of death which is generally at an early age, however some patients reach adulthood.[29] The neuroradiological hallmark for PCH2A is the 'dragonfly' pattern on MRI of the hypoplastic cerebellum (Figure 1e–f). In patients with a follow-up brain MRI, progressive cerebral atrophy is seen, with apparent ventricular enlargements accentuated by disproportionate affection of the caudate nuclear heads.[12] Neuropathological findings in PCH2A showed stunted folial growth, especially affecting the cerebellar hemispheres, and peculiar fragmentation of the dentate nuclei.[37] A breakthrough was achieved in 2008 with the discovery that mutations in the *TSEN54* gene underlie PCH2A. The mutation, p.(Ala307Ser), was initially found in a Dutch genetic isolate and is the most common cause of PCH2.[4] Patients with the same mutation p.(Ala307Ser) are also found on other continents suggesting that it is a very old mutation. *TSEN54* forms the TSEN complex with three other components: *TSEN34*, *TSEN2*, and *TSEN15*. Mutations in *TSEN2*, *TSEN34*, and *TSEN15* are very rare and result in PCH2B (MIM #612389), PCH2C (MIM #612390), and PCH2F (MIM #617026), respectively.[4,12,13] One patient with a TSEN34 mutation and four patients with *TSEN2* mutations have been reported.[12,38] *TSEN15* mutations are described in three PCH2F patients and have a milder phenotype with no visual impairments.[13] The two other PCH2 subtypes are not caused by aberration in the TSEN complex, namely PCH2D (MIM #613811) and PCH2E (MIM #615851). Both were previously described in literature as progressive cerebrocerebellar atrophies. PCH2D is caused by mutations in the O-phosphoserine tRNA-selenocysteine tRNA synthetase (*SEPSECS*) gene. Reported features of PCH2D are spasticity, optic atrophy, ataxia, and seizures. The MRI shows progressive cerebellocerebral atrophy with variable involvement of the pons.[14] PCH2D disease course is usually severe; however, a mild PCH2D phenotype has been recently reported.[15] PCH2E, caused by *VPS53* mutations, has been reported in twelve Jewish families of Moroccan origin.[16,39] All cases carried the same mutation, p.(Gln95Arg), suggesting a founder effect. Most patients appear healthy at birth but quickly develop hypotonia and delayed head growth. Subsequently, patients develop tonic-clonic -seizures, progressive spasticity, and progressive cerebrocerebellar atrophies on brain MRI.[16]

PONTOCEREBELLAR HYPOPLASIA 3

Only one family, from Oman, with PCH3 (MIM #608027) has been reported. All four affected children have a homozygous nonsense mutation in the *PCLO* gene.[17] Generalised hypotonia with hyperreflexia, progressive microcephaly, and seizures developed during their first year of life. The patients reported thus far all survived into puberty. MRI shows an atypical pattern with diffuse atrophy of the cerebellum and pons; however, not as drastic as a 'dragonfly' pattern. Supratentorial cerebral reduction of white matter with a thin corpus callosum was reported. *PCLO* is thought to play a role in regulation of proteins in neuronal synapses.[17]

PONTOCEREBELLAR HYPOPLASIA 4 AND 5

PCH4 (MIM #225753) describes a severe form of PCH2. Notably, patients present prenatal symptoms such as intrauterine seizures and polyhydramnios resultant of impaired fetal swallowing.[40,41] Neonates show massive un provoked myoclonus, hypertonia, hypoventilation, and sometimes seizures.[12] Patients usually require continuous mechanical ventilation and generally

die within the first month of life. Brain MRI shows subtotal destruction of cerebellar hemispheres, which are underdeveloped and atrophied with often mild-to-severe vermal folia atrophy (Figure 1g–h). In addition, cerebral atrophy is more apparent than in PCH2 (Figure 1g–h). Craniocerebral disproportion with a vastly increased subarachnoid space may be seen as the result of progressive loss of cerebral tissue with a prenatal onset (Figure 1h). Post-mortem examination revealed only remnants of the dentate nuclei and the inferior olive nucleus had a horseshoe shape, therefore PCH4 was originally named 'olivo-ponto-cerebellar-hypoplasia'.[37] Similar to that seen in PCH2A, *TSEN54* mutations also underlie PCH4 (MIM #225753). PCH4 patients are often compound heterozygous for the common p.(Ala307Ser) mutation in combination with a loss of function mutation. Although patients with homozygous null-alleles of *TSEN54* have not been described, the authors assume that this is incompatible with life. PCH5 (MIM #610204) was initially described as a distinct subtype based on the presence of intrauterine seizure-like activity in one family.[41] In retrospect, PCH2A, PCH4, and PCH5 represent mutations of the same gene where increasingly severe symptoms commensurate with the severity of mutation.[42]

PONTOCEREBELLAR HYPOPLASIA 6

Mutations in the autosomal gene encoding the mitochondrial argenyl tRNA synthetase (*RARS2*) cause PCH6 (MIM #611523). Characteristics of PCH6 include severe early onset seizures, CSF hyperlactataemia, mitochondrial respiratory chain defects, and later severe developmental delay with rapidly progressive microcephaly.[3] Neuroradiologic imaging reveals a global progressive atrophy of the brain with variable hypoplasia/atrophy of the cerebellum.[18,43] The basal ganglia were spared in some patients with severe cerebral atrophy. Neuropathological reports of two cases reveal an intact olive nucleus and relatively spared dentate nucleus.[43]

PONTOCEREBELLAR HYPOPLASIA 7

PCH7 (MIM #614969) is characterised by disorders of sexual development in addition to PCH.[19,44] Clinical symptoms are truncal hypotonia with hypertonic extremities, seizures, and increased tendon reflexes. Most described patients present with female external genitalia. Ten XY-patients are reported with a small or absent penis and undescended or absent testis. An ultrasound revealed ovaries in two of these patients. In one of the three XX-female patients, no ovaries could be found using ultrasound. Female external genitalia were normal; although one patient showed a prominent clitoris. A brain MRI revealed severely reduced cerebellar and pontine volumes with enlarged ventricles, diminished white matter, and a thin corpus callosum in some patients (Figure 1i–j). Patients carried homozygous or compound heterozygous mutations in the autosomal *TOE1* gene. The function of TOE1 remains elusive, although current evidence points to a role in snRNA metabolism in the nucleolus.[19]

PONTOCEREBELLAR HYPOPLASIA 8

CHMP1A mutations are rare and underlie PCH8 (MIM #614961).[20] Only three families and two different pathogenic mutations have been reported. In contrast to the other types of PCH, the disease course of PCH8 appears to be non-progressive. Patients have microcephaly, dystonia, and/or choreiform movements, and sometimes suffer from joint contractures and seizures. Developmental delay is severe, but some patients learn to walk and develop limited speech. Brain MRI shows PCH with reduced cerebral white matter volume and a thin corpus callosum. In patients with follow-up brain MRI, no progression of the atrophy was visible. Mechanistically, it is thought that *CHMP1A* aberrations disrupt the BMI1regulated proliferation of neuronal progenitor cells.[20]

PONTOCEREBELLAR HYPOPLASIA 9

PCH9 (MIM #615809) describes a broad disease spectrum with 25 affected individuals reported to date. Clinically, patients have progressive microcephaly, impaired swallowing, brisk tendon reflexes, spasticity, teeth abnormalities, and some patients display dysmorphic facial features.[21,22,45] While some patients survive into adulthood, most die early in life. In addition to the cerebellar hypoplasia, PCH9 patients displays a distinctive 'figure 8' shape of the midbrain on axial MRI (Figure 1l). Also, a hypoplastic or absent corpus

callosum has been reported. Mutations in the *AMDP2* gene are found in PCH9, but are also identified in one family with hereditary spastic paraplegia 63 (SPG63)[46] (MIM #615686). Aberrations in *AMDP2*, which regulates GTP levels, is thought to disrupt the initiation of protein translation.

PONTOCEREBELLAR HYPOPLASIA 10

PCH10 (MIM #615803) patients show dysmorphic facial features, cognitive impairment, seizures, and spasticity.[23,24] Brain MRI showed only mild atrophy of the cerebellum and the pons. Supratentorially, atrophy of the cortex, and a thin hypoplastic corpus callosum are reported. Furthermore, signs of progressive spinal motor loss are seen on electromyography. All PCH10 patients originated from Turkey and carried the same homozygous mutation in the *CLP1* gene, suggesting a founder effect. The CLP1 protein interacts with the TSEN complex and is involved in tRNA processing.[23,24]

PONTOCEREBELLAR HYPOPLASIA 11

PCH11 (MIM #617695) is reported in seven families who all carry biallelic mutations in the *TBC1D23* gene. Individuals are relatively mildly affected compared to other PCH types, and so far, a non-progressive nature of PCH11 is noted.[25,26] Patients present with dysmorphic facial features, microcephaly, hypotonia, and occasional ambulation. Brain MRI shows a reduced cerebellar volume and severe hypoplasia of the pons. The corpus callosum is hypoplastic in most cases. *TBC1D23* is involved in neuronal vesicular trafficking.[25]

PONTOCEREBELLAR HYPOPLASIA 12

The latest branch of the PCH tree is PCH12 (MIM #618266). PCH12 is a lethal disorder at the severe end of the PCH-spectrum, presenting with severe prenatal onset microcephaly, PCH, and congenital contractures.[27] Atrophy of the cerebrum and hypoplasia of the pons, cerebellum, brainstem, spinal cord, and basal ganglia were confirmed on postnatal brain MRI in one of the patients. Genetic analyses identified splice site and frameshift mutations in the *COASY* gene in affected individuals. *COASY* is responsible for the last two steps of coenzyme A (CoA) synthesis; the CoA synthesis pathway is highly conserved and essential for life. Earlier reports have also linked *COASY* mutations to neurodegeneration with brain iron accumulation 6 (MIM #615643) (NBIA6). Residual function of COASY appeared to be higher in NBIA6 patients than in the one tested PCH12 patient, which might explain the more severe phenotype in the latter.[47]

DIFFERENTIAL DIAGNOSIS

Several other diseases should be considered when a patient presents with PCH. The three most common diseases that mimic PCH phenotypes are caused by *CASK* mutations, *PMM2* mutations, and extreme prematurity. Mutations or microdeletions in the *CASK* gene cause mental retardation and microcephaly with pontine and cerebellar hypoplasia (MIM #300749) highly similar to PCH. In addition, neocortical malformations, hearing loss, and facial dysmorphic features can be present.[48] Overall, development delay is severe, but some patients reach some motor milestones, such as walking. In contrast to all PCH types, *CASK* mutations follow a X-linked dominant inheritance pattern and mutation are generally *de novo*.[49] Moreover, patients with heredity disorders of glycosylation type 1a (PMM2-CDG) (MIM #212065) can mimic PCH. Neuroimaging of PMM2-CDG patients reveals cerebellar hypoplasia/atrophy.[50] Clinically, the phenotype of PMM-CDG patients varies from mild intellectual disability and ataxia to a severe phenotype with antenatal onset multi-organ abnormalities and severe mental and motor impairments. Distinctive dysmorphic features include inverted nipples and abnormal fat distribution. PMM2-CDG can be diagnosed by detection of abnormal glycoproteins through isoelectric focussing of transferrin. Additionally, extreme prematurity (gestation age <32 weeks) can occasionally result in PCH.[51] The rapid growth of the cerebellum during the third trimester might increase vulnerability to disruption by very early prematurity.

When lissencephaly is noticed on brain MRI in addition to PCH, mutations in *RELN* (MIM #257320), *VLDLR* (MIM #224050), or α-dystroglycan related dystrophies should be considered.[52,53] Furthermore, patients at the severe end of the Hoyeraal-Hreidarsson syndrome (MIM #30500) spectrum and spinocerebellar ataxia 29 (SCA29) (MIM #17360) can resemble PCH.

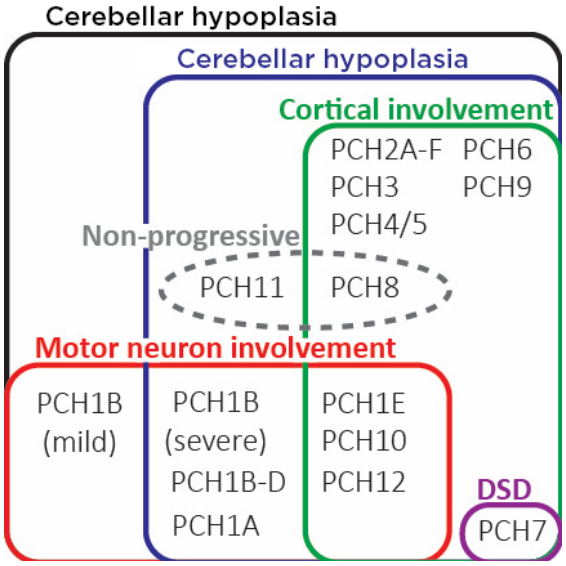

Figure 2: Depiction of pathological overlap between different PCH types.

In PCH2A-C, F, patient cortical involvement is not always present at birth but will always develop postnatally.

DSD: disorders of sexual development; PCH: pontocerebellar hypoplasia.

Patients with Hoyeraal–Hreidarsson syndrome usually present with a combination of progressive bone marrow failure and cerebellar hypoplasia.[54] Furthermore, a patient with an *ITPR1* mutation, normally resulting in SCA29, showed classical PCH on brain MRI.[55]

DIAGNOSIS OF PONTOCEREBELLAR HYPOPLASIA

The diagnoses of PCH is based on careful clinical examination and neuroimaging, followed by genetic testing. Genetic testing is required to confirm the diagnosis because differentiation between subtypes of PCH and other disorders is often not straightforward. Furthermore, for most PCH types the number of patients described is low (e.g., PCH3 or PCH8), and sometimes limited to a single mutation (e.g., PCH10), leading to an incomplete phenotypical characterisation.[15,56] Therefore, the notion should be taken that PCH phenotypes can deviate from reported descriptions.

PERSPECTIVES

PCH is a clinical diagnosis in patients with atrophy and hypoplasia of the pons and cerebellum. Unlike the suggestion implied by the name PCH, they represent a group of disorders that affect development of the structure and function of the whole brain. Progressive decline in structure and progressive microcephaly characterise the most common types. Identification of many different PCH genes and types resulted in a rapidly expanding classification. Initially described as a disease associated with defects in (t)RNA metabolism, the identification of *CHMP1A* and *VRK1* mutations challenged this hypothesis.[5,20] The classification became more complex when cases with milder disease manifestations, such as PCH1B and PCH10, were included.[7,23] This resulted in the current PCH classification as a collection of disorders that do not always share a common phenotype or affected pathway (Figure 2). Even the criteria of cerebellar and pontine atrophy are not always met; therefore, the authors propose that the classification should be revised, reserving the name PCH for the clinical description of patients with early onset atrophy of the cerebellum and often pons. The most obvious classification is based solely on clinical features, i.e., PCH with disorders of sexual development (PCH7), PCH with dystonia (PCH2), and PCH with spinal muscular atrophy (PCH1). Moreover, the disease course should be considered in the classification because it is important for counselling. The lack of knowledge about pathomechanisms in most forms currently

precludes a classification based on mechanism. The broad phenotypic spectrum within patients with mutations in the same PCH gene also precludes a purely genetic classification; therefore, the clinical symptoms and MRI should be leading in the diagnosis and the genetic diagnosis can be used for counselling and disease course prediction.

CONCLUSION

PCH remains a severe disease for which only symptomatic treatment is available. Preconception carrier screening in high-risk populations is successful and feasible for some subtypes.[57] It is likely that in the future more genes will be linked to PCH and new mutations in known PCH genes will broaden the phenotype further.

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Seizures in Childhood: Aetiology, Diagnosis, Treatment, and What the Future May Hold

Authors: *Sara Rubenstein[1], Andrew Levy[2]

1. Rutgers New Jersey Medical School, Newark, New Jersey
2. Technion Faculty of Medicine, Haifa, Israel
*Correspondence to sr1215@njms.rutgers.edu

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Abstract

Seizures are one of the most common medical problems affecting children, and epilepsy is the most common chronic neurological condition in children. Childhood epilepsy syndromes include a wide spectrum of disorders ranging from benign to life threatening. While there are many known epilepsy syndromes, there are many factors, which may lead to the development of seizures in children including infection, traumatic brain injury, or structural abnormality.

Up to 40% of childhood epilepsies are thought to have some component of genetic involvement. New genes, mutations, and variants involved in epilepsy are being identified continuously. Most of the genes which have been identified encode for neurotransmitter receptors, ion channels, molecules involved in intracellular signalling, or proteins involved in synaptic structure. As new candidate genes in epilepsy are identified, new technologies in genetic testing are becoming available and more accessible, making the molecular diagnosis of epilepsy increasingly relevant to researchers, physicians, patients, and their families.

The standard of care and first-line treatment is the use of antiepileptic drugs. For those patients with medication-refractory epilepsy other available therapies include ketogenic diet, vagal nerve stimulator, or epilepsy surgery. The newest advancement in the treatment of paediatric epilepsies is based around the idea of targeted therapy. These therapies incorporate pharmacogenomics, the principle that an individual's genetic background affects their response to specific drugs, as well as precision medicine, which identifies treatments for the damaged products resulting from specific gene mutations. Many of these therapies are still under research or in trial; however, there is much promise for the future of targeted medications.

INTRODUCTION

Seizures are one of the most common medical problems affecting children, and epilepsy is the most common chronic neurological condition in children. The incidence of epilepsy in children according to a 2012 cohort study is 144 per 100,000 person-years in the first year of life, and 58 per 100,000 person-years up to age 10.[1] The cumulative incidence of childhood epilepsy was

found to be 0.45% at the age of 5 and 0.66% at the age of 10.[1] In 2005, it was found that children below the age of 10 have a lifetime prevalence of epilepsy of 0.6%, and that up to 5.0% of children will have a febrile seizure by the age of 5.[2]

In 2014, the International League Against Epilepsy (ILAE) redefined epilepsy as meeting one of three inclusion criteria: 1) at least two unprovoked seizures occurring >24 hours apart; 2) one unprovoked seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; 3) the diagnosis of an epilepsy syndrome.[3] Recent advances in genetic testing, as well as increased awareness surrounding childhood epilepsy, have enabled diagnoses to be given earlier and with more accuracy.

