



Geneeskundige Stichting Koningin Elisabeth
Fondation Médicale Reine Elisabeth
Königin-Elisabeth-Stiftung für Medizin
Queen Elisabeth Medical Foundation

Publicaties – Publications – Publikationen – Publications

2021

G.S.K.E. – F.M.R.E. – K.E.S.M. – Q.E.M.F.

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Interuniversitaire onderzoeksprojecten
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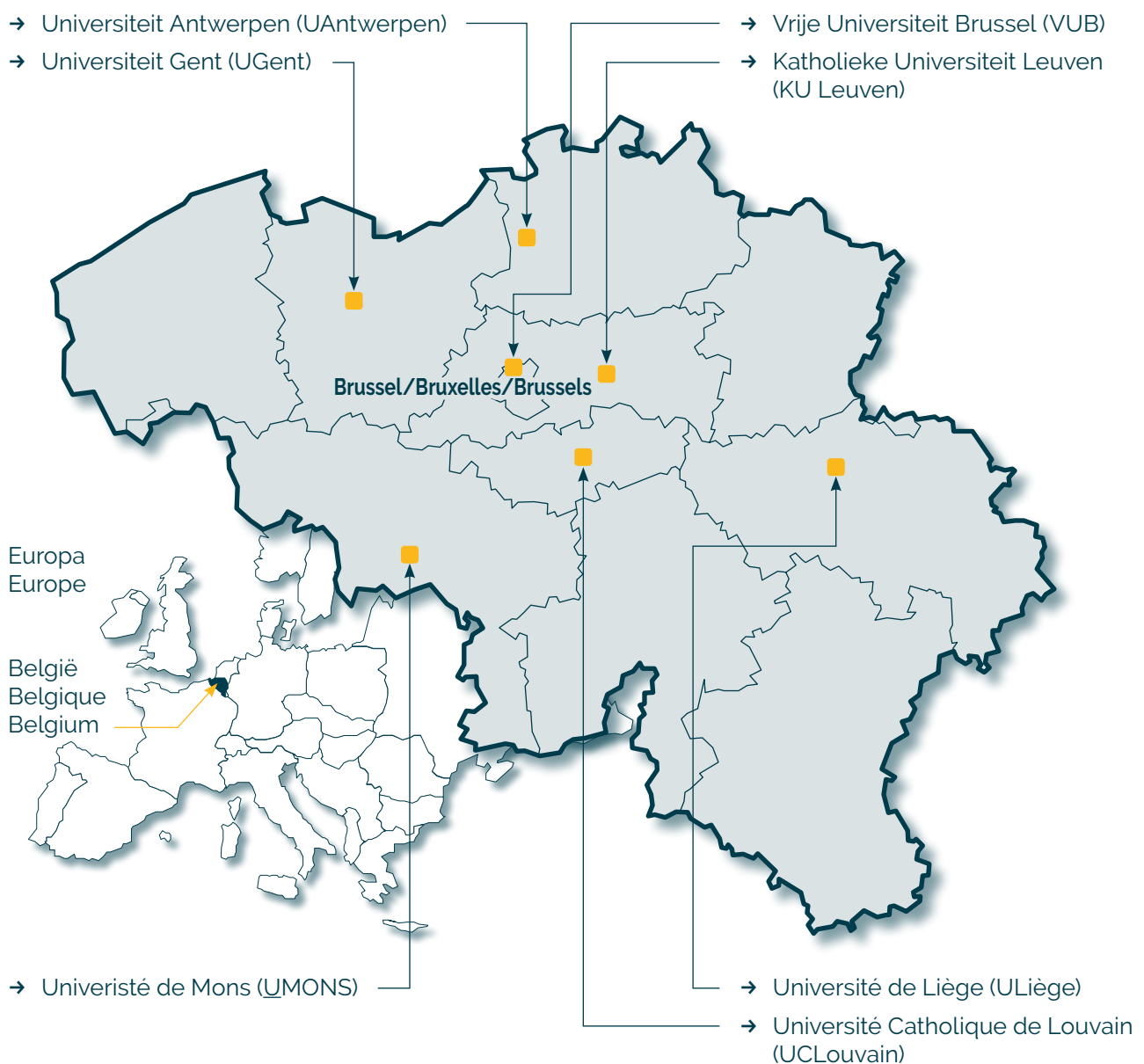
Projets de recherche interuniversitaire
2020–2022 subventionnés par la F.M.R.E.

Interuniversity research projects
2020–2022 funded by the Q.E.M.F.

Universiteiten met onderzoeksprogramma's die gesteund worden door de G.S.K.E.

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Publicaties – Publications –
Publikationen – Publications

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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Kreis A, Desloovere J, Suelves N, Pierrot N, Yerna X, Issa F, Schakman O, Gualdani R, De Clippele M, Tajeddine N, Kienlen-Campard P, Raedt R, Octave JN, Gailly P (2021)

Overexpression of wild-type human amyloid precursor protein alters GABAergic transmission
Scientific reports 11 (1), 1-18 - IF :6.5 - Q1

Abstract

The function of the amyloid precursor protein (APP) is not fully understood, but its cleavage product amyloid beta (A β) together with neurofibrillary tangles constitute the hallmarks of Alzheimer's disease (AD). Yet, imbalance of excitatory and inhibitory neurotransmission accompanied by loss of synaptic functions, has been reported much earlier and independent of any detectable pathological markers. Recently, soluble APP fragments have been shown to bind to presynaptic GABA β receptors (GABA β Rs), subsequently decreasing the probability of neurotransmitter release. In this body of work, we were able to show that overexpression of wild-type human APP in mice (hAPP_{wt}) causes early cognitive impairment, neuronal loss, and electrophysiological abnormalities in the absence of amyloid plaques and at very low levels of A β . hAPP_{wt} mice exhibited neuronal overexcitation that was evident in EEG and increased long-term potentiation (LTP). Overexpression of hAPP_{wt} did not alter GABAergic/glutamatergic receptor components or GABA production ability. Nonetheless, we detected a decrease of GABA but not glutamate that could be linked to soluble APP fragments, acting on presynaptic GABA β Rs and subsequently reducing GABA release. By using a specific presynaptic GABA β R antagonist, we were able to rescue hyperexcitation in hAPP_{wt} animals. Our results provide evidence that APP plays a crucial role in regulating inhibitory neurotransmission.

Funding: This work was supported by the Belgian Fund for Scientific Research (FNRS, Grant EQP U.N011.17 and CDR J.0065.21), the "Fondation pour la Recherche sur la Maladie d'Alzheimer" (SAO/FRA), the Queen Elisabeth Medical Foundation and the Concerted Research Action from the General Direction of Scientific Research of the French Community of Belgium (ARC17/22-083).

- Sáez-Orellana F, Leroy T, Ribeiro F, Kreis A, Leroy K, Lalloyer F, Baugé E, Staels B, Duyckaerts C, Brion JP, Gailly P, Octave JN, Pierrot N (2021)

Regulation of PPAR α by APP in Alzheimer disease affects the pharmacological modulation of synaptic activity.

Journal of Clinical Investigation Insight 6 (16) JCI : e150099

IF : 8.35 - Q1 (not directly related to the project)

Abstract

Among genetic susceptibility loci associated with late-onset Alzheimer disease (LOAD), genetic polymorphisms identified in genes encoding lipid carriers led to the hypothesis that a disruption of lipid metabolism could promote disease progression. We previously reported that amyloid precursor protein (APP) involved in Alzheimer disease (AD) physiopathology impairs lipid synthesis needed for cortical networks' activity and that activation of peroxisome proliferator-activated receptor α (PPAR α), a metabolic regulator involved in lipid metabolism, improves synaptic plasticity in an AD mouse model. These observations led us to investigate a possible correlation between PPAR α function and full-length APP expression. Here, we report that PPAR α expression and activation were inversely related to APP expression both in LOAD brains and in early-onset AD cases with a duplication of the APP gene, but not in control human brains. Moreover, human APP expression decreased PPARA expression and its related target

genes in transgenic mice and in cultured cortical cells, while opposite results were observed in APP-silenced cortical networks. In cultured neurons, APP-mediated decrease or increase in synaptic activity was corrected by a PPAR α -specific agonist and antagonist, respectively. APP-mediated control of synaptic activity was abolished following PPAR α deficiency, indicating a key function of PPAR α in this process.

Acknowledgements: We thank the Fondation Louvain for support to NP and the Chilean ANID "Becas Chile" for support to FSO and the Netherlands Brain Bank for providing us with human brain samples. This work was supported by the Belgian Fonds pour la Recherche Scientifique, the Concerted Research Actions, the Belgian Fonds de la Recherche Scientifique Médicale, the [Queen Elisabeth Medical Foundation](#), and the Fondation pour la Recherche sur la Maladie d'Alzheimer.