CHILDHOOD EPILEPSY SYNDROMES

Childhood epilepsy syndromes include a wide spectrum of disorders ranging from benign to life-threatening. Self-limited epilepsy with centrotemporal spikes accounts for 15–25% of all childhood epilepsies and is the most common idiopathic childhood epilepsy syndrome in children.[4] Peak onset is between the ages of 7 and 8 years and usually resolves by age 16. It has the best prognosis of all epilepsy syndromes.[5] Seizures typically present at night and are usually focal involving one side of the face, lips, or tongue.[4] Recent studies have shown, that although self-limited epilepsy with centrotemporal spikes has a very favourable prognosis, some children may have long-term cognitive deficits, including language, memory, and auditory processing impairments.[6]

Self-limited occipital epilepsy is another childhood epilepsy syndrome and accounts for 5–10% of epilepsies.[4,7] There are two types, early onset which is known as Panayiotopoulos syndrome and late onset, which is known as Gastaut type. In Panayiotopoulos syndrome, the age of onset is between the age of 1 and 6, and seizures typically resolve within 3 years. It presents as a triad of symptoms of nocturnal seizures, tonic eye deviation, and vomiting.[7] Prognosis is very good and antiepileptic drugs (AED) are usually not needed. The Gastaut type begins later in childhood with peak incidence at 8 years old. It is characterised by brief seizures with visual symptoms, such as simple visual hallucinations, followed by hemiclonic seizures.[7] Treatment with AED is necessary, and 50–60% of children become seizure free within 2–3 years.[4]

Childhood absence epilepsy accounts for 8–15% of all childhood epilepsies.[8] Age of onset is typically between 4 and 10 years old. Absence seizures are very brief, usually lasting only seconds; however, they can occur up to hundreds of times a day. They are characterised by behavioural arrest and impaired consciousness. Approximately 60% of children will have associated automatisms including eye blinking or lip smacking. The classical electroencephalogram (EEG) for absence epilepsy is 3 Hz spike and wave complexes. Many of these children have associated behavioural and cognitive impairments, including an increased prevalence of attention deficit hyperactivity disorder (ADHD).[8] In total, 70% of cases respond to ethosuximide.[8] In 60–80% of patients, remission will be achieved within 2–5 years of onset. Fifteen percent of children with absence epilepsy will develop juvenile myoclonic epilepsy (JME).[4]

JME includes 5–10% of epilepsy syndromes. It usually peaks between the ages of 13 and 15.[8] Presentation includes multiple different seizure types including myoclonic jerks, generalised tonic-clonic seizures, and absence seizures. Seizures typically occur in the mornings, and sleep deprivation is a common trigger. JME is a lifelong syndrome with most patients needing to remain on AED indefinitely.[8]

Epileptic encephalopathy with continuous spike and wave in sleep (CSWS) presents in children 2–4 years old as focal seizures and developmental regression.[4,5] The EEG is classic for generalised spike and wave during slow wave sleep. The seizures and abnormal EEG typically resolve by age 9.[4] The severity of cognitive decline and developmental regression is variable; however, it can affect behavioural, cognitive, language, and social modalities. Aetiology remains largely unknown, with genetic mutations accounting for approximately 17% of cases.[4]

Landau Kleffner syndrome (LKS) is an unusual childhood epilepsy syndrome which is described as an acquired epileptic aphasia. It typically presents in previously healthy children between

the ages of 3 and 10 years old as significant, expressive, and receptive language regression. Children also develop seizures, which typically resolve by the age of 15.[5] Recent research has supported a missense mutation in the *GRIN2A* gene, involved in NMDA receptor activation, as a possible aetiology for the syndrome.[9]

Lennox Gastaut syndrome (LGS) is one of the most devastating childhood epilepsy syndromes, and one of the most variable. LGS is characterised by multiple types of seizures, including drop attacks, cognitive and developmental regression, and generalised slow spike and wave complexes on EEG.[10,11] Given the variable presentation of LGS, there are likely many underlying aetiologies. Infantile spasm, one of the most common epilepsy syndromes in the first year of life, is characterised by clusters of spasms, developmental regression, and hypsarrhythmia on the EEG; it may develop into LGS in approximately one third of cases.[11]

Dravet syndrome, also known as severe myoclonic epilepsy of infancy (SMEI), is an intractable epilepsy syndrome which usually presents in the first year of life.[11] Infants will typically present with febrile seizures and then proceed to develop frequent afebrile seizures, developmental delays, and cognitive impairments.[10,11] Approximately 80% of cases are caused by mutations in the *SCN1A* gene, which is involved in the voltage-gated sodium channel NaV1.1.[10,11] Generalised epilepsy with febrile seizures plus (GEFS+) may also be caused by mutations in the *SCN1A* gene and is characterised by febrile seizures which continue into older childhood, and then progress into afebrile generalised seizures in adolescence.[5]

Ohtahara syndrome, also known as early infantile epileptic encephalopathy (EIEE), is one of the earliest epilepsy syndromes to present and can be diagnosed as early as the first week of life. The EEG is characterised by a burst suppression pattern. Seizures are typically intractable and do not respond to AED.[10,11] EIEE has a very poor prognosis, associated with severe global developmental delay.[10]

OTHER AETIOLOGIES OF SEIZURE IN CHILDREN

While there are many known epilepsy syndromes, there are a multitude of factors which may lead to the development of seizures in children. Outside of epilepsy there is about a 1% lifetime risk of a single unprovoked seizure, and about a 6% lifetime risk of a provoked seizure.[12] Typically, provoked seizures have little chance of recurring; however, febrile seizures may be an exception. Febrile seizures are the most common type of seizure, affecting 2–6% of the population.[13] Three to four percent of children will have at least one febrile seizure and 20–40% of children will have a recurrence.[12, 13] Febrile seizures are thought to be multifactorial, provoked by fever, genetic susceptibility, and the stage of brain maturation in young children.[12] The risk of developing epilepsy after a simple febrile seizure is approximately 2% and up to 6% after a complex febrile seizure.[13,14]

Seizure following traumatic brain injury (TBI) is another example of provoked seizure and is a common complication after severe TBI in children. Post traumatic seizure (PTS) occurs in roughly 16% of 14–17-year-olds with accidental severe TBI and in up to 36% of children <2 years old.[15] PTS increases up to 57% in the setting of subdural haemorrhage (SDH) and non-accidental trauma in younger children. Both SDH and non-accidental trauma have each been shown to increase the risk of PTS 2-fold.[15] The risk of PTS decreases with age.[15] A provoked seizure can be caused by any interruption to the functioning of the cerebral cortex, and the triggers are many. These include metabolic abnormalities such as electrolyte derangements, chemical or inflammatory irritation in the setting of meningitis or encephalitis, ischaemia, haemorrhage, or even concussion.[16] Seizures can be caused by progressive neurological disorders or tumours. They can also result from perinatal asphyxia, hypoxic insult, or from abnormal neuronal proliferation and migration.[16] Neuronal migration disorders such as heterotopia, lissencephaly, schizencephaly, and polymicrogyria have all been shown to cause seizures.[17]

The incidence and prevalence of epilepsy is higher in developing countries than in more developed countries.[18,19] In sub-Saharan Africa and in rural India, it has been shown that the most common risk factors for seizure include family history of seizure, trauma or asphyxia at birth, head trauma, and central nervous system infection.[18,19] In sub-Saharan Africa, parasitic infections, such as neurocysticercosis, cysticercosis, and toxocariasis are the most common infectious cause of

seizures.[1] Roughly 80–85% of epilepsy patients in the developing world are not treated effectively. This is largely because of limited financial resources, as well as cultural and social stigmas surrounding seizure disorders.[18]

It is widely accepted that the risk of recurring seizures after a first unprovoked seizure is between 32% and 69%.[20] Recently, six studies were analysed, which included 815 neurologically and developmentally normal children, and the rate of seizure recurrence was found to be 45% within 3 years.[20] Many causes of epilepsy have been identified; however, in at least 50% of children with epilepsy the cause remains unknown.[12] It has been suggested that epilepsies can be divided into three categories based on aetiology: unknown, genetic, and structural/metabolic.[21] In recent years the genetic basis of epilepsy has garnered much attention and research.

GENETIC BASIS OF EPILEPSY

New genes, mutations, and variants involved in epilepsy are being identified almost daily.

Table 1: Ion channel mutations identified in epilepsy syndromes.[23-29]

Ion channel	Gene	Function	Epilepsy syndrome	Potential targeted therapy
Potassium	*KCNQ2*	M-channel subunits of potassium channels.	BNFS	Potassium channel openers retigabine (or ezogabine).
	KCNQ3	M-channel subunits of potassium channels.	BNFS	Potassium channel openers retigabine (or ezogabine).
AChR	*CHRNA2*	α2-subunit of neuronal nicotinic AchR.	Partial seizures in ADNFLE.	Nictotinic AChR antagonists.
	CHRNA4	α2-subunit of neuronal nicotinic AChR.	Partial seizures in ADFNLE.	Low-dose $GABA_A$ receptor antagonists.
	CHRNB2	β2-subunit of neuronal nicotinic AChR.	Partial seizures in ADFNLE.	Nicotinic AChR antagonists.
Sodium	*SCN1A*	Sodium channel Nav1.1.	GEFS+; SMEI	Avoid sodium channel blockers.
	SCN1B	β1-subunit of sodium channel Nav1.1.	GEFS+	
	SCN2A	α subunit Nav1.2 of voltage-gated sodium channels.	Benign familial neonatal-infantile convulsions; GEFS+.	Phenytoin, sodium channel blockers.
	SCN8A	NaV1.6 subunit sodium channel protein.	Early onset seizures and intellectual disability.	Phenytoin, sodium channel blockers.
Calcium	*CACNA1A*	Calcium channel subunit gene.	Idiopathic generalised epilepsy.	
	CACNA1H	Subunit of T-type calcium channel.	Childhood absence epilepsy; idiopathic generalised epilepsy.	

AChR: acetylcholine receptor; ADFNLE: partial seizures in autosomal dominant nocturnal frontal lobe epilepsy; BNFS: benign familial neonatal seizures; $GABA_A$: γ-aminobutyric acid A; GEFS+: generalised epilepsy with febrile seizures plus; SMEI: severe myoclonic epilepsy of infancy.

Most of the genes identified encode for neurotransmitter receptors, ion channels, molecules involved in intracellular signalling, or proteins involved in synaptic structure.[22] Up to 40% of childhood epilepsies are thought to have some component of genetic involvement.[23] Mutations in genes, leading to dysfunction in sodium, chloride, and potassium channels in neurons, have been well established to have a role in many types of epilepsy.[24] Multiple epilepsy syndromes have been identified which result from mutations causing gain or loss of function in ion channels (Table 1).[23-29] As new candidate genes in epilepsy are identified, new technologies in genetic testing are becoming available and more accessible, making the molecular diagnosis of epilepsy increasingly relevant to researchers, physicians, patients, and their families. Next-generation sequencing has allowed for the rapid sequencing of large amounts of DNA, making it possible to test many genes at once, or even the entire genome.[30] Comparative genomic hybridisation (CGH) is used to detect copy number variants (CNV), including deletions, duplications, and complex rearrangements.[31] In patients with epilepsy and developmental delay, abnormalities are found with CGH in up to 23.5% of cases.[30] It has been shown that CNV involving chromosomal regions 1q21.1, 15q11.2, 15q13.3, 16p13.11, 16p11.2, and 22q11.2 are associated with epilepsy, intellectual disability, and autism.[32,33] Recently, a large cohort study looked at CNV data of 1,255 patients with pre-existing CGH or single nucleotide polymorphism array, who had epilepsy in addition to other comorbid conditions such as intellectual disability, autism, or other neurological features. Of the 1,097 patients included in the study, 12.7% were identified as having pathogenic or possible pathogenic mutations.[34] CGH is relatively inexpensive and results are usually available within 2–4 weeks, increasing the value in clinical settings.

Diagnostic sequencing typically involves single gene sequencing, a gene panel, or whole exome sequencing (WES). In most epilepsy syndromes, gene panels are of high diagnostic value given the involvement of multiple genes, variable expression of single genes, and the number of genes that may cause one phenotype. There are multiple epilepsy gene panels and selecting the appropriate panel is determined by the seizure type, family history, age, and suspected gene involvement.[31] WES allows for analysis of all genes encoding for proteins in the genome, which account for approximately 85% of pathological mutations.[33] WES is useful for cases in which a genetic disease is suspected but there are no specific gene tests available, the presentation is not consistent with known genetic epilepsy syndromes, or if prior genetic testing has been negative.[30,31] WES has a diagnostic rate of close to 25% in patients previously undiagnosed.[33] The largest caveat with WES is the data returned and the significance behind it. While pathogenic variants are often identified, there is a high incidence of benign variants and variants of unknown significance. There is also the increased risk of incidental findings and further implications for the patients and their families. If prior workup and genetic tests have been unrevealing, the pros and cons must be considered in pursuing WES.

TREATMENT

With increased availability of genetic testing and new mutations being identified, there is hope for new and more effective treatment of epilepsy in children. The standard of care and first-line treatment is AED therapy. There are currently >20 available AED, and choosing the appropriate medication largely depends on age and seizure type (Table 2).[35-37] Approximately half of the patients become seizure-free on their first AED, and about two thirds will achieve seizure freedom with the use of AED.[37] This leaves approximately 30% of patients with medication-refractory epilepsy. The ILAE defines drug-resistant epilepsy as "failure of adequate trials of two tolerated, appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom."[38] In addition to AED therapy there are other modalities available in the treatment of seizures.

The ketogenic diet has been proven to be an effective treatment for children with drug-resistant epilepsy.[37,39] The classical ketogenic diet consists of a diet composed of 90% fats, 7% proteins, and 3% carbohydrates.[40] Today, there are multiple variations of the ketogenic diet consisting of varying ratios of fats to proteins and carbohydrates. A 2006 review of 15 studies on the ketogenic diet found a >50% reduction in seizure frequency in approximately one third

of children with medication-refractory epilepsy on the ketogenic diet.[39] For patients with glucose transporter type1 deficiency syndrome (Glut1DS), in which a mutation in the *SLC2A* gene causes impaired transport of glucose across the blood-brain barrier resulting in seizures; and in those with pyruvate dehydrogenase deficiency (PDHD), in which pyruvate cannot be metabolised into acetyl-CoA also resulting in seizures, the ketogenic diet is first-line therapy.[37,40] In most patients with intractable epilepsy, the mechanism by which the ketogenic diet decreases seizure activity remains largely unknown.

Table 2: Commonly used antiepileptic drugs in paediatric patients.[35-37]

AED	Paediatric dosing	Mechanism of action	Seizure type
Carbamazepine	10–35 mg/kg/day	Sodium channel blocker targeting voltage gated Na channels.	Focal seizures.
Clobazam	<2yo: 0.5–1 mg/kg/day >2yo: 10–20 mg/day	Activation of $GABA_A$ receptors.	Add on AED for seizures associated with LGS.
Ethosuximide	15–40 mg/kg/day	Targets low voltage Ca channels.	Absence Seizures.
Eslicarbazepine	400–1,200 mg/day based on weight	Sodium channel blocker targeting voltage-gated Na channels.	Focal seizures.
Felbamate	15–45 mg/kg/day	Thought to act on voltage-gated Na channels, high voltage Ca channels, and $GABA_A$ receptors.	Focal seizures, drop attacks in LGS.
Lacosamide	11–29 kg: 6–12 mg/kg/day 30–49 kg: 4–8 mg/kg/day	Sodium channel blocker targeting voltage-gated Na channels.	Focal seizures.
Lamotrigine	Monotherapy: 4.5–7.5 mg/kg/day Add on: 5–15 mg/kg/day	Targets voltage-gated Na channels and possibly acts on high voltage Ca channels.	Focal and most generalised seizures.
Levetiracetam	20–60 mg/kg/day	Targets synaptic vesicle protein 2A, exact mechanism is unknown.	Most seizure types.
Oxcarbazepine	30–40 mg/kg/day	Sodium channel blocker targeting voltage-gated Na channels.	Focal seizures.
Phenytoin	4–8 mg/kg/day, up to 600 mg/day for adolescents.	Sodium channel blocker targeting voltage-gated Na channels.	Focal seizures and generalised seizures.
Phenobarbital	Infants: 5–6 mg/kg/day Children <5: 6–8 mg/kg/day	Activation of $GABA_A$ receptors.	Most seizure types.
Primidone	10–25 mg/kg/day	Activation of $GABA_A$ receptors.	Most seizure types.
Rufinamide	45 mg/kg/day	Sodium channel blocker targeting voltage-gated Na channels.	Focal seizures, drop attacks in LGS.
Tiagabine	<12yo: 4–8 mg/kg/day >12yo: max 32 mg/day	Increases GABA concentration by blocking synaptic GABA reuptake.	Focal seizures.
Topiramate	5–9 mg/kg/day	Thought to act on voltage-gated Na channels, HVA Ca channels, and $GABA_A$ receptors.	Most seizure types.
Valproate	30–60 mg/kg/day	Thought to target Na and Ca channels.	All seizure types.
Vigabitran	Infants: 50–150 mg/day Older children: 500–3,000 mg/day	Increases GABA concentration by inhibition of the mitochondrial enzyme GABA-transaminase.	Infantile spasms, focal seizures.
Zonisamide	5–8 mg/kg/day	Targets voltage-gated Na channels and possibly acts on low voltage Ca channels.	

AED: antiepileptic drug; $GABA_A$: γ-aminobutyric acid A; HVA: high voltage activated; LGS: Lennox Gastaut syndrome; yo: years old.

Neuromodulation is another treatment modality available to children with medication-refractory epilepsy. In 1997 the U.S. Food and Drug Administration (FDA) approved the vagal nerve stimulator (VNS) as an adjuvant treatment for patients aged 12 years and older with refractory epilepsy.[41] In 2017, the VNS was approved by the FDA for children age 4 years and older with medication-refractory focal onset seizures.[41] A number of studies have shown that about half of paediatric patients achieve >50% reduction in seizure frequency.[37,41] However, <5% of patients with a VNS became seizure-free.[37] In addition to VNS, there are newer available neurostimulation therapies, which include deep brain stimulation of the anterior nucleus of the thalamus, responsive neurostimulation, trigeminal nerve stimulation, and transcutaneous vagus nerve stimulation.[37,41,42]

Another available treatment modality for intractable epilepsy is surgery. Over the past decade, there has been significant progress in the imaging and surgical techniques available. The most basic type of epilepsy surgery is the lesionectomy. Candidates for lesionectomy must have failed two or more AED and have epilepsy that is localised to a well-defined region based on EEG and radiological findings.[43] Other available surgical treatments include lobectomy, multilobar resection, hemispherectomy, or disconnection of hemispheres.[43] Recently there have been some trials and advancements in minimally invasive surgical techniques. MRI-guided laser interstitial thermal therapy, radiofrequency thermocoagulation, and magnetic resonance guided focussed ultrasound surgery are all relatively new techniques which allow for the ablation of a desired area of tissue.[44-46] Further studies are needed to validate the efficacy and safety of these new non-invasive techniques.

In recent years, cannabidiol (CBD) has emerged as a new potential AED. CBD has been found to be most effective in refractory epilepsy syndromes such as Lennox Gastaut and Dravet syndromes.[47] Epidiolex is the first CBD medication approved by the FDA for treatment of LGS and Dravet.[41] Some of the suspected mechanisms by which CBD controls seizures include modulating neuronal excitability, antioxidant activity, and anti-inflammatory effects;[47,48] however, the exact mechanism of action remains unknown. Although CBD may provide a promising alternative treatment for refractory epilepsy, recent studies have shown that up 55% of patients discontinue treatment and up to 94% report unwanted side effects, such as somnolence, decreased appetite, diarrhoea, and vomiting.[49,50] The antiseizure activity of CBD has been proven; however, the long-term safety and efficacy of the medication is still under investigation.

TARGETED THERAPIES

The newest advancement in the treatment of paediatric epilepsies is based around the idea of precision medicine or targeted therapy. The continued identification of new genes and mutations underlying these syndromes is allowing for the development of individualised therapies for patients based on the molecular pathophysiology of the mutation found in a specific child. Targeted treatment can range from the use of ketogenic diet in Glut1DS, avoiding AED that act on sodium channels in patients with mutations in *SCN1A*, or the use of a specific drug that targets a specific mutation.

Pharmacogenomics uses the principle that an individual's genetic background affects their response, including efficacy and adverse reactions, to a specific drug.[28,37] There has been progress in identifying genomic predictors of response to various AED, which also has a role in individualising treatment for epilepsy patients. It has been established that cytochrome P450 enzymes are involved in the metabolism of many AED. In particular, it has been shown that polymorphisms in *CYP2C9, CYP2C19*, *CYP2D6*, and *CYP3A4* can affect individual AED metabolism.[28,51]Patients with pathogenic *CYP2C19* polymorphisms can have reduced clearance of phenobarbital, and the metabolism of valproic acid is altered with polymorphisms in *CYP2C9*.[28,52] It has also been shown that the *CYP3A4*1B* variant in Mexican children with epilepsy resulted in multidrug resistance to AED.[51] There is also evidence that a specific G to A polymorphism in the *SCN1A* gene correlates with needing higher doses of carbamazepine for therapeutic effect.[28,53]

The most recent advances in precision medicine are those which are identifying treatments for the damaged products resulting from specific gene mutations. Many of these therapies are still under research or in trial; however, there is much promise for the future of

targeted medications. Everolimus is a medication which targets the mTOR signalling pathway, which regulates cell growth and differentiation in the brain.[48] Tuberous sclerosis has been identified as resulting from mutations in the regulator proteins of mTOR. In both mouse models and clinical trials, everolimus was shown to be effective in treating seizures in tuberous sclerosis.[48] As discussed above, mutations in *GRIN2A*, which encodes for a subunit of the NMDA receptor, have been identified as epilepsy syndromes such as LKS. There have been multiple open label trials investigating the effects of NMDA receptor antagonists as a possible seizure treatment.[28,48] Memantine resulted in reduced seizure activity in a child with early-onset epilepsy with a *GRIN2A* missense mutation;[28,54] however, in another child with a different missense mutation in *GRIN2A* memantine was not effective.[28] A gain of function mutation in the potassium channel gene *KCNT1* was noted to cause the early-onset epilepsy syndrome: epilepsy of infancy with migrating focal seizures. The anti-arrhythmic drug quinidine is a partial antagonist of KCNT1, and has been shown in laboratory studies of *Xenopus* oocytes, and in one confirmed case of a child with migrating focal epilepsy, to significantly reduce seizure activity.[28,48,54] Mutations in *KCNQ2* have been identified in a wide range of childhood epilepsies. Retigabine (or ezogabine) is a drug which acts as a positive modulator of KCNQ2 potassium channels, and it is the first neuronal potassium channel opener used in the treatment of epilepsy. [28,54] As new genes are identified the opportunity for new and individualised treatment grows. Recently, loss of function mutations in *DEPDC5* (Disheveled, Egl-10, and Pleckstrin Domain Containing Protein 5) have been reported in a variety of genetic focal epilepsy syndromes.[54] Research has also shown that mutations in PRICKLE proteins, which are involved in cell polarity signalling pathways, cause seizures in humans, mice, zebrafish, and flies.[54] These newly identified genes may provide additional targets in the development of new precision therapies. The number of genes yet to be identified in paediatric epilepsy is unlimited. Large cohort studies, such as EpiPGX, are using genome-wide analysis in large populations of patients with epilepsy syndromes to identify genetic biomarkers with the goals of improving use of our current AED and identifying new targets for therapies.[54,55]

CONCLUSION

Paediatric epilepsy is one of the most common neurological disorders affecting children across the world. While almost two thirds of paediatric epilepsy patients are efficiently managed with available AED, the remaining one third rely on alternative therapies such ketogenic diet, neuromodulators, and surgery. The ongoing research in genetics and neurobiology holds promise for the development of individualised treatment with optimisation of our current AED, and the development of new targeted therapies. The possibilities remain endless; however, determining the safety and efficacy of new therapies may prove to be challenging and costly.

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At Physiologically Relevant Concentrations, Valproic Acid and Lithium Carbonate Reduce Oxidative Stress in Human Astrocytoma Cells

Authors: *Joana GC Rodrigues, Havovi Chichger

Anglia Ruskin University, Cambridge, UK
*Correspondence to joana.rodrigues1@aru.ac.uk

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Abstract

Background: The pathophysiology of bipolar disorder is largely unknown; however, recent studies have suggested that metabolic dysfunction, particularly at the mitochondrial level, may represent a previously unexplored pathway. Lithium carbonate, valproic acid, and a combination of these represent the mainstay of treatment for bipolar disorder; however, the mechanisms underpinning the drugs' clinical efficacy are not well characterised. At present, such mechanistic studies use concentrations which widely differ from the known bioavailability, thus, there is a need to establish the effect of lithium carbonate, valproic acid, and combination therapy at physiologically relevant doses.

Methods: Human astrocytoma 1321N1 cells were treated for 4, 24, and 48 hours. The MTT method was used to detect cytotoxicity upon drug treatment. Reactive oxygen species (ROS) production was quantified by dichlorofluorescin diacetate fluorescence.

Results: Upon H_2O_2-induced cellular stress, cell viability was significantly reduced; however, lithium exhibited a protective effect. In the absence of the stressor, the drugs had no negative effect on 1321N1 cellular viability. All the drug treatments exhibited protection against H_2O_2-induced ROS accumulation with lithium, bringing it closer to the control baseline.

Conclusion: The findings contribute to the understanding of the drugs' biological effects, particularly as oxidative stress reducers. Furthermore, it highlights the need for research using comparable physiologically relevant models. This may advance the discovery of diagnostic biomarkers and new research approaches to the diagnosis of bipolar disorder.

INTRODUCTION

There is growing evidence for the connection between mitochondrial alterations in psychiatric disorders and psychiatric symptoms in mitochondrial disorders. There are high degrees of overlapping comorbidities and drug efficacies, suggesting that mood affective disorders

incorporate components of mitochondrial dysfunction in their pathogenesis. Research has described mitochondrial structural abnormalities in patients with bipolar disorder (BPD) such as smaller surface areas and abnormal cellular distribution,[1] mitochondrial DNA mutations, and polymorphisms,[2,3] as well as widespread brain energy metabolism abnormalities.[4] It is also common for *bona fide* mitochondrial disorders to be comorbid with psychotic symptoms (71% of patients[5]), which are often misdiagnosed as BPD and/or schizophrenia.[6,7] All of these observations support the role of mitochondria in the clinical presentation of psychosis. Mitochondrial pathology can be a consequence of genetic susceptibility,[7] dysregulation of neurotransmitter systems,[8] or environmental insults such as exposure to famine, infections, toxins, or substance abuse,[9,10] all of which are also risk factors for BPD.[11]

Bipolar Disorder

BPD is a chronic mood affective disorder characterised by episodic behavioural disturbances of mania (or hypomania) and depression. The current International Classification of Diseases (ICD) nosological classification of the disorder divides it into BPD type I (BPD-I), BPD type II (BPD-II), cyclothymic disorder, and recurrent manic episodes not otherwise specified (NOS).[12] While BPD-I is the most severe type, with alternating episodes of mania and depression which often lead to hospitalisation, BPD-II is characterised by hypomania and depression.[12,13] The lifetime prevalence is 0.4–1.0% for BPD-I and 3.0–4.0% for BPD-II.[13] When compared to the general public, a person with BPD has a 20-fold higher risk of suicide.[13] Individuals with BPD often require long-term drug treatment aimed at preventing relapses and recurrences. In the UK, lithium, valproic acid (VPA), olanzapine, and quetiapine are the main therapeutic drugs used for BPD.[14]

Lithium

Lithium is a mood stabiliser, used as a prophylactic agent in BPD as well as in the management of manic or hypomanic episodes. It is a naturally occurring water-soluble ion which does not bind to plasma proteins and is characterised by linear pharmacokinetics (proportional amount of drug administered and its blood concentration). Only lithium carbonate (LICA) and lithium citrate integrate the British National Formulary (BNF). Although the bioavailability of the two preparations is similar,[15] peak lithium concentration is 10% higher in LICA preparations[16] thus, this is most widely prescribed.[17] Lithium does not undergo metabolism and is almost exclusively excreted by the kidneys (renal clearance values of 10–40 mL/minute, strongly correlated with renal function).[18] Lithium is able to cross the blood–brain barrier (BBB) and its brain concentration after therapeutic dosing has been reported to sit between 0.5–1.3-fold the serum concentration, peaking after 24 hours of administration.[19-22] With that, brain concentrations at therapeutic LICA dosing sit between 0.20–1.30 mM; however, currently available *in vitro* studies have not significantly addressed this. For instance, Lopes-Ramos et al.[23] used 2.50–7.50 mM LICA in pheochromocytoma PC12 rat cell culture, De-Paula et al.[24] used 0.02–2.00 mM LICA, and Kurauchi et al.[25] used 1.0–5.0 mM LICA in primary rat hippocampal neuronal cultures.

Valproic Acid

VPA is an anticonvulsant drug, generally used in the treatment of epilepsy. In addition to this, VPA has other clinical indications, including BPD, given its mood-stabilising properties. The absorption of VPA is complete and rapid from the gastrointestinal tract, reaching peak plasma concentration within 4 hours of oral tablet administration.[26] Its metabolism is complex and the majority of the administered dose is excreted in urine in the form of various metabolites.[26,27] Unlike lithium, VPA binds well to plasma proteins (>90%), particularly albumin.[26,28] This binding is saturable, with free VPA fraction higher when total plasma concentrations are elevated.[28] The unbound portion is considered pharmacologically-active and able to cross the BBB, with reports of brain to serum ratios varying from 0.068–0.540 after VPA administration.[29-32] As such, it is estimated that, at therapeutic doses, brain concentration sit between 20–325 µM; however, available *in vitro* studies have not actively addressed this: Tan et al.[33] used 1 mM VPA in primary rat cortical cells; Wang et al.[34] used primary rat astrocyte cells and 0.3–1.2 mM VPA; and Zhang et al.[35] used human glioblastoma U87 cell line and 2–16 mM VPA.

Thus, the aim of this study was to investigate the putatively protective effects of physiologically

relevant concentrations of LICA and VPA on cytotoxicity and oxidative stress in astrocytoma 1321N1 cells. Findings from this study are the first to identify the biological effects of these drugs, alone and in combination, on astrocytoma cellular biology using physiologically relevant doses.

METHODS

Reagents

LICA, VPA, and all other chemicals were procured from Sigma-Aldrich (Haverhill, UK), unless otherwise specified. All chemicals were of high commercial grade.

Cell Culture

1321N1 (human astrocytoma) cells were cultured in complete growth medium made from Dulbecco's modified Eagle's medium high glucose GlutaMAX™ plus 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin. The cells were maintained at 37 °C in a humidified 5% carbon dioxide atmosphere. Approximately $1x10^4$ cells were harvested per assay and incubated for 24 hours in complete growth medium before proceeding. Cells from passage 39–49 were used.

Drugs

LICA (Li_2CO_3, 73.89 g/mol) and VPA ($C_8H_{15}NaO_2$, 166.19 g/mol) were resuspended in water and filter sterilised before use. LICA stock was prepared at a concentration of 10 mM at room temperature, whereas VPA stock was 3 mM and stored at 4 °C, as per the manufacturer's recommendations. The drugs were warmed to 37 °C for successive experiments. The physiologically relevant working concentrations of LICA and VPA were selected on the basis of previously mentioned pharmacokinetic evidence.[19-22,29-32]

Morphology Studies

Cell morphology was monitored at various time points during drug treatment by obtaining brightfield microscopy pictures using the ZOE™ Fluorescent Cell Imager (Bio-Rad). All images were obtained at 175x magnification.

Measurement of Cell Viability

To evaluate the effects of LICA and VPA on cell viability levels, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide MTT assays were used. Cells seeded in 96-well plates for 24 hours were treated with 100 µL/well of treatment and control solutions. The cells were exposed for different periods of time (4, 24, and 48 hours) before 40 µL MTT (5 mg/mL) were added to each well. The cells were then incubated for 2 hours at 37 °C, followed by the addition of 80 µL dimethyl sulfoxide (DMSO) to solubilise the purple formazan crystals. The amount of dye released from metabolically active cells was measured at 620 nm with a microplate reader (Tecan Sunrise™) immediately after DMSO addition. Viability was determined by dividing the absorbance of treated cells by that of untreated (vehicle control) cells.

Quantification of Intracellular Reactive Oxygen Species Levels

Intracellular ROS production was assessed using a ROS-sensitive fluorescent probe, 2',7'-dichlorofluorescin diacetate (DCFDA). Briefly, cells were incubated in black-walled 96-well plates with DCFDA (10 µM/well) for 30 minutes, in dark conditions and at 37 °C. The fluorescent dye-loaded cells were washed once in Dulbecco's phosphate-buffered saline before the addition of 100 µL/well of treatment and control solutions. ROS production was detected as an increase in fluorescence, using the VICTOR™ X3 microplate reader (PerkinElmer) set to do 1-second reads/well at 485 nm excitation and 535 nm emission.

A Model of Cytotoxic and Oxidative Stress Conditions

1321N1 cells were exposed to two concentrations of H_2O_2 (120 and 480 µM), which were co-administered with LICA and/or VPA and incubated for 24 hours prior to MTT or DCFDA assay. This dismutated, non-radical, semi-stable ROS served as stimuli mimicking physiological cytotoxic and oxidative stress conditions in BPD.[36,37]

Statistical Analysis

Unless otherwise specified, all data is expressed as mean ± standard error of the mean (SEM). Data from at least three independent triplicate experiments was analysed using GraphPad to give n=1. Grubb's outlier test was used for all studies to establish any significant outliers. The variance in data sets was analysed using the Mann–Whitney test followed by the T-test. For three or more

groups, variance was assessed by using Bartlett's test with data sets not reaching significance studied by Kruskal–Wallis test followed by Dunn's test. Significance was reached when $p<0.05$.

RESULTS

Lithium Carbonate and Valproic Acid have no Effect on Cell Viability Following Exposure in 1321N1 Cells

The effect of physiologically relevant concentrations of LICA and VPA on cell survival was firstly investigated. MTT analysis indicated that after exposure, neither 1.3 mM LICA, 325 µM VPA, nor their combination (COMBO) significantly affected 1321N1 cell viability (Figures 1a, 1b, and 1c). At 48 hours of exposure, there is a trend for cell viability decrease with COMBO treatment; however, it is not statistically significant (Figure 1c). This is further evidenced by the accumulation of debris observed with brightfield imaging (Figure 1d). At 48 hours, untreated 1321N1 cells are non-confluent and well-organised in layers; however, upon LICA treatment numerous cells appear rounded, withered, disconnected, and floating in the media (Figure 1d). Upon VPA treatment, there is a noteworthy observation of distinctively thinner and longer cells at 48 hours of treatment, which may be indicative of cytoskeletal changes rather than cell death-related pathways (Figure 1d). Therefore, use of these drugs at physiologically relevant concentrations does not affect cell viability but do appear to impact on cellular morphology.

Valproic Acid, but not Lithium Carbonate, Decreases Reactive Oxygen Species Accumulation in 1321N1 Cells

The next study investigated the mechanism through which this cellular stress could be regulated. The possibility that LICA and VPA generated free radicals was assessed using the DCFDA assay. During the time of incubation, it was shown that VPA altered baseline intracellular ROS production by significantly reducing it from 100.0±1.3% to 69.3±2.3% (Figure 1e). Conversely, neither LICA (99.8±3.1%) nor COMBO (99.6±4.7%) affected DCFDA fluorescence (Figure 1e). Thus, VPA exposure, but not LICA or a combination of therapies, decreases ROS accumulation in human astrocytoma cells.

Lithium Carbonate, but not Valproic Acid or a Combination Treatment, Significantly Protects 1321N1 Cells from H_2O_2-Induced Cell Death

Using H_2O_2 as a physiological cellular stress model,[36,37] the effect of LICA and VPA on cell viability was assessed. At 480 µM H_2O_2 treatment, the three drug treatments displayed significantly reduced 1321N1 cell viability, being unsuccessful at restoring cell viability compared to their respective drug-only counterpart (Figure 2a). Only LICA significantly protected 1321N1 from cellular death when compared to H_2O_2-only treatment (Figure 2a). Cell viability in the absence of LICA was 65.6±0.8% for 120 µM H_2O_2 and 50.1±1.7% for 480 µM H_2O_2. In contrast, in the presence of LICA, cell viability was maintained near baseline at 94.8±2.3% for 120 µM H_2O_2 and 71.6±3.4% for 480 µM H_2O_2. Notably, COMBO did not produce any statistically significant protective effect when compared to H_2O_2-only treatment or its drug-only equivalent (at 120 µM H_2O_2). Microscopically, an apparent increase in cell number is present upon drug treatment when compared to H_2O_2-only (Figure 2c). This cellular stressor also triggers cell ballooning ameliorated by all treatments tested presently (Figure 2c).

Lithium Carbonate, Valproic Acid, and their Combination Significantly Attenuate H_2O_2-Induced Reactive Oxygen Species Accumulation in 1321N1 Cells

To evaluate the effect of LICA and VPA on the H_2O_2-induced formation of ROS, DCFDA fluorescence was assayed. LICA significantly reduced ROS production compared to H_2O_2 treatment, bringing it to a comparable level to its drug-only counterpart thus exhibiting a protective effect (Figure 2b). Conversely, VPA and its co-treatment with 120 µM H_2O_2 significantly reduced ROS and are comparable to each other (Figure 2b). Interestingly, VPA co-treatment with 480 µM H_2O_2 resulted in significantly higher ROS build-up than drug-only but close to the 100% baseline. These findings may relate to the relative DCFDA fluorescence of 1321N1 cells, given their significantly decreased viability upon said co-treatment (Figure 2a). That said, there was a decrease in cell viability from 65.6±0.8% in 120 µM H_2O_2-treated cells to 50.1±1.7%

following exposure to 480 µM H_2O_2. This was coupled with a decrease in ROS accumulation from 138.3±3.4% in 120 µM H_2O_2-treated cells to 108±1.9% following exposure to 480 µM H_2O_2. Lastly, COMBO was effective at reducing ROS production both when compared to H_2O_2 and drug treatment (Figure 2b). Taken together, these findings demonstrate that, at physiologically relevant concentrations, all three drug treatments attenuate ROS build-up with LICA being most effective at returning it to a vehicle-comparable baseline.

DISCUSSION

The long-term management of BPD is complex. Both LICA and VPA are recommended by the National Institute for Health and Care Excellence (NICE) as first-line treatment and, indeed, these are the most commonly prescribed drugs for long-term maintenance.[38] In the present study, the cytotoxic effects of LICA and VPA at physiologically relevant concentrations were investigated in human astrocytoma 1321N1 cells.