- *Gualdani R, Yuan JH, Effraim PR, Di Stefano G, Truini A, Cruccu G, Dib-Hajj SD, Gailly P, Waxman SG (2021)*

Trigeminal Neuralgia TRPM8 Mutation: Enhanced Activation, Basal [Ca²⁺]_i and Menthol Response

***Neurology Genetics* 7 (1) - IF = 3.5 - Q1**

Abstract

Objective: To assess the functional effects of a variant, c.89 G > A (p.Arg30Gln), in the transient receptor potential melastatin 8 (TRPM8) cold-sensing, nonselective cation channel, which we have previously identified in a patient with familial trigeminal neuralgia.

Methods: We carried out Ca²⁺ imaging and whole-cell patch-clamp recording.

Results: The TRPM8 mutation enhances channel activation, increases basal current amplitude and intracellular [Ca²⁺]_i in cells carrying the mutant channel, and enhances the response to menthol.

Conclusions: We propose that Arg30Gln confers gain-of-function attributes on TRPM8, which contribute to pathogenesis of trigeminal neuralgia in patients carrying this mutation.

Funding: This work was supported in part by Merit Award (I01 RX003201) from the Rehabilitation Research and Development Service, U.S. Department of Veterans Affairs; by the [Queen Elisabeth Medical Foundation](#) (Belgium); and the Concerted Research Action from the General Direction of Scientific Research of the French Community of Belgium (ARC17/22-083).



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Publicaties – Publications –
Publikationen – Publications



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- Florian Perrin, Nicolas Papadopoulos, Nuria Suelves, Remi Opsomer, Devkee M. Vadukul,³ Céline Vrancx,³ Steven O. Smith,⁴ Didier Vertommen, Pascal Kienlen-Campard, and Stefan N. Constantinescu¹

Dimeric Transmembrane Orientations of APP/C99 Regulate gamma-Secretase Processing Line Impacting Signaling and Oligomerization.

Science 2021; 24(2): 102057. (IF 5.08)

Summary

Amyloid precursor protein (APP) cleavage by the γ -secretase produces the C99 transmembrane (TM) protein, which contains three dimerization-inducing Gly-x-x-x-Gly motifs. We demonstrate that dimeric C99 TM orientations regulate the precise cleavage lines by γ -secretase. Of all possible dimeric orientations imposed by a coiled-coil to the C99 TM domain, the dimer containing the ³³Gly-x-x-x-Gly³⁷ motif in the interface promoted the Ab₄₂ processing line and APP intracellular domain-dependent gene transcription, including the induction of BACE1 mRNA, enhancing amyloidogenic processing and signaling. Another orientation exhibiting the ²⁵Gly-x-x-x-Gly²⁹ motif in the interface favored processing to Ab_{43/40}. It induced significantly less gene transcription, while promoting formation of SDS-resistant "Ab-like" oligomers, reminiscent of Ab peptide oligomers. These required both Val24 of a pro-b motif and the ²⁵Gly-x-x-x-Gly²⁹ interface. Thus, crossing angles imposed by precise dimeric orientations control γ -secretase initial cleavage at Ab₄₈ or Ab₄₉, linking the former to enhanced signaling and Ab₄₂ production.

Acknowledgements: We thank Dr. Jean-Philippe Defour for initial lentiviral cloning of coiled coil dimeric proteins, Joanne Van Hees and Lidvine Genet for expert technical assistance and Dr. Nicolas Daguët and Dr. Xavier Cahu for Flow Cytometry support. SNC is Honorary Research Director at FRS-FNRS Belgium. Funding to SNC is acknowledged from Ludwig Institute for Cancer Research, Fondation contre le cancer, Salus Sanguinis and projects Action de recherche concertée (ARC) 16/21-073 and WelBio F 44/8/5 - MCF/UIG - 10955. Funding to PKC is acknowledged from SAO-FRA Alzheimer Research Foundation, Fondation Louvain and Queen Elisabeth Medical Research Foundation (FMRE). FP was supported by ARC 16/21-073 (to SNC) and ARC14/19-059 (to PKC). Support funds from FNRS grant PDRT.0177.18 to PKC, from NIH 27317 to SOS and subcontract to NIH AG27317 to SNC are acknowledged.

- Devkee M. Vadukul, Céline Vrancx, Pierre Burguet, Sabrina Contino, Nuria Suelves, Louise C. Serpell, Loïc Quinton & Pascal Kienlen-Campard

An evaluation of the self-assembly enhancing properties of cell-derived hexameric amyloid-beta.

Sci Rep 2021; 11(1): 11570. (IF4.13)

Summary

A key hallmark of Alzheimer's disease is the extracellular deposition of amyloid plaques composed primarily of the amyloidogenic amyloid- β (A β) peptide. The A β peptide is a product of sequential cleavage of the Amyloid Precursor Protein, the first step of which gives rise to a C terminal Fragment (C99). Cleavage of C99 by γ -secretase activity releases A β of several lengths and the A β ₄₂ isoform in particular has been identified as being neurotoxic. The misfolding of A β leads to subsequent amyloid fibril formation by nucleated polymerisation. This requires an initial and critical nucleus for self assembly. Here, we identify and characterise the composition and self-assembly properties of cell derived hexameric A β ₄₂ and show its assembly enhancing

properties which are dependent on the A β monomer availability. Identification of nucleating assemblies that contribute to self-assembly in this way may serve as therapeutic targets to prevent the formation of toxic oligomers.

Acknowledgements: The authors thank Esther Paître and Pierre Burguet for their technical support. We also thank Loïc Quinton for kindly providing us with the Gel Eluted Liquid Fraction Entrapment Electrophoresis (GELFrEE) 8100 system. Funding to P.K.C is acknowledged from SAO-FRA Alzheimer Research Foundation, Fondation Louvain and Queen Elisabeth Medical Research Foundation (FMRE to P.K.C and LQ). The work was supported by funds from FNRS grant PDRT.0177.18 to P.K.C and LQ.

- Céline Vrancx · Devkee M. Vadukul · Nuria Suelves · Sabrina Contino · Ludovic D'Auria
Florian Perrin · Vincent van Pesch · Bernard Hanseeuw · Loïc Quinton · Pascal Kienlen
Campard

Mechanism of Cellular Formation and In Vivo Seeding Effects of Hexameric beta-Amyloid Assemblies.

Mol Neurobiol 2021; 58(12): 6647-69. (IF4.95)

Abstract

The β -amyloid peptide (A β) is found as amyloid fibrils in senile plaques, a typical hallmark of Alzheimer's disease (AD). However, intermediate soluble oligomers of A β are now recognized as initiators of the pathogenic cascade leading to AD. Studies using recombinant A β have shown that hexameric A β in particular acts as a critical nucleus for A β self-assembly. We recently isolated hexameric A β assemblies from a cellular model, and demonstrated their ability to enhance A β aggregation *in vitro*. Here, we report the presence of similar hexameric-like A β assemblies across several cellular models, including neuronal-like cell lines. In order to better understand how they are produced in a cellular context, we investigated the role of presenilin-1 (PS1) and presenilin-2 (PS2) in their formation. PS1 and PS2 are the catalytic subunits of the γ -secretase complex that generates A β . Using CRISPR-Cas9 to *knockdown* each of the two presenilins in neuronal-like cell lines, we observed a direct link between the PS2-dependent processing pathway and the release of hexameric-like A β assemblies in extracellular vesicles. Further, we assessed the contribution of hexameric A β to the development of amyloid pathology. We report the early presence of hexameric-like A β assemblies in both transgenic mice brains exhibiting human A β pathology and in the cerebrospinal fluid of AD patients, suggesting hexameric A β as a potential early AD biomarker. Finally, cell-derived hexameric A β was found to seed other human A β forms, resulting in the aggravation of amyloid deposition *in vivo* and neuronal toxicity *in vitro*.

Conclusions: Altogether, our findings have shed light on a particular cell-derived A β assembly that corresponds to an A β_{42} hexamer. An insight in cellular mechanisms at stake suggests a strong contribution of PS2 to the formation of this particular A β oligomer, which is released in the extracellular milieu inside vesicles. This in line with the previous reports linking the restricted location of PS2 in acidic compartments of the endocytic pathway, from which extracellular vesicles can form, to the production of more aggregation-prone A β . Combining *in vitro* and *in vivo* approaches, we have revealed an absence of detrimental effects of cell-derived hexameric A β by itself, but its capacity to aggravate amyloid deposition and induce cytotoxicity when there is A β to seed at disposal.