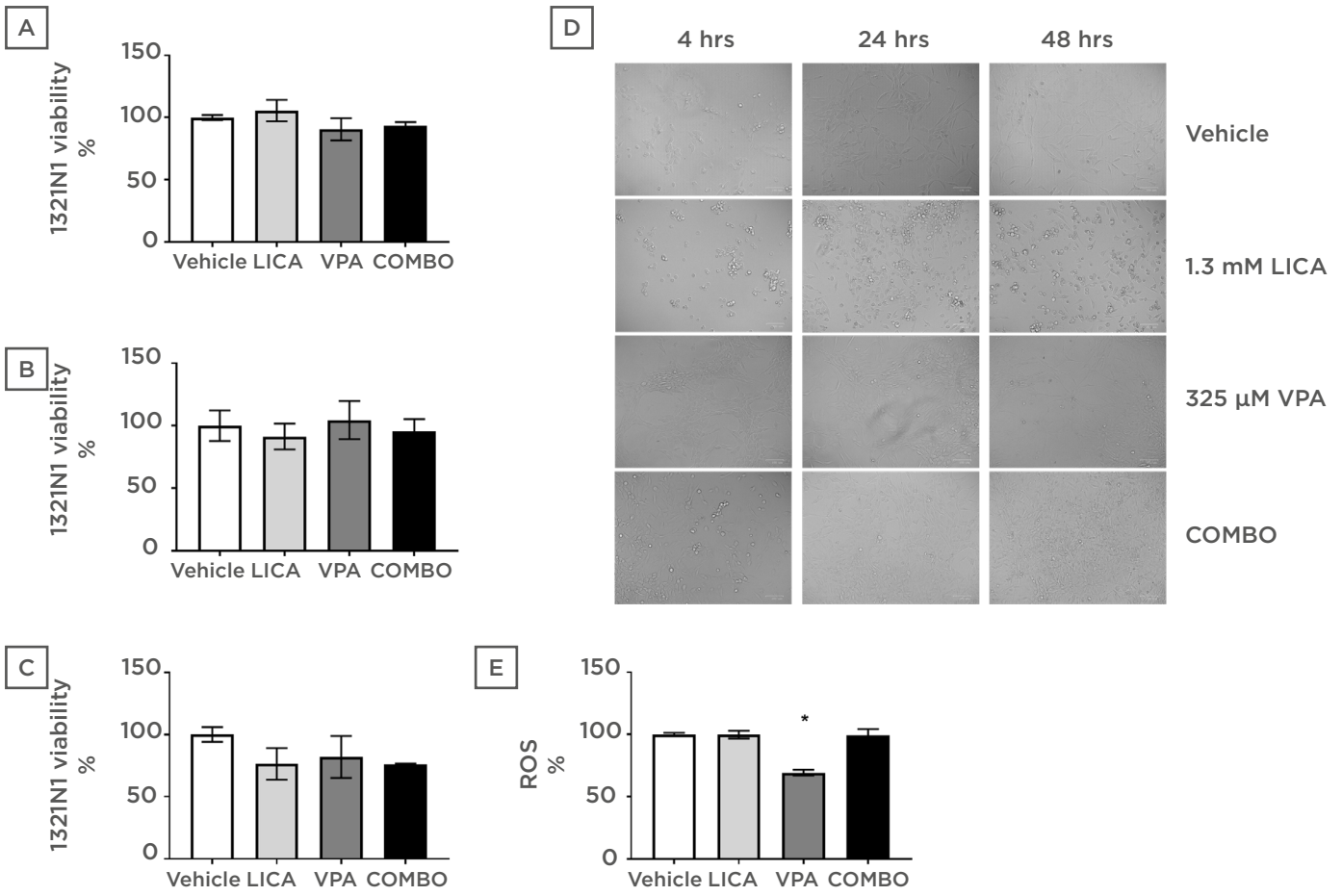

Figure 1: Physiologically relevant concentrations of valproic acid, but not lithium carbonate, significantly reduce oxidative stress in astrocytoma cells.

1321N1 astrocytoma cells were exposed to LICA (1.3 mM), VPA (325 µM), or a combination of the two (COMBO) for 4 (a), 24 (b), and 48 (c) hours. A-C: Cell viability was assessed by MTT assay. D: Morphology was assessed by microscopy with brightfield images. Scale bar represents 100 µm, magnification 175x. E: Oxidative stress was assessed by ROS accumulation using DCFDA dye for 1 hour. Data are presented as mean ± S.E.M with n of 3-4. Data were analysed using one-way ANOVA and compared to vehicle control with significance observed for ROS accumulation in VPA-treated cells compared to vehicle-treated cells. *p<0.05 versus vehicle for LICA and VPA.

DCFDA: 2',7'-dichlorofluorescin diacetate; LICA: lithium carbonate; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide; ROS: reactive oxygen species; VPA: valproic acid.

The present findings demonstrated that in the absence of cellular stressors, none of the presently tested drug treatments affected cellular viability at 4, 24, or 48 hours (Figures 1a, 1b, and 1c). Furthermore, only VPA significantly affected intracellular ROS accumulation, reducing it below baseline levels (Figure 1e). The findings were consistent with the proven safety of these drug treatments, making them the most commonly prescribed medication for BPD.[14]

The present study used a generally unreactive endogenous ROS, H_2O_2, to mimic physiological conditions in BPD. This ROS is present in very low intracellular concentrations under normal physiological conditions but can considerably increase via feed-forward systems under pathological settings.[36,37] The cell viability study demonstrated that only LICA significantly restored cell viability when compared to H_2O_2 (Figure 2a). Nevertheless, all drug treatments significantly ameliorated cell ballooning triggered by H_2O_2 (Figure 2c). This may be indicative of a time-dependent response to the stressor which has not been investigated in the present study.

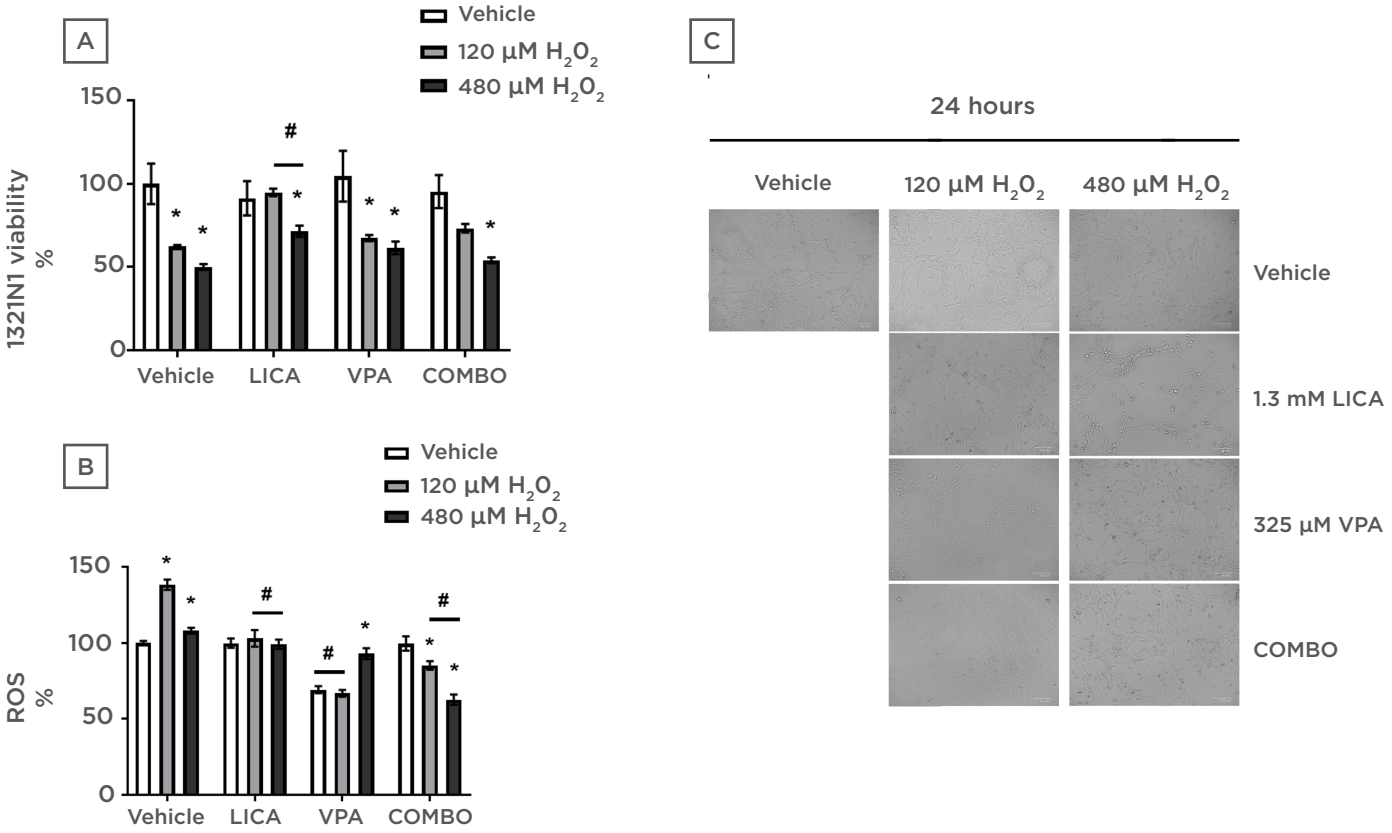

Figure 2: Physiologically relevant concentrations of both lithium carbonate and valproic acid attenuate oxidative stress in astrocytoma cells whilst only lithium carbonate protects against H_2O_2-induced cell death.

1321N1 astrocytoma cells were exposed to LICA (1.3 mM), VPA (325 µM), or a combination of the two (COMBO) in the presence and absence of hydrogen peroxide (120 and 480 µM H_2O_2) for 24 hours. A) Cell viability was assessed by MTT assay. B) Oxidative stress was assessed by ROS accumulation using DCFDA dye for 1 hour. C) Morphology was assessed by microscopy with brightfield images. Scale bar represents 100 µm, magnification 175x. Data are presented as mean ± S.E.M with n of 3-4. Data was analysed using two-way ANOVA and compared to vehicle control for drugs or vehicle for H_2O_2. Significance was observed for cell viability and ROS accumulation when treated 120 and 480 µM H_2O_2 versus the vehicle. *p<0.05 versus vehicle for H_2O_2. Significance was also observed in cell viability and ROS accumulation measurements for LICA-treated and COMBO-treated cells with H_2O_2 co-treatment (120 and 480 µM) versus vehicle-treated cells with H_2O_2 co-treatment (120 and 480 µM H_2O_2). Significance was also observed for ROS accumulation studies for VPA-treated cells treated with vehicle or 120 µM H_2O_2 versus the vehicle for the drug. #p<0.05 versus vehicle for LICA and VPA.

Furthermore, a growing body of research has demonstrated that oxidative damage from ROS is involved in neuropsychiatric disorders.[39] Previous studies have demonstrated that astroglial cells are more susceptible to oxidative stress because of poor antioxidant protection.[40] To test the protective effect of physiologically relevant concentrations of LICA and VPA on H_2O_2-induced oxidative stress, intracellular ROS in 1321N1 cells was monitored by DCFDA assay. At 120 µM H_2O_2, H_2O_2-induced ROS was significantly reduced by the three tested drug treatments (Figure 2b). This suggests that these treatments could also be effective against downstream targets of ROS responsible for astroglial death such as lipid peroxidation.[40] This, allied to the reduced ROS production, would be in accordance with inhibition of oxidative stress and prevention of cell damage. However, the mechanism through which this may occur remains inconclusive in this study, as it may be a result of free radical-scavenging activity (for instance, catalase, glutathione peroxidase, or superoxide dismutase), altered mitochondrial membrane permeability, or a combination of these. At 480 µM H_2O_2, H_2O_2-induced ROS was significantly reduced by LICA and COMBO (Figure 2b). In these conditions, although VPA reduced ROS levels, this was not significant when compared to H_2O_2-only treated cells (Figure 2b). Furthermore, COMBO not only significantly prevented ROS build-up caused by 480 µM H_2O_2 co-treatment, it also reduced this accumulation below its drug-only baseline. This unexpected finding likely correlates to the reduction in cell viability present in this treatment (Figure 2a).

Previous studies have shown that H_2O_2 regulates the balance between bcl-2 and bcl-2-associated X protein (Bax), altering mitochondrial membrane permeability, activating caspase cascades, and upregulating p53, ultimately leading to apoptosis.[11,40] Furthermore, the bcl-2 family of regulator proteins plays an important role in apoptosis and cellular injury during neuropsychiatric pathology progression, including BPD.[11] The process of apoptosis is characterised by morphological changes including decrease in cell volume and chromatin condensation in the nucleus. The morphological findings of treatment with LICA and, to a lesser extent, VPA, could be indicative of this (Figure 2c). Further studies should aim to confirm whether the treatments were inducers of apoptosis in 1321N1 cells, by use of staining agents such as Hoechst 33342.[41] Such methodology would allow for determination of the suggestive apoptogenic effect. Another noteworthy observation was the presence of dark intracellular inclusions after COMBO treatment and, to a lesser extent, VPA (Figure 2c). These could be significant misfolded protein aggregations, which often underlie the toxicity associated with neurodegenerative and neuropsychiatric disorders.[42]

Additionally, the findings in this study should be contemplated within the context of practical limitations. The 1321N1 cell line is commonly used in basic molecular and cellular biology research relating to astroglial cell function[43,44] but is derived from U-118MG cells which are human malignant glioblastomas.[45] The oncogenic modifications associated with such may have altered cellular regulation processes, differing from normal astroglial cells.[46,47] Yet, immortal cancerous cell lines such as 1321N1 are often used in research as they provide several advantages such as being cost-effective, easy to use, and providing a virtually unlimited supply of materials, whilst bypassing ethical concerns associated with animal and human testing.[46] They do, however, hold the limitation of suffering genotypic and phenotypic variation over serial sub-culturing events, therefore meaning they may not adequately represent primary cells.[46] Nevertheless, cell culture models are well-established, frequently used tools in both basic research and drug testing.[43,44,48] To surpass any limitations, further *in vitro* work could aim to confirm the findings using primary brain endothelial cells[43,44] or immortalised brain-endothelial co-cultured cell lines, two recognised BBB models for neuropharmaceutical screening.[47,49] Ultimately, to accurately address the organisational sophistication and myriad interactions between systems in the whole organism, *in vivo* studies would be necessary.

Due to the heterogeneity of BPD and lack of understanding of its pathophysiology, there is no specific biological marker which diagnoses the disease, thus current clinical practice relies on studying the patient's medical history and thorough behavioural assessment.[50] It is possible that the increasingly available research, such as that presented, will lead to a new focus on BPD within which the disease is seen as metabolic leading to new research approaches to

treatment and diagnosis. Importantly, said research should take into consideration the pharmacokinetic evidence available on these drugs, addressing physiologically relevant hypotheses such as that presented.

CONCLUSION

In summary, based on the data provided by the present study, it has been demonstrated that physiologically relevant concentrations of LICA, VPA, and COMBO do play a role on the metabolic hypothesis of BPD by affecting cell viability and ROS production thus further elucidating their clinical efficacy mechanisms. It remains to be explored the exact mechanisms by which these protective effects act on 1321N1 cells; however, this study draws attention to the different trending hypotheses that view BPD as systemic and requiring different clinical approaches as well as further research addressing treatment resistance and mechanisms of efficacy.

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A Possible Explanation For Neurodegenerative Disease

Authors: Richard A. Armstrong

1. Vision Sciences Building, Aston University, Birmingham, UK
Correspondence to R.A.Armstrong@aston.ac.uk

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Abstract

This review proposes an explanation for the pathogenesis of those neurodegenerative diseases which result in dementia and the resulting diversity of their disease phenotypes. The explanation is based on five principal observations, specifically: 1) neurodegenerative disease may be the direct consequence of neural ageing; 2) ageing may cause differential degeneration of neuroanatomical pathways; 3) breakdown of anatomical pathways may result in the formation of 'reactive' proteins; 4) these proteins may exhibit 'prion-like' behaviour and spread along anatomical pathways; and 5) neurodegenerative disease may be characterised by heterogeneity, overlapping phenotypes, and multiple pathology. The explanation proposes that genetic and environmental risk factors act cumulatively over a lifetime to increase an individual's 'allostatic load', which determines the overall rate of neural ageing. This process results in the differential breakdown of neuro-anatomical pathways, influenced by their relative use or disuse during life, the consequence being the formation of one or more reactive proteins. Many of these proteins may spread through the brain from initial sites of ageing along neuro-anatomical pathways to affect specific neural networks. Variation in the proteins formed and in pathways of their spread result in the observed clinical and pathological diversity of disease phenotypes. Hence, minimising the factors that contribute to the allostatic load, together with cognitive and physical exercise to counter disuse of specific anatomical pathways over a lifetime, may be necessary to reduce the incidence of neurodegenerative disease.

INTRODUCTION

In 2015, 46.8 million individuals worldwide had a neurodegenerative disease, with 4.6 million new cases being recorded each year.[1] Many of these neurodegenerative diseases result in dementia and it is these disorders that are largely addressed in this review. The overall prevalence of dementia, calculated by the European Dementia Meta-analysis (EURDEM) of all European studies, is 1.6% and 1.0% for males and females, respectively, in the 65–69 year age class, rising to 11.0% and 12.6% in the 85–89 year age class.[2] Approximately 62% of dementia cases are attributable to Alzheimer's disease (AD), 17% to vascular dementia (VaD) alone, 10% to a combination of VaD and AD, 4% to dementia with Lewy bodies

(DLB), 2% for frontotemporal dementia (FTD), 2% for Parkinson's disease dementia (PDD), and the remaining 3% of dementias for all other causes collectively.[2,3]

Normal physiological ageing often consists of the same changes in the nervous system that can be observed in neurodegenerative disease but at significantly reduced levels.[4-7] Therefore, normal and pathological ageing results in brain atrophy and the formation of proteins in the form of 'signature' pathological lesions. Originally, the majority of neurodegenerative disorders were classified into two major molecular groups: 1) the tauopathies, including AD, Pick's disease, argyrophilic grain disease, progressive supranuclear palsy (PSP), and corticobasal degeneration associated with the microtubule associated protein tau; and 2) the synucleinopathies, including PDD, DLB, and multiple system atrophy associated with the synaptic protein α-synuclein.[8] Subsequently, cases that did not possess either tau or α-synuclein-immunoreactive inclusions were described. First, a proportion of FTLD cases were shown to have inclusions that were immunoreactive to the product of the transcriptor repressor gene (*TARDP*), specifically a transactive response DNA-binding protein of 43 kDa (TDP-43) (FTLD-TDP).[9] Second, neuronal intermediate filament inclusion disease was shown to be associated with the product of the 'fused in sarcoma' (*FUS*) gene.[10] Many of these diseases are therefore characterised by specific neuronal cytoplasmic inclusions, such as neurofibrillary tangles (NFT), and/or protein deposits such as the β-amyloid (Aβ) deposits in the form of senile plaques (SP) in AD and prion protein (PrP^{sc}) deposits in Creutzfeldt–Jakob disease.