Funding: This work was supported by a grant of the Belgian F.N.R.S FRIA (Fonds National pour la Recherche Scientifique) and a grant of UCLouvain Fonds du Patrimoine to CV. Funding to PKC is acknowledged from SAO-FRA Alzheimer Research Foundation, Fondation Louvain and Queen Elisabeth Medical Research Foundation (FMRE to PKC and LQ). The work was supported by funds from FNRS Grant PDRT.0177.18 to PKC and LQ.

- Anna Kreis, Jana Desloovere, Nuria Suelves, Nathalie Pierrot, Xavier Yerna, Farah Issa, Olivier Schakman, Roberta Gualdani, Marie de Clippele, Nicolas Tajeddine, Pascal KientenCampard, Robrecht Raedt, Jean-Noël Octave & Philippe Gailly

Overexpression of wild-type human amyloid precursor protein alters GABAergic transmission.

Sci Rep 2021; 11(1): 17600 (IF 4.13)

Summary

The function of the amyloid precursor protein (APP) is not fully understood, but its cleavage product amyloid beta (A β) together with neurofibrillary tangles constitute the hallmarks of Alzheimer's disease (AD). Yet, imbalance of excitatory and inhibitory neurotransmission accompanied by loss of synaptic functions, has been reported much earlier and independent of any detectable pathological markers. Recently, soluble APP fragments have been shown to bind to presynaptic GABA β receptors (GABA β Rs), subsequently decreasing the probability of neurotransmitter release. In this body of work, we were able to show that overexpression of wildtype human APP in mice (hAPP_{WT}) causes early cognitive impairment, neuronal loss, and electrophysiological abnormalities in the absence of amyloid plaques and at very low levels of A β . hAPP_{WT} mice exhibited neuronal overexcitation that was evident in EEG and increased longterm potentiation (LTP). Overexpression of hAPP_{WT} did not alter GABAergic/glutamatergic receptor components or GABA production ability. Nonetheless, we detected a decrease of GABA but not glutamate that could be linked to soluble APP fragments, acting on presynaptic GABA β Rs and subsequently reducing GABA release. By using a specific presynaptic GABA β R antagonist, we were able to rescue hyperexcitation in hAPP_{WT} animals. Our results provide evidence that APP plays a crucial role in regulating inhibitory neurotransmission.

Funding: This work was supported by the Belgian Fund for Scientific Research (FNRS, Grant EQP U.N011.17 and CDR J.0065.21), the "Fondation pour la Recherche sur la Maladie d'Alzheimer" (SAO/FRA), the Queen Elisabeth Medical Foundation and the Concerted Research Action from the General Direction of Scientific Research of the French Community of Belgium (ARC17/22-083).

- Gettemans J.

Site-Specific Fluorescent Labeling, Single-Step Immunocytochemistry, and Delivery of Nanobodies into Living Cells

Methods Mol Biol: 2446:373-393.doi: 10.1007/978-1-0716-2075-5_19. IF 38,81 - Q3

Abstract

The smallest natural antibody fragments currently available are single-domain antibodies obtained from camelid species and sharks (variable new antigen receptors). These molecules consist of a single amino acid chain of ~120 amino acids that adopts a typical immunoglobulin fold. Single-domain antibodies (nanobodies) are monovalent and can be isolated from immunized animals, from naïve libraries, or from synthetic libraries. Importantly, their complete DNA sequences are readily obtained by default, which greatly facilitates their rapid manipulation for various applications. Here, a PCR-based protocol for inserting a sortase A recognition sequence at the carboxy-terminus of a nanobody is described. Subsequently, a sortase A-catalyzed biochemical reaction results in tagging of the nanobody with a short carboxy-terminal amino acid sequence that carries a non-canonical residue (propargyl glycine). This allows click chemistry to be performed with an azido-derivatized fluorophore, with the ensuing fluorescent nanobody being covalently and site-specifically labeled. The labeled nanobody can be used directly for immunocytochemistry, omitting the classical secondary antibody step. Also described are methods for delivery of fluorescent nanobodies into the cytoplasm of mammalian cells by photoporation, a very low-toxicity approach involving laser light and

graphene quantum dots. The combined protocol embodies a novel route for studying protein function in living cells at high resolution.

Acknowledgements

This work was supported by the Stichting Alzheimer Onderzoek (Fondation Recherche Alzheimer) and the [Queen Elisabeth Medical Foundation for Neurosciences](#).



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Publikationen – Publications

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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Wiels, W. A., Wittens, M. M. J., Zeeuws, D., Baeken, C., & Engelborghs, S. (2021).