In most neurodegenerative disorders, there are small numbers of cases linked specifically to gene mutations and a larger number of sporadic cases not directly linked to genetics. Quantitative studies have demonstrated considerable similarities in the pathology of familial and sporadic forms of various diseases. Hence, variation in Aβ deposition was studied across several disorders including familial and sporadic AD using principal components analysis.[11] Aβ deposition varied continuously across these disorders and did not distinguish between the familial and sporadic forms. In addition, there were no essential differences in the spatial patterns of Aβ deposits in familial and sporadic AD, both being distributed in regularly spaced clusters.[12] There were no differences either in the spatial patterns of AD cases expressing or not expressing the apolipoprotein E (APOE) allele ε4, a major risk factor for AD.[13,14] Furthermore, laminae distributions of Aβ deposits, which indicate the pattern of cortical degeneration, were similar in familial and sporadic AD, and the cortical layer at which Aβ deposits reached maximum density and the maximum density were also similar.

Similar results have been reported for familial and sporadic cases of FTLD with TDP-43-immunoreactive pathology (FTLD-TDP) although some differences in familial and sporadic FTLD-TDP have been reported. Hence, cases with and without progranulin (*GRN)* mutations have similar demographics, but *GRN* cases often have greater language deficits.[15] Pathologically, cases lacking *GRN* mutations may have a less severe pathology affecting the neocortex and striatum.[16] By contrast, a quantitative study of 94 cases of FTLD-TDP using principal components analysis suggested that the familial cases as a whole did not have a pathological phenotype that was distinct from the sporadic cases. In addition, the frequencies of the different laminar distributions in FTLD-TDP associated with *GRN* mutations[17] were similar to those previously reported in sporadic FTLD-TDP,[18] suggesting that the *GRN* mutations do not determine a specific pattern of laminar degeneration in FTLD-TDP. Hence, an explanation for neurodegenerative disease needs to explain the similarity of its familial and sporadic subtypes.

Given the present and future potential burden on health systems worldwide and the absence of widespread effective therapies, explanations are needed to account for the pathogenesis of neurodegenerative disease that collectively can lead to new treatment strategies. Based on interpretation of the literature, this review proposes an explanation of the pathogenesis of those neurodegenerative diseases resulting in dementia and attempts to account for the diversity of disease phenotypes. The explanation is based on five principal observations: 1) neurodegenerative disease may be the direct consequence of neural ageing; 2) ageing may cause differential degeneration of neuroanatomical pathways; 3) breakdown of anatomical pathways may result in the formation of 'reactive' proteins; 4) these

proteins may exhibit 'prion-like' behaviour and spread along anatomical pathways; and 5) neurodegenerative disease may be characterised by heterogeneity, overlapping phenotypes, and multiple pathology.

THE FIVE OBSERVATIONS

Neurodegenerative Disease May be the Direct Consequence of Neural Ageing

Epidemiological studies frequently agree that the greatest factor associated with neurodegenerative disease is age.[4] In addition, in AD[6] and PDD,[5,7] there is direct evidence that neurodegeneration may be an accelerated form of ageing. Thus, most if not all AD neuropathological change (ADNC)[19] also occurs in normal aged brains,[20] including the enlargement of ventricles and loss of synapses and dendrites,[21] together with the 'signature' histological features of AD (SP[22] and NFT[23,24]). In addition, using Pittsburgh compound-B PET, a specific marker for Aβ deposition and therefore SP, Aβ was observed in 10–30% of healthy elderly patients.[25] There is often considerable overlap in Aβ deposition in the normal elderly and in disorders such as AD and DLB (Figure 1), such that some control cases have greater densities of Aβ deposits than AD and DLB and some cases of dementia have very low densities. Other molecular markers of neurodegeneration also occur in a normal brain; phosphorylation and truncation of α-synuclein, which are characteristic of the 'synucleinopathies' Parkinsons's disease (PD), PDD, DLB, and multiple system atrophy, are also normal events in the adult human brain.[26] Moreover, in 110 cognitively normal individuals, 36% exhibited TDP-43,[27] the pathological hallmark of a common subtype of FTLD.[28] These data suggest that normal physiological ageing and neurodegenerative disease essentially share common cellular and molecular processes.

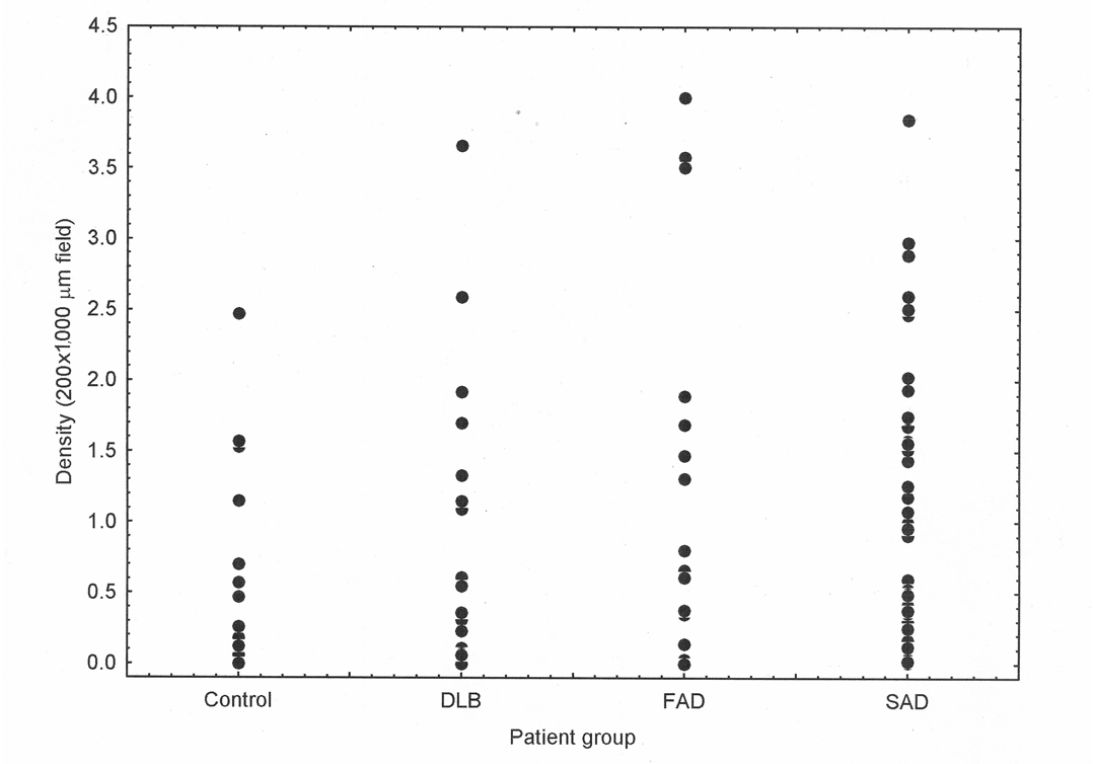

Figure 1: There is considerable overlap in the density of β-amyloid deposits between control and disease groups.

β-amyloid (Aβ) deposition in cases of normal elderly (control) brain, dementia with Lewy bodies, familial Alzheimer's disease, and sporadic Alzheimer's disease showing considerable overlap in the density of Aβ deposits between control and disease brains, between DLB and AD, and between FAD and SAD.

DLB: dementia with Lewy bodies; FAD: familial Alzheimer's disease; SAD: sporadic Alzheimer's disease.

Ageing May Differentially Affect Anatomical Pathways

The efficiency of brain function depends on its long and short-range anatomical connections, there being fewer long-range connections as greater resources are required to maintain them.[29] Normal adolescence is characterised by selective strengthening of the long-range connections, while ageing is associated with marked structural changes in the brain, including cortical thinning, degradation of myelin, and reduced connectivity. These changes especially affect the long-range connections, including those involving the basal forebrain, substantia nigra, locus caeruleus, and raphe nucleus.[30] This reduced connectivity often results in a functional reorganisation later in life to compensate for the structural losses attributable to ageing.[30] The pathways vulnerable to ageing include the structural covariance networks that subserve the language-related semantic network; the executive control network; the default-mode networks;[31] the hippocampal network, which can affect memory function;[32] and the resting state motor network.[33] Hence, the selective disruption of anatomical pathways observed in different neurodegenerative disorders could result from the differential effects of ageing.[34] Relative use or disuse during a lifetime could determine this selective disruption. Therefore, in individuals that suffer early blindness, there is significant reduction in white matter volume in the optic tracts, and radiation and significant loss of grey matter in the visual cortex.[35] By contrast, physical activity may maintain or even restore pathways degraded by ageing.[30,36-39]

Breakdown of Anatomical Pathways Attributable to Ageing May Result in the Formation of 'Reactive' Proteins

Abnormally aggregated or misfolded proteins in the form of cellular inclusions have played a key role in the diagnosis, classification, and studies of pathogenesis.[40] An important question is whether the deposition of abnormal proteins is a causal factor or a consequence of neurodegeneration.[41] This question is controversial because protein aggregates may be either non-toxic (i.e., found in normal cells) or toxic, thus contributing directly to both primary and secondary phases of degeneration. The major evidence for a direct causative effect of aggregated proteins comes from studies of familial disease, their pathological phenotypes being similar, apart from age of onset, to those of sporadic forms of the same or related diseases.[42,43] As a result, studies of gene mutation have had a major influence on the development of theories as to the pathogenesis of neurodegenerative disease as a whole. In familial disease, the major molecular constituent of a lesion is regarded as the residue of a direct or indirect effect of a pathogenic gene mutation that, via the accumulation of an insoluble protein aggregate, directly leads to cell death. This type of theory is best exemplified by the 'amyloid cascade hypothesis' proposed to explain the pathogenesis of AD, in which deposition of Aβ is the primary pathological event resulting in NFT, cell death, and eventually dementia.[44]

Nevertheless, a number of observations also suggest aggregated proteins are 'reactive' and a consequence of neurodegeneration. Firstly, the morphology and molecular constituents of cellular inclusions are dependent on cell type and location; cortical and subcortical NFT in AD, for example, comprise morphologically similar but antigenically different paired helical filaments.[45] By contrast, cortical and brain stem Lewy bodies (LB) are morphologically different but antigenically similar,[46] brainstem LB having an electron-dense core with radially oriented filaments differing significantly from cortical LB. Secondly, in cases of traumatic brain injury, amyloid precursor protein (APP) occurs in neuronal perikarya and in the dystrophic neurites surrounding Aβ deposits suggesting the production of APP as a response to neuronal injury.[47] Specific neurons in the medial temporal lobe also secrete large quantities of APP and more APP-immunoreactive neurons present in these areas in cases of traumatic brain injury.[48] Consequently, increased expression of APP after head trauma could be an acute-phase response to neuronal injury,[49] with the overexpression of APP leading to increased deposition of Aβ. Thirdly, experimental damage to the nucleus basalis in rats decreased cortical choline acetyltransferase, elevated cortical peptides such as somatostatin and neuropeptide Y,[50] and caused neuronal loss and the formation of SP in the cortex. Lesions of the nucleus basalis also elevated APP synthesis in the cerebral cortex suggesting a specific response to loss of functional

innervation.[51] Finally, the formation of NFT in AD may also be part of the neurons response to injury;[52] thus, denervation of dopamine pathways and septal lesions affect both the cholinergic system and GABA neurons projecting to the dentate gyrus, and result in a loss of dendritic MAP2 and the appearance of tau-immunoreactive dentate gyrus granule cells.[53]

Proteins May Spread Along Anatomical Pathways

Studies suggest an association between neurodegenerative disease and the breakdown of specific neuroanatomical pathways.[54] Populations of neurons lost in a particular disease often share a common metabolic abnormality and, therefore, neuronal connections between different regions could specify the pattern of cell losses in each disease.[55] Research has confirmed these ideas and suggests that pathogenic proteins, including tau; α-synuclein, the disease form of PrP^{sc}; and Aβ may be secreted from cells, enter other cells, and seed small intracellular aggregates within these cells.[56,57] Pathological proteins, such as tau and α-synuclein, could exit cells via exocytosis or secretion and enter a new cell by endocytosis or by interactions with membrane lipids. Transfer may also occur via tunnelling nanotubes, which connect various neurons.[57] Much of the support for pathological spread comes from *in vitro* experiments and there is less evidence from anatomical studies of neurodegenerative disease. However, if proteins spread from cell to cell in the cortex, the resulting inclusions may exhibit a spatial pattern that reflects this spread. Previous studies have suggested non-random distributions of the inclusions in the cerebral cortex in various disorders, the inclusions often exhibiting a distinct clustering pattern consistent with their spread via the cortico-cortical pathways.[58]

Neurodegenerative Disease may be Characterised by Heterogeneity, Overlap, and Multiple Pathology

Neurodegenerative disease comprises a wide diversity of clinical and pathological phenotypes.[59] First, there is considerable variation in the severity and distribution of the pathology within many individual disorders, most notably in AD[60] and FTLD.[28,61] Second, many studies report 'overlap' between closely related disorders (i.e., coexistence of clinical and/or pathological features of more than one disorder in the same case).[62] Third, many examples of more extensive 'multiple pathology' have been reported.[63] In the parkinsonian syndromes, 38% of cases of PD have ADNC, 9% have PSP, 25% have argyrophilic grains, and 24% have congophilic amyloid angiopathy; in DLB, 89% have ADNC pathology, 1% have PSP, 21% have argyrophilic grains, and 25% have congophilic amyloid angiopathy.[7] In addition, in a comparative survey of 1,032 cases representing ten different disorders, 361 cases (approximately 35% of the sample) were excluded because of multiple pathology.[27] Multiple pathology is a consequence of either the co-occurrence of different pathologies by chance or the induction of one pathology by another. Hence, the coexistence of AD and PD is common because both disorders show a rapid increase in incidence with age and there is a high probability that both could coincide in the same individual.[64] Alternatively, the presence of one type of pathology may encourage or induce the formation of another; e.g., the amyloid cascade hypothesis proposes that the formation of Aβ is the initial pathological event in the cascade directly leading to the formation of NFT.[43]

A POSSIBLE EXPLANATION

The explanation proposed in Figure 2 is that neural ageing is the initial trigger to neurodegenerative disease and is mediated by the 'allostatic load', i.e., the degree of lifetime stress experienced by an individual. The brain is the ultimate mediator of stress-related mortality through hormonal changes resulting in hypertension, glucose intolerance, cardiovascular disease, and immunological problems.[65] Henderson[4] also concluded that it was unlikely that genetic or environmental factors act directly, but that they accentuate some general process that occurs in the brain with age. It was postulated that the common feature associated with many of the risk factors is that they act by promoting the increasing liberation of oxygen free radicals, which exacerbates the rate of normal ageing, ultimately resulting in neurodegenerative disease. Second, this process results in synaptic disconnection and the differential breakdown of neuroanatomical pathways related, in part, to their degree of use or disuse during life. Third, synaptic

disconnection results in the upregulation and deposition of various 'reactive' proteins such as Aβ, tau, α-synuclein, TDP-43, and FUS.[51,66,67] Fourth, once a protein is formed, cell-to-cell transfer among interconnected neuroanatomical regions may occur, which results in recruitment of further pathogenic protein,[56,57,68] as well as disruption of the blood–brain barrier resulting in an immunological response.

The most overt manifestation of this process is in individuals with specific gene mutations that directly influence the outcome of age-related degeneration by determining the solubility and/or toxicity of the molecular products, and which rapidly overwhelm the cellular protection systems causing early-onset disease. By contrast, in individuals without a specific genetic mutation, the outcome is mainly soluble and smaller quantities of insoluble proteins that are degraded by the cellular protection systems and do not significantly accumulate to form pathogenic lesions until much later in life, causing late-onset sporadic forms of disease: often phenotypically similar to their familial counterparts.[55] Variation in the observed disease phenotype results from differential vulnerability of specific neural pathways to the accumulating allostatic load and the effects of oxidative damage; genotypic variation, which determines the outcome of cellular degeneration and, therefore, the number, type, and frequency of proteins formed; and variations in the pathways of spread of various proteins along neuroanatomical pathways. The ultimate result of these processes is the complex overlap of many different pathologies, cases of neurodegenerative disease essentially forming a 'spectrum' or 'continuum' (Figure 3).[59] In this hypothesised scheme, APOE genotype largely determines the degree of Aβ deposition with individuals expressing alleles ε2 or ε3 being associated with lower levels of Aβ deposition compared with those expressing allele ε4.[69] Cases are further defined by which reactive proteins are formed: tau, α-synuclein, TDP-43, PrP^{sc}, and FUS; each of these categories of disease may or may not be associated with Aβ depending on APOE genotype. Hence, cases in which Aβ and tau are predominant are 'typical' of AD and cases with tau deposition but little Aβ of the 'classical' tauopathies such as PSP, CBD, or AGD.

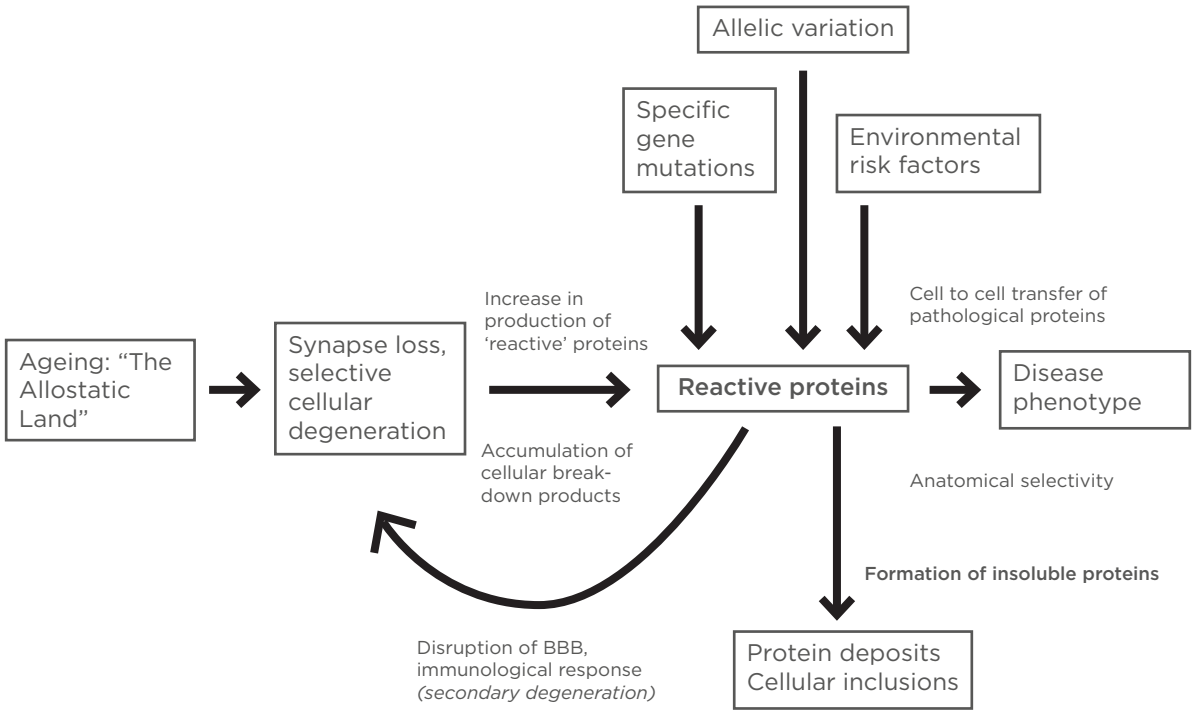

Figure 2: An explanation of the pathogenesis of neurodegenerative disease based on the five principal observations.

BBB: Blood–brain barrier.

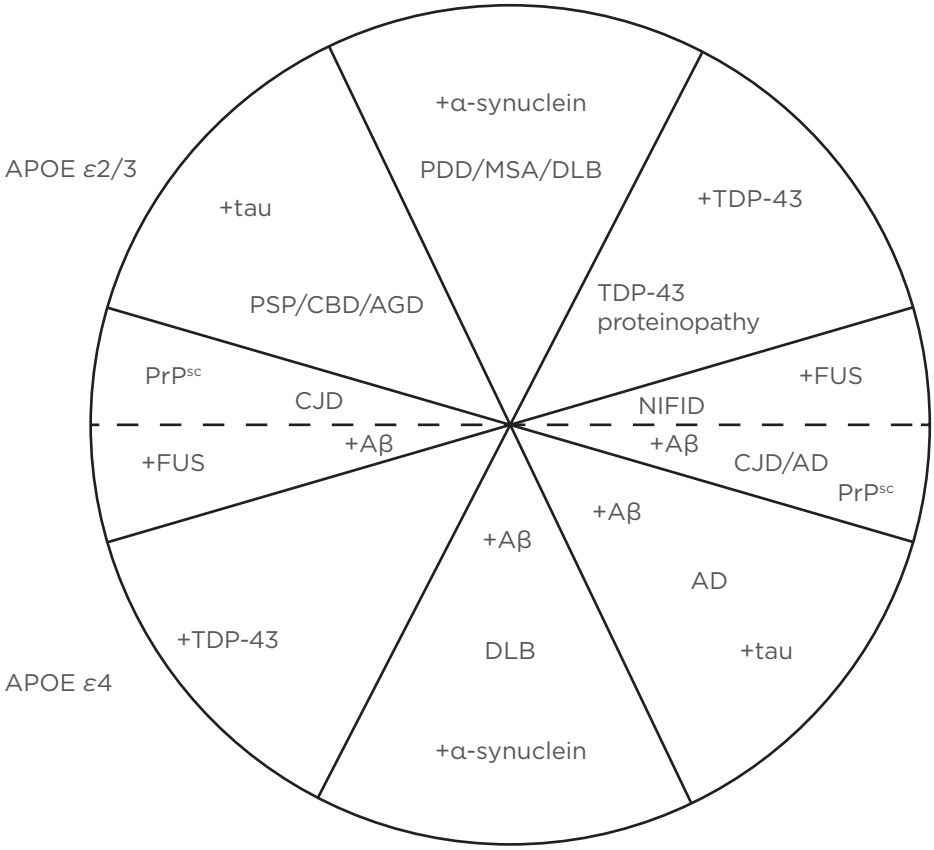

Figure 3: The 'spectrum' of neurodegenerative disease resulting from the proposed explanation in Figure 2.

Upper quadrants represent cases that express apolipoprotein E (APOE) genotypes ε2 or ε3 and are associated with low levels of β-amyloid (Aβ) deposition, while lower quadrants express APOE ε4 and are associated with significantly higher levels of Aβ deposition. Upper and lower quadrants are also defined by the formation of other major 'reactive' proteins, i.e., tau, α-syn (α-synuclein), prion protein, TDP-43, and FUS. Each of these categories of disease may or not be associated with Alzheimer's disease neuropathologic change in the form of Aβ depending on APOE genotype. Hence, in cases located in the bottom right quadrant, Aβ and tau are predominant which is typical of Alzheimer's disease, whereas cases located in the upper left quadrant are characterised by tau deposition but little Aβ typical of the 'classical' tauopathies, e.g., progressive supranuclear palsy, corticobasal degeneration, and argyrophilic grain disease. Boundaries between these groupings are unlikely to be distinct and there is continuous variation in disease phenotype both around the circumference and along the radii.

AD: Alzheimer's disease; AGD: argyrophilic grain disease; APOE: apolipoprotein E; CBD: corticobasal degeneration; CJD: Creutzfeldt–Jakob disease; DLB: dementia with Lewy bodies; FUS: 'fused in sarcoma'; MSA: multiple system atrophy; NIFID: neuronal intermediate filament inclusion disease; PDD: Parkinson's disease dementia; PrPsc: prion protein; PSP: progressive supranuclear palsy.

Predictions

First, this explanation predicts that many risk factors would be associated with neurodegenerative disease; in fact, any factor that can be shown to enhance the allostatic load is a potential risk factor. Several studies have confirmed this prediction, with the seminal review by Henderson,[4] for example, identifying >20 different risk factors in AD, and several risk factors have also been identified in PDD.[27] Second, there are individuals that reach considerable age without exhibiting neurodegenerative disease and, therefore, may represent a 'survival elite'.[4] The explanation predicts that such individuals would carry a low allostatic load. Third, as neural ageing is predicted to be the initial trigger of neurodegenerative disease, the effect of a gene mutation in transgenic experiments should be age-dependent, which has been demonstrated in a number of experiments.[70-73] In addition, in the animal model for *TgF344-AD*, which incorporates mutant *APP* and *PS1* genes, age-dependent

amyloidosis, tauopathy, gliosis, apoptotic loss of neurons in the cortex and hippocampus, in addition to cognitive disturbance, was observed and may offer a more complete model of AD.[74] Fourth, significant signs of neuronal degeneration as a result of ageing should precede the deposition of pathological proteins especially in sporadic disease. This statement is controversial and needs investigation because there are few current observations that indicate neuronal degeneration occurs prior to aggregated protein formation. Fifth, all 'classical' forms of neurodegenerative disease should exist with and without ADNC, a prediction already borne out by many disorders, such as AGD, CBD, Creutzfeldt–Jakob disease, DLB, PDD, and VaD, but less evident in PSP and MSA.[62] Sixth, familial and sporadic forms of the same disease should have essentially the same phenotypes, an observation borne out by several studies.[42,43]

Limitations

The explanation presented has a number of limitations and also relies on controversial assumptions. First, there is limited data on the changes in brain connectivity with age leading to mild cognitive impairment and dementia. Second, there is limited evidence for the spread of aggregated proteins in cases of neurodegenerative disease, especially involving TDP-43 and FUS. Third, transgenic experiments do not always examine the influence of age on the developing pathology and, although pathological changes may be age-dependent, it is unclear whether they are a consequence of ageing as well as the genetic changes. Fourth, whether there are specific differences between familial and sporadic forms of the same disease is controversial. Fifth, whether the formation of aggregated proteins is a primary ('causal') or secondary ('reactive') process is an important element of the explanation and remains to be elucidated. The explanation assumes that the proteins are largely reactive and spread, 'prion-like', along neuroanatomical pathways. Further data on all these aspects are required to fully test the proposed explanation.

Implications

Given this explanation, it is less likely that many forms of neurodegenerative disease can be effectively treated by simple pharmacological intervention.[75] Instead, the explanation suggests that attention should also be directed to reducing those factors that contribute to the life-time cumulative effects of allostatic load and oxidative damage[52] and to encourage activity that contributes to exercising both cognitive and motor pathways throughout life. Reducing the allostatic load will require the identification of modifiable lifestyle and health-related variables to identify optimal combinations of such factors that could slow down the development of dementia.[75] Current evidence is controversial and does not provide a sound basis for making specific recommendations and this question awaits further detailed study. The explanation suggests that exercise of a specific brain pathway may reduce the risk of a particular disease, such as cognitive exercise reducing AD and motor exercise PD. Some studies suggest that moderate intensities of physical activity over a lifetime may protect against volumetric brain loss most commonly affecting the prefrontal cortex and the hippocampus.[37] In a further study, regular physical activity resulted in pathways less affected by typical age-related decline in cognitive function.[19] In addition, individuals who exercised regularly reduced the risk of AD, the beneficial effect mediated by brain-derived neurotrophic factor acting on neuroplasticity and stress resistance,[76] results not necessarily consistent with the explanation suggested in this review. In PD, however, there is evidence that heavy leisure-time physical activity may lower the risk of disease consistent with the hypothesis that continued exercise of the motor pathways may reduce their rate of aging.[38] In addition, treadmill exercise in a murine model of PD improved motor performance and reduced α-synuclein expression while promoting tyrosine hydroxylase, dopamine transfer, and plasma dopamine levels.[28] Thus, differential ageing resulting from variations in level of activity could be an important factor influencing the anatomical selectivity observed in neurodegenerative disease.[39]

CONCLUSIONS

Based on the literature, this review proposes an explanation of the pathogenesis of neurodegenerative disease and the diversity of its disease phenotypes based on 5 principal

observations: 1) neurodegenerative disease may be the direct consequence of neural ageing; 2) ageing may cause differential degeneration of neuroanatomical pathways; 3) breakdown of anatomical pathways may result in the formation of 'reactive' proteins; 4) many of these proteins may exhibit 'prion-like' behaviour and spread along anatomical pathways; and 5) neurodegenerative disease may be characterised by heterogeneity, overlapping phenotypes, and multiple pathology. The explanation suggests that reducing the extent of the allostatic load over a lifetime and encouraging activity to exercise both motor and cognitive brain pathways may be necessary to reduce the incidence of neurodegenerative disease.

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The Autonomic Nervous System: Delineating Historical Landmarks and Their Translation to Target Autonomic Dysfunctions in Multiple Sclerosis

Authors: Uwe Ernsberger

Neuroscience Center, Institute for Clinical Neuroanatomy, Johann – Wolfgang – Goethe University, Frankfurt/Main, Germany

*Correspondence to ernsberger@outlook.de

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Abstract

The integrative action of the autonomic nervous system (ANS) and its cellular constituents promotes the harmonic cooperation between different organs and functional units of the body. Research into its structure and physiology has promoted key advances in system, cellular, developmental, and clinical neurosciences and brought forward a range of milestone concepts central to neuroscience.

ANS dysfunction in multiple sclerosis entails a range of prominent challenges concerning cardiovascular control, thermoregulation, and pelvic organ function. They not only derail the smooth interaction of bodily functions but also negatively impact the course of the disease. With the resolution of central questions on ANS function, different strategies and pharmaceutical approaches emerged to handle these autonomic dysfunctions.

INTRODUCTION

Multiple sclerosis (MS) is first perceived as a disease affecting voluntary motor and sensory systems. The autonomic dysfunctions associated with the disease receive less publicity yet pose a major burden because they critically affect the daily lives of patients and deteriorate the course of the disease. Because of the tight interplay between the study of the autonomic nervous system (ANS), the development of major concepts in neuroscience, and the progress in drug development, their comparative discussion makes a showcase for the progress and the shortcomings of battling such a complex disease.

A Short History of the Autonomic Nervous System

With the monographs of John Newport Langley (1921)[1] and Walter Holbrook Gaskell (1920),[2] the autonomic or involuntary nervous system became a well delineated target of exploration in a range of biomedical sciences. They addressed the questions of how this system operates, how it develops, and how it is altered in disease.

Physiological and pharmacological studies throughout the 20th century shaped the picture of the ANS as a key to the neurobiology of homeostasis.[3] In addition, developmental studies promoted insight into the generation and integration of its cellular elements into autonomic neural circuits.[4] Moreover, molecular analysis reshaped current understanding of the distinct ANS domains into developmentally and molecularly distinct branches.[5]

Already in the 18th century, Jaques–Benigne Winslow (1732)[6] attributed the sympathies of the body to a set of nerves, including the great sympathetic, then called the intercostal nerve, and the medium sympathetic nerve, the par vagum. The subdivision of the ANS into sympathetic and parasympathetic domains was then established on anatomical, physiological, and pharmacological grounds by Langley[1] and Gaskell.[2] The demonstration of the interaction in the regulation of the heart and other organs resulted in a picture of two largely antagonistic nerve nets whose integrative power adjusts the organs performance to the actual bodily requirements.

The cardiovascular control in fact constitutes one essential task of the ANS appropriating the local blood supply while synchronising blood flow and temperature control. The ground-breaking work by Otto Loewi and Henry Hallett Dale paved the way for understanding this communication between the nervous system and target organs such as the heart. In his landmark study on the action of the vagus and sympathetic nerves on frog hearts, Loewi in 1921[7] demonstrated the release of soluble substance(s) mediating the nervous information to the target organ. The Vagus Stoff, the mediator of vagus nerve stimulation that was postulated by Loewi, was characterised by Dale as acetylcholine released by the nerve.[8] Only later, the active substance released by sympathetic nerves was identified as noradrenaline by von Euler.[9,10] Consequently, the antagonistic interaction of the sympathetic and parasympathetic system (not only into the heart) became understood in an initial, oversimplified model as the balance between noradrenergic and cholinergic transmission to the target organs.

A Short Glimpse at the Autonomic Dysfunctions in Multiple Sclerosis

Since the publication by Wilhelm Uhthoff in 1890[11] on visual disturbances in patients with 'multiple Herdsklerose' as a consequence of physical exercise and increases in body temperature, heat intolerance and thermoregulatory dysfunction[12,13] are recognised as key pathophysiological features in MS.

The delineation of the precise causes of heat intolerance poses a complex enterprise as disturbances in the peripheral sensory, central processing, and peripheral efferent pathways may be involved. Alterations in sweat production, one regulatory arm operated directly by the sympathetic nervous system (SNS), in individuals who are experiencing relapsing remitting MS[14] point to a compromised integration of the SNS outflow as contributing to the compromised thermotolerance under load.[15]

In addition, orthostatic intolerance is frequently detected in MS patients[16] by heart rate and blood pressure measurements, albeit cardiovascular autonomic dysfunctions figure less prominent than, for example, in Guillain-Barré disease. Yet, the lower than normal SNS activity[17] is considered to contribute not only to cardiovascular problems but also to immune inflammatory and neurodegenerative processes, promote osteoporosis, and favour fatigue and depressive episodes.[18,19] Thus, sympathetic dysfunction may not only be a consequence of the disease but also affect its course. While sympathetic dysfunction correlates with the clinical activity of MS, parasympathetic dysfunctions are indicators of the progression of disability.[20]

Despite the limitations posed by compromised cardiovascular and temperature regulation, dysfunctions of the pelvic organs may have more dramatic effects on the lives of individuals with MS. Disturbed control of bladder[21-23] and bowel[24,25] may not only have serious consequences on the daily lives but also result in secondary

complications, in particular urinary tract infections. In addition, sexual dysfunction may be a feature in MS.[26,27] The lumbar and sacral ANS is central to the co-ordination of these pelvic organs' function. Developmental analysis of the sacral pre and post-ganglionic components demonstrated close relationship to the thoracolumbar domains and reclassification of this entire region as sympathetic.[5]

THE HISTORY OF SCIENTIFIC CONCEPTS ON AUTONOMIC NERVOUS SYSTEM FUNCTION AND THE ADVANCEMENT OF THE THERAPEUTIC OPTIONS FOR AUTONOMIC NEUROGENIC DYSFUNCTION

The Search for the Transmitting Principle

With the work of Langley[1] at the turn to the 20th century, a decisive change in the experimental approaches to ANS function was introduced in the use of diverse purified natural substances such as adrenaline, nicotine, curare, or pilocarpine. One critical observation was that stimulation by injection of adrenaline in an experimental animal elicited effects similar to those of sympathetic nerve stimulation by brief tetanising inductive shocks, whereas application of pilocarpine provoked effects similar to stimulation of parasympathetic nerves. In addition, Langley observed that the 'end apparatus'[1] of the nerve fibre was responsible for paralysing effects of curare acting on preganglionic fibres to then raise the question for the 'receptive substance'[1] that mediates the action in the target tissue. Thus, Langley's work opens the search for the transmitting principles of neurones which is to become one key topic in the neurosciences as well as a main road in drug development. This entails the question for the 'receptive substance' as well as the 'end apparatus'.

Proof that the transmitting principle may be a soluble substance released from the nerve was provided by Loewi[7] at the same time that Langley published his monograph. The identification of this substance by Dale[8] as acetylcholine resolved the molecular identity of the first classical neurotransmitter molecule. In addition, Loewi provided evidence for the release of an adrenaline-like substance as a result of sympathetic nerve stimulation. Although the opinion that adrenaline is the sympathetic mediator held until the late 1930s, a range of discrepancies, in particular pharmacological in nature, ultimately culminated in the demonstration of noradrenaline as the sympathetic neurotransmitter.[10]

The Quest for the Receptive Substance

At the turn to the 20th century the structure of adrenaline, the active principle of the adrenal medulla, became uncovered due to the work of Abel, Takamine, Fuerth, Aldrich, and others (see references).[28] At the same time, structurally related substances became available for physiological experimentation. The studies demonstrated that 'an action simulating that of the true SNS is not peculiar to adrenine, but is possessed by a large series of amines' and coined the term 'sympathomimetic'.[28] The comparison of the effects of these sympathomimetic substances on different cardiovascular parameters in a range of mammalian species provided evidence that there are 'two types of adrenotropic receptors'.[29] 'The alpha[...]receptor is associated with most excitatory functions (vasoconstriction...) whereas the beta receptor is associated with most of the inhibitory functions (vasodilation...)'.[29]

Further characterisation, isolation, and cloning unfolded the diversity of the receptor family members involved in adrenergic signalling. With the purification and isolation, by affinity chromatography with conjugates, of noradrenaline linked to agarose beads from canine ventricular myocardium, the hypothetical 'receptive substance'[1] or 'adrenotropic receptor'[29] turned into a 'physicochemical entity', the β-adrenergic receptor.[30,31] The molecular cloning of the receptors, starting with the mammalian β2 adrenergic receptor,[32] turned into the identification and structural characterisation of the two families of α and β adrenoceptors[33] underlying adrenergic and noradrenergic regulatory signalling. Also, the muscarinic receptors mediating acetylcholine signalling in autonomic target tissues constitute a gene family, and comparison with adrenoceptor genes demonstrated that the gene families are closely related.[34]

The Diversity of Transmitter Receptor Molecules – A Road to Improve Sympathomimetic Specificity

The observation of different types of adrenergic receptors in the diverse autonomic target tissues[29] was complemented by the demonstration of tissue-specific expression patterns as described already for the first cloned receptor genes.[32] The findings allowed plausible explanation of the wide ranging effects of sympathomimetic drugs characterised by a combination of desired therapeutic actions and a rich spectrum of side effects. Together, with the reports of different actions of distinct adrenaline-related amines on various target organs,[28] they fed the vision that structural modification of sympathomimetics would allow the generation of more specific drugs. This is well illustrated with the development of sympathomimetic bronchodilators,[35] starting from the 'original autonomic drug' adrenaline.[36]

With the development of salbutamol by the group of David Jack in 1968, a key drug in respiratory medicine became available[37] that acts as specific β-2 adrenoceptor agonist. In addition to the effects of β adrenoceptor agonists on asthma and obstructive airway disorder, β-2 agonists are discussed as add-on therapy in MS. Apart from improving sympathovagal balance and cardiovascular dysfunction in MS, the use of sympathomimetics goes beyond treatment of these basic autonomic functions.[38,39] The involvement of adrenoceptors in the neuro–immune connection and the role of β-2 adrenoceptors in inflammatory demyelination and axonal degeneration point out different alleys along which modulation of the ANS and/or adrenoceptor function may impact the course of the disease.

Interestingly, antagonists for muscarinic acetylcholine receptors were also used early on for purposes overlapping with the bronchodilatory effects of the adrenoceptor agonists. Due to the general spasmolytic effects, much effort has been devoted to the development of specific antimuscarinergic drugs targeting the individual members of the receptor family in different organs. In the case of asthma and obstructive pulmonary diseases, a set of substances is available with increased specificity for M3 muscarinic receptors[40] that may be combined with adrenergic agonists. Most relevant for individuals with MS is the anticholinergic therapy for the treatment of lower urinary symptoms.[23] In MS with diverse manifestations of the symptoms[24] this figures prominently to treat overactive bladder syndromes.[22] A number of different drugs are available with some preference for M3 receptors.

The Signal Transduction Mechanisms – the Advent of the Second Messengers

The purification and identification of the adrenoceptor as 'physicochemical entity'[31] merged with the discovery of cyclic adenosine monophosphate (cAMP) and characterisation of cAMP as 'second messenger'.[41] This fusion of knowledge between physiology and biochemistry had a major impact on drug development well into the 21st century.

The reconstitution of adenylate cyclase (AC) activation upon adrenoceptor activation in heterologous and depleted systems[42] demonstrated that the signal transduction initiated by a neurotransmitter and its receptor could be deciphered at the level of single protein interactions. This observation paved the way to deconstruct the cellular signal transduction pathways down to intricate molecular details.

The picture diversified into different directions. In the 1980s, it was recognised that AC may be under dual control,[43] paying tribute to the diversity of signalling molecules and their receptors, i.e., adrenergic and muscarinic. The characterisation of different GTP-binding proteins (G proteins) that provide the connection between receptor and AC explains part of the activating versus inhibiting mechanisms.[44] The diversity of AC encoded by a gene family poses another level of complexity.[45]

Thus, in a long-standing effort it was shown that activation of AC by adrenoceptors constitutes the primary mechanism to increase cardiac performance in the heart under load,[46] where the equilibrium between AC and phosphodiesterases (PDE) dictates cAMP/PKA activities.[47,48]

Roughly a decade after the characterisation of cAMP,[49] cyclic guanosine monophosphate (cGMP), and a guanylate cyclase were described.[50,51] Consequently, transduction of hormonal and neurotransmitter signalling in cells was recognised to rest on the cyclic nucleotides cAMP and cGMP. The regulation of

their synthesising enzymes and the degrading PDE turned into a key focus of basic science and drug development. These scientific milestone discoveries were awarded the Nobel prizes in Physiology or Medicine for Earl Wilbur Sutherland in 1971, for Alfred Goodman Gilman in 1994, and in Chemistry for Robert Joseph Lefkowitz in 2012.

Additional Transmitting Substances

In addition to the two classical transmitters noradrenaline and acetylcholine, a range of neuromodulators have been described to fine-tune autonomic neurotransmission. Already in the 1970s, purines like ATP and peptides like neuropeptide Y, somatostatin, and vasoactive intestinal polypeptide were recognised to play important roles in autonomic neurotransmission.[52,53] Importantly, receptors for the nonclassical neurotransmitters turned out to, at least in part, couple to G proteins.[52]

A most surprising member of the neuromodulator group, nitrogen monoxide (NO), a substance originally known as a caustic pollutant in the earth's atmosphere, was characterised to exert dramatic effects on guanylate cyclase activity in target cells, in particular vascular smooth muscle.[54] Starting with the observation that NO is able to activate the cytosolic form of guanylate cyclase,[55] it could be shown that NO is able to relax vascular smooth muscle cells by affecting cGMP levels.[56] In the following, the field of cardiovascular regulation by cyclic nucleotide second messengers turned into the most rapidly expanding field in biomedical sciences.[54] The balancing of cGMP and cAMP and the role of their degrading enzymes, the cyclic nucleotide PDE, as therapeutic targets became a focus of interest.

With the emergence of inhibitors of the PDE, a quantum leap was achieved in the treatment of male erectile dysfunction (ED). This autonomic dysfunction also affects patients with MS.[26,27] In an attempt to develop inhibitors against PDE5 for the treatment of hypertension and angina pectoris, a substance, discarded for its lack of the desired effects, attracted attention for a side effect. Sildenafil, a highly specific PDE5 inhibitor,[57] revolutionised the treatment of ED by supporting the desired vasodilatory effect in the appropriate contextual setting and with high tolerability.[58] With the excellent efficacy and tolerability profile, a series of drugs with comparable effects were rapidly developed. Among the autonomic dysfunctions, in particular of the pelvic organs, the success in the treatment of ED is outstanding.

The Search for the End Apparatus: A Highly Complex Target for Toxins

With the breakthroughs demonstrating how information propagation between neuron and target cell is mediated by soluble transmitter molecules and the understanding of the receptive process on the target cell being mediated by receptor proteins, the release of the transmitter from a neuron remained a key question in cellular and molecular neuroscience. Refined electrophysiological techniques opened the window into the nature and kinetics of the release process. Modern methods of protein purification in combination with molecular cloning, targeted inactivation, and expression techniques enabled biochemists to characterise the molecular players of the synapses at the nerve terminal.[59]

Various toxins such as the clostridial tetanus toxin and botulinum toxin (BoTX)[60] supported the characterisation of individual proteins in the synaptic protein complexes. These toxins degrade specific proteins associated with presynaptic plasma membrane and synaptic vesicles required for calcium-induced fusion of the transmitter-containing vesicle and consequent neurotransmitter release. The molecular methods to express and/or manipulate individual synaptic proteins allowed to describe their specific functions[61,62] and to understand the vesicle cycle as the interaction of a diverse set of synaptic proteins.

In a surprising conversion of toxic to beneficial function,[63,64] controlled local application turned BoTX into a therapeutic tool. Taking advantage of the specific uptake of toxin subunits into nerve terminals and degradation of individual SNARE complex proteins critically involved in calcium-dependent synaptic vesicle fusion,[65] BoTX can turn down the release of neurotransmitters. In this manner, activity of selected targets can be reduced or blocked by localised application of the toxin. For MS patients, overactivity of the detrusor muscle can be reduced in some manifestations of the neurogenic bladder.[66] This allows an approach to the voiding function in cases where dysfunction is refractory to anticholinergic therapy or when its side effects prevail.

THE INTERDIGITATION OF AUTONOMIC NERVOUS SYSTEM AND IMMUNE SYSTEM FUNCTION AND THE IMPACT OF IMMUNE MODULATORY THERAPIES

An issue of particular relevance is the interaction of the ANS and the immune system and its disturbances in MS.[19,67,68] This entails a range of different aspects concerning the functional interaction in the two systems where the sympathetic nerve plays a critical role as 'integrative interface'.[69] Here, the autonomic innervation of primary, secondary, and tertiary lymphoid organs is of essence.[70,71] Unbalanced sympathetic activity in the spleen is discussed to drive immune-mediated inflammatory activity.[72] Disease-mediated alterations in the properties of lymphocytes and associated cell types in lymphatic tissues include changes in adrenoreceptor expression on lymphocytes in MS.[73-75] The combined effects of such processes at the meninges, a gathering place for diverse types of immune cells richly innervated by autonomic neurons,[76] is still unresolved. In addition, challenges secondary to the immediate consequences of the dysfunction in nervous and immune system may be critical. Of particular interest are disturbances in the voiding functions, particularly lower urinary tract symptoms and associated bacterial infections.[77] How significantly the alterations in gut motility and function imposed by autonomic dysregulation add to this complexity[78] is a lasting question.

A critical issue in this context concerns the effect of immune modulatory therapies on the functions exerted by the ANS. Treatment of acute exacerbations in MS with high doses of methylprednisolone has the potential to induce cardiac arrhythmias and conduction disturbances.[79] For immune modulatory approaches to manage long-term course and morbidity after MS diagnosis, the picture is as diverse as the arsenal of substances. The first significant step forward has been achieved with β interferon preparations which, during the initial years of searching, were praised for their absence of important side effects.[80] Indeed, no differences were found in autonomic scores for cardiovascular functions in a placebo-controlled study.[20]

A different situation is found for the immune modulatory drugs mitoxantrone and fingolimod that may compromise cardiovascular function on different timescales.[81] Via its action on sphingosine 1-phosphate receptors (S1PR), fingolimod may result in the desired action on lymphocyte sequestration via subtype 1 S1PR expressed in lymphocytes. In addition, transient, occasionally severe, cardiac side effects may be elicited via other receptor subtypes.[82,83] Parasympathetic as well as sympathetic cardiac drive may be affected.[84] Differently, long-term treatment with mitoxantrone, a DNA topoisomerase inhibitor, may lead to cardiomyopathy associated with mitochondrial dysfunction.[85] As such, different immunotherapies acting via entirely distinct mechanisms may induce off-target effects on autonomic functions of very different type and scale.

CONCLUSIONS

Since the description of a conspicuous heat intolerance by Uhthoff in 1890[11] in patients with sclerotised lesions in central nervous system white matter, autonomic dysfunctions emerged as a frequently observed catalogue of symptoms severely impacting quality of life in people affected by MS. During the very same time at the end of the 19th century, Langley and Gaskell performed their ground-breaking anatomical, physiological, and pharmacological studies on the ANS that were summarised in their monographs on the 'Autonomic Nervous System' by Langley in 1921[1] and the 'Involuntary Nervous System' by Gaskell in 1920.[2]

At that time, the pharmacological approach used by Langley opened a series of key questions shaping the molecular neurosciences during the 20th century: the imminent problem of the information transmitting principle of autonomic neurones, the *in vivo* pendants to adrenaline and pilocarpin, the nature of the receptive substance, and the properties of the end apparatus responsible for communicating neural activity to the target organ. Each of these key problems yielded rich molecular knowledge on basic mechanisms in nervous system function resulting in a detailed picture of the working of cellular and molecular autonomic neuroscience (Table 1).

Table 1: The evolution of key concepts on autonomic neurotransmission and the development of drugs affecting these transmission pathways in multiple sclerosis.

Basic problem	Experimental approach	Emerging substance or entity	Therapeutic options in MS	Nobel prize (year)
Transmitting principle	Frog heart preparation[7]	Humoral (soluble) transmitter		Otto Loewi (1936) P/M
	Purification and physiology[8]	Acetylcholine as para-sympathetic trans-mitter		Henry H Dale (1936) P/M
	Purification, pharmacology[10]	Noradrenaline as sympathetic transmitter	Noradrenaline precursor[39]	Ulf von Euler, Julius Axelrod, Bernard Katz (1970) P/M
	Comparison of related amines[28]	Sympathomimetic substances		
Receptive substance[1]	Adrenoceptor phar-macology[29]	Alpha and beta 'ad-renotropic' receptors		
	Biochemical purification from canine ventricular myocardium,[30] molecular cloning[32]	Beta-adrenergic receptor protein, activation of AC Multi-gene family, convey AC specificity	Sympatho – mimetics[38,39]	Robert J Lefkowitz, Bri-an Kobilka (2012) Chem
	Biochemical purification, cloning[34]	Muscarinic receptors	Anti-muscarinergic[22,23]	
Nonadrenergic, non-cholinergic transmission	Physiology and biochemistry, vascular smooth muscle cells[56]	NO as neuromodu-lator		Louis J Ignarro, Ferid Murad, Robert F Furchgott (1998) P/M
	Physiology, bio-chemistry, and histology in different targets	Neuromodulation: purinergic,[52] peptidergic,[53] opioid[86]	Cannabinoids for LUTS[86]	
Signal transduction	Biochemistry dog liver[49]	cAMP as second messenger		Earl W Sutherland (1971) P/M
	Diverse mammalian tissues[50,51]	cGMP as second mes-senger		
	Biochemistry rat tissues[47]	Phosphodiesterases	PDE5 inhibitors for ED[26,27]	
	Biochemical analysis and reconstruction of signalling[42]	Coupling of transmitter receptors to G proteins		Alfred G Gilman, Martin Rodbell (1994) P/M
End apparatus[1]	Biochemistry and toxicology[60]	Blockade of transmitter release by TeTX, BoTX	BoTX for OAB[66]	
	Biochemistry, physiology, molecular biology[59]	Calcium-dependent vesicle exocytosis and endocytosis		Thomas C Suedhof, Randy W Schekman, James E Rothman (2013) P/M

BoTX: botulinum toxin; Chem: chemistry; LUTS: lower urinary tract symptoms; MS: multiple sclerosis; NO: nitrogen monoxide; OAB: overactive bladder; P/M: physiology or medicine; TeTX: tetanus toxin.

The impact of the discovery of the endogenous neurotransmitters and neuromodulators, the characterisation of the transmitter receptor molecules, and the deciphering of intracellular signal transduction on drug development manifested in selected substance groups relevant in MS therapy.

While compromised cardiovascular function and temperature control remain to a large extent subject to individual adaptations in nutrition, clothing, and behaviour, disease-related dysfunctions of the pelvic organs are frequently approached by pharmaceutical intervention in addition to nutritional and behavioural adaptations. Classical antimuscarinic therapy has dominated treatment of overactive bladder syndromes, yet counterproductive effects on bowel activity as well as cognitive side effects remain a problem in addition to incomplete relief of the voiding symptoms. The application of BoTX to suppress neurotransmitter release may help in some cases.

The amazing success in the treatment of male sexual dysfunctions with PDE 5 inhibitors at the start to the 21st century sets new standards concerning efficacy and tolerability. Comparable success with respect to efficacy and tolerability of treatment in the case of bladder and bowel dysfunction would benefit not only both sexes of patients with MS, but also a large number of people affected by neurogenic and age-related disorders.

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Acquired Language Disorders in Bilinguals

Authors: *Elisa Cargnelutti,[1] Barbara Tomasino,[1] Franco Fabbro[2,3]

1. Scientific Institute, IRCCS E. Medea, Dipartimento/Unità Operativa Pasian di Prato, Udine, Italy
2. Cognitive Neuroscience Laboratory, DILL, University of Udine, Italy
3. PERCRO Perceptual Robotics Laboratory, Scuola Superiore Sant'Anna, Pisa, Italy

*Correspondence to elisa.cargnelutti@lanostrafamiglia.it

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Abstract

The literature reports an increased number of aphasias involving bilingual people. Dealing with bilingual aphasia requires particular attention from the diagnostic to the therapeutic phase. In this review, the authors describe the possible impairment patterns, which could be different between the two languages and be characterised by specific deficits and sometimes unexpected profiles. The role of some crucial factors in determining the observed deficits and impairment patterns is illustrated, for instance age of appropriation and proficiency. An early versus late language appropriation recruits different brain processes and hence different brain structures. In general, a greater vulnerability is observed for the late-learned languages, although a high proficiency or use and exposure appear to prevent language impairment even in the case of late appropriation. The authors also discussed the role of other intervening factors, such as emotional–motivational aspects, which could explain unusual profiles. Furthermore, language deficits specific to bilingualism, such as pathological mixing and switching and translation problems were described. In this respect, the authors underlined the fundamental involvement of cognitive control mechanisms and of the brain structures associated with this. Lastly, the clinical practice issues in bilingual aphasia were outlined, underlining the need for a careful diagnosis. This should take into account the patient's language history in order to avoid biased assessments and instead promote the setup of effective intervention programmes.

INTRODUCTION

The cases of bilingual aphasia are increasing worldwide, as they also reflect the globally increasing number of individuals speaking more than two languages (representing more than half of the population),[1,2] who are referred to as bilinguals, irrespective of the number of known languages. Bilinguals differentiate one another under multiple aspects and their clinical language profiles may differ. In this review, the authors provide an overview of the different bilingual aphasia profiles and the factors associated with these different conditions, providing hints for their understanding and treatment.

In relation to the different patterns of language impairment or recovery, Paradis[3] proposed, in

1977, the first structured classification: a) parallel impairment, in which the languages are similarly compromised; b) differential impairment, in which one language is more affected than the other; c) selective impairment, in which only one language is affected and the other is spared; d) blended or mixed impairment, in which there is interference between the languages and the patient cannot keep them separated; e) antagonistic, in which improvement in one language is associated with an increased impairment in the other and vice versa; and f) successive recovery, characterised by improvement in one language taking place only after the complete recovery of the other.

These impairment patterns reflect the interplay between many factors. These include first the clinical parameters that shape aphasia in monolinguals as well, such as lesion volume or patients' age, but, crucially, also the patients' language background. To this regard, the age at which the patients were exposed to the non-native or second language (L2) is also crucial.[4,5] Age of acquisition or appropriation (AoA) is critical as it influences the way the language is represented in the brain. Neuroimaging studies in healthy bilinguals usually take the age of 6 as the cut-off to differently investigate the brain networks associated with an early versus late L2 appropriation, because around this age crucial developmental changes occur in the brain and in the learning mechanisms. Indeed, up to this age, language appropriation takes place in the form of acquisition, meaning an almost unconscious process supported by implicit mechanisms. Otherwise, late appropriation is defined in terms of learning, which instead relies on explicit processes.[6-8]

According to authors such as Paradis and Ullman,[6-9] the role of AoA differs based on the considered language structural domain. It is particularly crucial for morpho-syntax and phonology/articulation. Internalisation of the related processes and, hence, native-like proficiency can only be achieved with early acquisition, relying on implicit mechanisms. On the other hand, lexical knowledge depends more on the degree of language use and exposure, as it is supported by explicit memory. Besides AoA, other factors also influence language mastery and related brain representation, with the chief role of proficiency.[10] The following paragraphs illustrate these main factors and relate them to the impairment patterns.

PATTERNS OF LANGUAGE IMPAIRMENT IN BILINGUAL APHASIA

Cases of either parallel or differential impairment are reported in many studies as the sole conditions, indicating their higher incidence with respect to the other impairment patterns. For instance, Fabbro described 20 bilinguals (AoA up to 7) with left-hemisphere damage, of whom 65% manifested parallel impairment and the remaining differential impairment. In these, either the native, first language L1 (15%), or non-native, L2 (20%), were affected the most.[11] According to Paradis, the overall most frequent condition is parallel impairment, although it is underrepresented in literature, probably because it appears less appealing and therefore less worthy to be reported.[6,7]

Parallel impairment is frequently observed between non-native languages (for patients knowing more than two languages) when these had similar AoA, in particular when they shared the same learning modality (e.g., formal instruction).[12] However, parallel impairment was also observed irrespective of AoA. For instance, Green et al.[13] reported a parallel impairment between L1 and English, the L2, in patients having lived in the UK for many years, indicating the fundamental role of language use and exposure.[13] The authors, however, attributed this condition to impaired control abilities, which is discussed further.

Concerning differential impairment, many patients follow either the so-called Ribot's rule, postulating better preservation of L1,[14] or the Pitres' rule, according to which it is the most familiar language to be better preserved.[15] In fact, although it is more intuitive to hypothesise greater resistance to damage for the language learned first, in many cases L1 was instead the most affected. This occurred, for instance, when the patients had a premorbid high level of L2 proficiency and frequency of use, although a recent systematic review seemed to restrict this possibility to early bilinguals.[16]

The patient described by Samar and Akbari[17] had more preserved L2, which she learned at school, then studied at university and taught there

as a teacher for 18 years. In this case, the high and deep knowledge of the language together with its constant use reduced the impairment severity. In healthy adults, the language networks appeared highly similar between L1 and L2 when proficiency is high.[10] The learning method, represented by formal instruction, also had a possibly relevant role. In fact, as long as the brain lesion spares the explicit learning system and therefore the consciously learned meta-linguistic skills, formal knowledge, which relies on them, can potentially promote language recovery. This view is supported by the cases of an apparently paradoxical profile in which the patients were impaired in their native language but retained the use of an only-formally learned language, including the dead languages, such as Latin.[18]

Nevertheless, main exposure alone can not assure the language preservation. Impairment can indeed occur in cases of L2 learning that took place recently[19] or in adulthood,[20] when the brain is less prone to remodelling and language brain representation, therefore results can be less sound, hence more vulnerable to damage.

All the aforementioned language background factors indeed shape the language brain representation. Tangible information about the bilingual patients' brain networks mainly comes from intraoperative stimulation studies. In a large-cohort study, Roux and Trémoulet[21] observed that only two patients displayed solely common stimulation sites between the two languages, whereas the majority of them had both common and language-specific sites. Interestingly, language-specific sites were observed even in early bilinguals and, secondly, no additional cortical sites were found for the less-proficient language. There were similar results from a subsequent study on late but proficient bilingual patients, which further reported 'L2-restricted zones' in the perisylvian cortex, i.e., sites associated exclusively with L1.[22] Although these findings were limited to the scouting of the affected region and its surroundings, they show that even close brain sites may be dedicated to different languages and that sometimes neither AoA nor proficiency can predict the extent of different representation between the two languages.

The depicted language brain representation leads us to suppose that a brain lesion, unless it is small and circumscribed, hardly affects one language while completely sparing the other. Nevertheless, a few cases of selective language impairment have been reported. This phenomenon was described in patients with epilepsy, with a selective postictal temporary loss of either L1[23,24] or L2.[25] This specific type of impairment may hence have a neurofunctional rather than a neuroanatomical substrate. Changes in the normal brain electrical activity may temporarily inhibit the circuits associated with a specific language, which is recovered when the normal brain functioning is restored. In this vein, selective recovery might also result from impairment in control functions, which is illustrated in the following chapter.

The reversible inhibition of one language characterises another apparently odd condition, naming the alternating antagonism. This phenomenon is characterised by phases in which only one language seems to be accessible, whereas the other is apparently lost, and usually takes place in the immediate post-event period.[26] This confirms the hypothesis that the impaired language is not lost, but inhibited, and that when this inhibition resolves, either spontaneously or throughout rehabilitation programmes, the language may recover.

Alternating antagonism is likely to take place when there is an underlying deficit in the regulation processes, which caused competition even between structurally distant languages, such as Farsi and German, as seen in the patient described by Nilipour and Ashayeri.[27] The patient also manifested a successive recovery, with English (L3) recovering only after the complete recovery of the other two languages.

ROLE OF COGNITIVE CONTROL IN BILINGUAL APHASIA

Bilingualism indeed entails the need to coordinate language use to activate the proper language according to the context, while suppressing the irrelevant. This entails the constant recruitment of cognitive control functions and a certain degree of cognitive flexibility to properly shift from one language to the other.[28] This could be the reason of the cognitive advantage some studies

observed in bilinguals with respect to monolinguals, even in presence of aphasia.[29]

According to the neural convergence hypothesis,[30] as bilinguals become more familiar with the new language, its brain representation converges with that of the native language. In this perspective, differences rather rely on the diverse recruitment of the cognitive control resources. Moreover, Radman et al.[31] observed language improvement following stroke to be associated with increased connectivity between language areas and those devoted to cognitive control.[31] However, they noticed this phenomenon was restricted to the language that improved the most, therefore suggesting that, at least in some cases (e.g., different AoA or proficiency), differences in the neural representation between the languages can actually be present.

Control deficits were observed in both parallel[13,32] and differential or selective language impairment.[33] Recently, many studies aimed to understand whether possible cognitive control deficits in bilingual aphasia. Some studies seem to suggest a language-specific control deficit,[32] although problems in general control were also observed, and the interplay between other intervening factors, such as task complexity or lesion site were proposed to modulate the relation between language and control deficits.[13,34]

In their recent review on the neuroimaging of language control, Abutalebi and Green[28] recapitulated their previous studies on the topic by illustrating the specific role of the brain structures associated with the language control network. These include both cortical (i.e., prefrontal cortex, dorsal anterior cingulate cortex/pre-supplementary motor area, and inferior parietal cortex, with the involvement of both hemispheres) and subcortical regions (i.e., left basal ganglia and thalamus and right cerebellum). These regions are deputed to specific functions within the language control process and lesions at their level might cause different control deficits.

Among these regions, extensive literature has highlighted the crucial role of the basal ganglia, and particularly the left (head of the) caudate and putamen, which are involved in appropriate language selection. Aglioti and Fabbro[35,36] reported the case of a woman who lost her ability to speak her first language (an Italian dialect), but surprisingly began to speak Italian, which she had learned at school but rarely spoke throughout her life.[35,36] She also began to speak with a strong German accent, a phenomenon known as foreign accent syndrome and described even in monolingual patients (Figure 1).

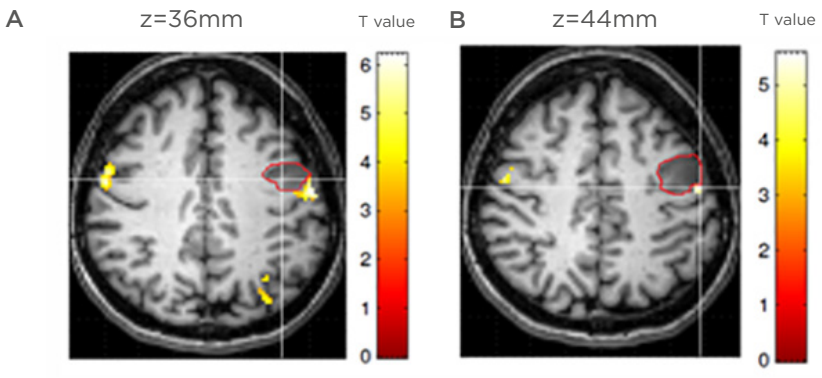

Figure 1: Foreign accent syndrome.

Foreign accent syndrome is a rare acquired motor speech variation, which has been reported in about 60 cases in literature.[37] Patients suddenly exhibit a seemingly 'strange' accent and are perceived as having a foreign accent by listeners of the same speech community. Tomasino et al.[38] reported a tumoral patient developing foreign accent syndrome following a small and circumscribed lesion in the left precentral gyrus. The patient, an Italian native speaker, developed altered speech rhythm and melody. During pronunciation of words and pseudowords in fMRI tasks, the patient showed a hyperactivation, compared to the control group, in areas around the pre/postcentral gyrus corresponding to those involved in phonation (i.e., larynx motor area).

The fMRI cluster related to mouth (A) and tongue (B) movements located behind the patient's lesion (indicated by the red circle).

This is a case of paradoxical use of one language and can be explained in light of the lesion location, which affected the left basal ganglia,[39] and also stresses the role of the subcortical structures in implicit memory processes, which normally support the early acquired languages.

For the role the basal ganglia plays in implicit memory processes, a lesion at this level is likely to predominantly affect the mopho-syntactic processes. This was reported even for late-learned languages of high proficiency, for which even these linguistic processes could have become automated.[40] This result indicates the crucial role proficiency may have in promoting language representation reshaping throughout life.

An illustrative case of the association between lesion site and impaired language was described by Moretti et al.[41] The patient manifested impairment in the native language following an infarct in the left caudate nucleus and then, when a lesion affected the left frontotemporal cortex, she developed deficits in her late-learned L2. This supports the predominant cortical representation of languages learned through explicit processes, although an opposite trend was also described.[42]

OTHER CLINICALLY RELEVANT FACTORS

Recovery patterns may also be modulated by factors other than AoA, proficiency, and cognitive control deficits (see Figure 2 for an overview). Bilinguals learning a new distant language try to adopt the same L1 processes, but when these turn out to be unsuitable, they need to develop new processes (assimilation-accommodation processes).[43] Evidence is however lacking regarding the possibly greater impairment in L2 when structurally distant from L1.[16]

Recorded difficulties reflect the cognitive demands required to process a given language, for instance when reading a transparent versus opaque language. This point is tricky as these differences might bias the diagnosis between the languages,[44] and therefore call for a language assessment respectful of language complexity.

Other factors contributing to the definition of a given impairment pattern include the language spoken in the environment, namely in the hospital, in the period immediately following the clinical event. This factor can also be relevant from the rehabilitation viewpoint, as lower-than-expected improvements in the treated language were attributed to the fact that the patient was constantly exposed to another language outside the rehabilitation setting.[45]

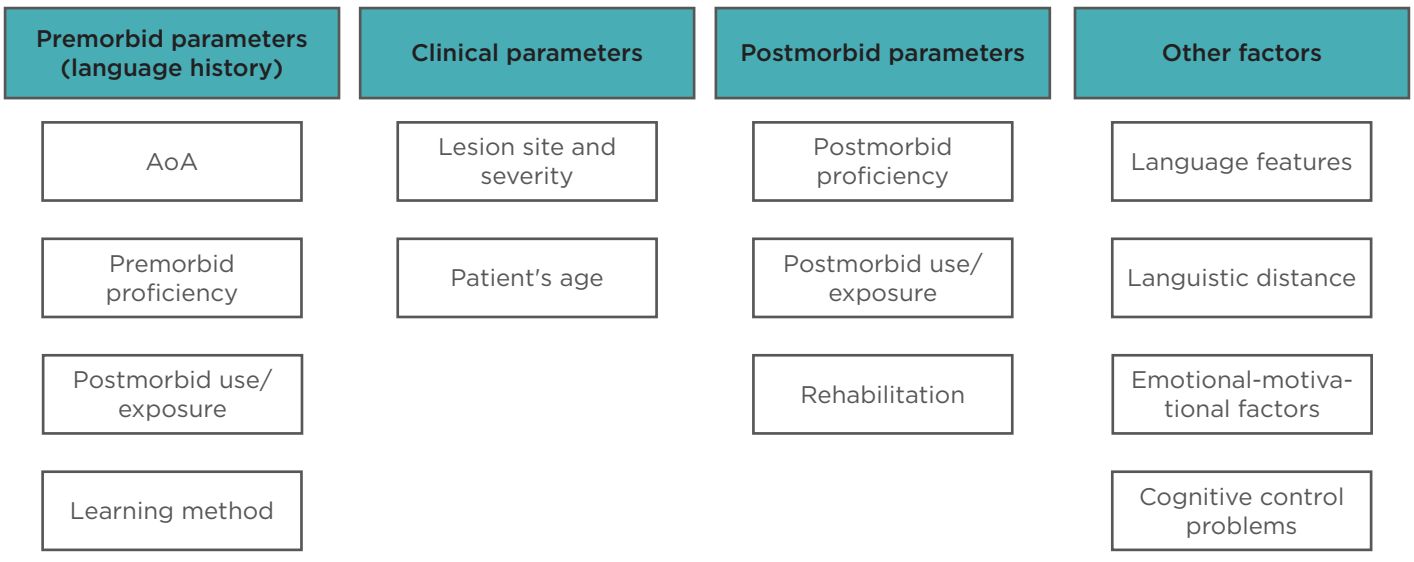

Figure 2: Overview of the factors contributing to the different language recovery patterns.
AoA: age of acquisition or appropriation.

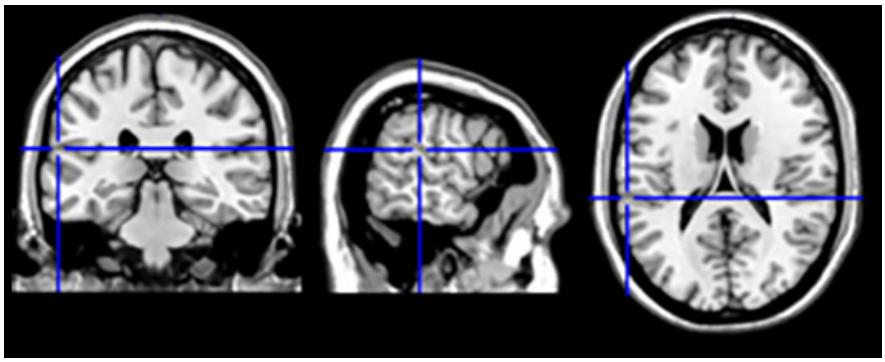

Figure 3: Switching from L2 to L1.

In neurosurgical patients, Penfield W and Roberts L[54] largely documented language switching during electro-cortical stimulation mapping as an automatic mechanism that turns off one language when the other language is on. Tomasino et al.[55] described involuntary language switching from L2 (Italian) to L1 (Serbian) evoked by electro-stimulation in the left superior temporal gyrus/supramarginal gyrus during awake brain surgery. The language switching site belonged to an fMRI cluster in the area Stp (in the planum temporale) which has a role in phonological processing and was found to activate for both L1 and L2 during language tasks.

The language switching site (MN1 coordinaltes: x=-61, y=-30, z=18)

Finally, even the affective factors can assume a fundamental role. Emotionally relevant episodes may induce the release of the apparently lost language[46] and the willingness to recover a given language may actually prompt its improvement.[47]

SPECIFIC LANGUAGE DEFICITS IN BILINGUAL APHASIA

Differently from monolinguals, bilingual patients may develop deficits characterising specifically the bilingual condition and concerning the reciprocal use of the two languages. These symptoms include mixing (i.e., recourse of words or other elements of one language during the use of the other), switching (e.g., shift from one language to the other), and problems in translation.

Frequently, these events arise from lesions in the mentioned areas involved in regulating the proper language use,[48,49] with greater interference frequently observed between two structurally close languages.[19,50] Fabbro et al.[51] described the case of a patient with a glioma in the left prefrontal and cingulate cortices who involuntary switched to his native language, even talking to people he knew could not understand it, indicating the inability to inhibit the process.

Lesions in other areas were observed to pathologically induce or prevent language switching and include the inferior parietal lobe[52] and the fronto-temporal cortex.[53] Interestingly, switching was observed during direct electrostimulation of specific brain sites (Figure 3), including the white-matter tracts connecting control and language-specific brain areas.[56]

In some other cases, these phenomena reflect language impairment, as they occurred to compensate for anomia or other difficulties in the more impaired language.[57,58] This can also be the case of translation deficits, which often reflect the general impairment in a specific language, with greater difficulty in translating from better preserved to more impaired languages than vice versa. Sometimes, this language deficit occurs selectively, despite spared ability in naming[59] or in recognising translation equivalents across the languages.[60] In other cases, in which naming abilities were impaired, translation processes were preserved, and further employed to recover word finding difficulties through translation from the preserved languages, therefore preventing switching.[49]

However, some patients were observed to paradoxically translate to the most affected language while unable to translate to the spared language,[61] for instance when antagonistic recovery occurred.[26] Sometimes, the patients instead manifest the compulsive tendency to

translate from one language to the other, while not being able to prevent this automatic behaviour.[39]

ISSUES IN BILINGUAL APHASIA

Dealing with bilingual aphasia requires specific precautions from the diagnostic to the therapeutic phase. Firstly, for a proper diagnosis, it is fundamental to take into account the level of premorbid language proficiency, which was observed to be one of the most important factors in predicting the postmorbid level of deficit.[62] Hence, it is fundamental to first thoroughly inspect the language history (e.g., AoA, proficiency, frequency, and context of use) by means of structured questionnaires.

Secondly, clinical assessment should ideally be performed in all the languages the patient knows, even though, especially for immigrated people, clinicians may not master the patient's L1. When testing the different languages, it is also fundamental to take into account the structural differences between them. To this aim, Paradis and Libben developed the Bilingual Aphasia Test (BAT), now available in >70 languages, with items for each language matched in complexity and selected for their cultural adequacy.[63] The battery is structured in three parts: the first inspecting the language history, the second making a comprehensive assessment of each language skills, and the last addressing specific language pairs, offering an understanding of which language was affected the most. A proper diagnosis is fundamental for setting the rehabilitation programme. Ideally, each impaired language should be treated. When this is not possible, therapists should train the language that could have more beneficial effects on the untreated language, therefore promoting cross-linguistic transfer. Although not univocally, many studies have observed that treatments focussing on the weaker language, meaning a non-native language[64] or, in the case of comparable AoA, a lower-proficiency language[65] are more likely to boost improvements in the untreated language.[62,63] With regard to naming training, this trend can be explained in light of the revised hierarchical model by Kroll and Stewart,[66] according to which L1 words are tightly linked to their correspondent meaning, whereas L2 word semantic access occurs via translation to L1. Consequently, semantic access after L2 training likely takes place by passing through the L1 lexicon, which recovers in turn. In the case of difficulty in properly regulating the language use, rehabilitating general cognitive functions is recommended first.[67]

Gil and Goral,[68] however, qualitatively observed beneficial transfer effects following treatment in each language, in spite of the structural distance between them (i.e., Russian and Hebrew, for which transfer was poor only in/for writing, which differs substantially between them). However, they tested the patient in the subacute phase, when spontaneous recovery was also taking place. The majority of the reported studies, however, took charge of the patients in the chronic phase and documented positive treatment effects as well. Language skill improvement and associated brain reorganisation were observed to occur just 10 days after intensive training, indicating the high brain plasticity potential even many months post-onset.[69]

According to Kiran et al.,[70] all the parameters that have been described, such as AoA, language use, and proficiency before and after the clinical event, are relevant for the prediction of the recovery profile following treatment, although their interplay and the intervention of additional factors undermines an accurate prediction. In some instances, lack of transfer can be attributed to factors other than the treated language. These can include the choice of inappropriate rehabilitation strategies,[71] the impossibility to practice the treated language outside the rehabilitation setting,[45] the fact that the unimproved language had already reached its highest recovery level.[72] Lastly, some partly unexpected improvements might be attributed to the willingness to recover a given language, highlighting the emotional valence the languages can take on.[47]

CONCLUSION

In conclusion, although it is difficult in clinical practice to concretely take account of the bilingual patients' languages, some attempts should be made to achieve an accurate diagnosis and guarantee the most effective possible therapeutic intervention, as impairments in a given language can have relevant consequences for the patients' life on social, affective, and working levels.

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