Neuropsychiatric Symptoms in Mild Cognitive Impairment and Dementia Due to AD: Relation with Disease Stage and Cognitive Deficits.

Frontiers in Psychiatry, Vol. 12, p. 1322. <https://doi.org/10.3389/fpsyt.2021.707580>

Background: The interaction between neuropsychiatric symptoms, mild cognitive impairment (MCI), and dementia is complex and remains to be elucidated. An additive or multiplicative effect of neuropsychiatric symptoms such as apathy or depression on cognitive decline has been suggested. Unraveling these interactions may allow the development of better prevention and treatment strategies. In the absence of available treatments for neurodegeneration, a timely and adequate identification of neuropsychiatric symptom changes in cognitive decline is highly relevant and can help identify treatment targets.

Methods: An existing memory clinic-based research database of 476 individuals with MCI and 978 individuals with dementia due to Alzheimer's disease (AD) was reanalyzed. Neuropsychiatric symptoms were assessed in a prospective fashion using a battery of neuropsychiatric assessment scales: Middelheim Frontality Score, Behavioral Pathology in Alzheimer's Disease Rating Scale (Behave-AD), Cohen-Mansfield Agitation Inventory, Cornell Scale for Depression in Dementia (CSDD), and Geriatric Depression Scale (30 items). We subtyped subjects suffering from dementia as mild, moderate, or severe according to their Mini-Mental State Examination (MMSE) score and compared neuropsychiatric scores across these groups. A group of 126 subjects suffering from AD with a significant cerebrovascular component was examined separately as well. We compared the prevalence, nature, and severity of neuropsychiatric symptoms between subgroups of patients with MCI and dementia due to AD in a cross-sectional analysis.

Results: Affective and sleep-related symptoms are common in MCI and remain constant in prevalence and severity across dementia groups. Depressive symptoms as assessed by the CSDD further increase in severe dementia. Most other neuropsychiatric symptoms (such as agitation and activity disturbances) progress in parallel with severity of cognitive decline. There are no significant differences in neuropsychiatric symptoms when comparing "pure" AD to AD with a significant vascular component.

Conclusion: Neuropsychiatric symptoms such as frontal lobe symptoms, psychosis, agitation, aggression, and activity disturbances increase as dementia progresses. Affective symptoms such as anxiety and depressive symptoms, however, are more frequent in MCI than mild dementia but otherwise remain stable throughout the cognitive spectrum, except for an increase in CSDD score in severe dementia. There is no difference in neuropsychiatric symptoms when comparing mixed dementia (defined here as AD + significant cerebrovascular disease) to pure AD.

Funding. WW was an FWO (Fonds voor Wetenschappelijk Onderzoek Vlaanderen) Fundamental Research Fellow, Grant No. 11E8620N. This work was supported by the [Geneeskundige Stichting Koningin Elisabeth](#)/Fondation Médicale Reine Elisabeth.



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Publicaties – Publications –
Publikationen – Publications

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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Janssens I, Campillo-Davo D, Van den Bos J, De Reu H, Berneman ZN, Wens I, Cools N (2022) ***Engineering of regulatory T cells by means of mRNA electroporation in a GMP-compliant manner.***

Cytotherapy; accepted for publication. IF 5.414

Abstract

Regulatory T cells (Tregs) are crucial in inducing and maintaining tolerance. This unique capacity of Tregs, in combination with proof-of-principle in preclinical studies, highlights the potential clinical use of Tregs for the treatment of autoimmunity and transplant rejection. Although proven to be safe and well tolerated in the first clinical trials, only modest clinical results were observed. In this regard, it has been hypothesized that current challenges lie in the development of antigen-specific Tregs. Here, we present an innovative, good manufacturing practices (GMP)-compliant manufacturing protocol for Tregs applicable in a clinical-grade setting, allowing efficient and safe redirection of Treg specificity. First, a soluble polymer conjugated with antibodies to CD3 and CD28 and high amounts of exogenous IL-2 for *in vitro* Treg expansion resulted in a >70-fold and 185-fold increase of a pure population of CD4⁺CD127⁻CD25^{hi} Tregs and CD4⁺CD127⁻CD25⁺CD45RA⁺ Tregs, respectively. Next, as a proof-of-principle, expanded Tregs were engineered by means of TCR-encoding mRNA electroporation to generate antigen-specific Tregs. This resulted in an expression of the newly introduced TCR in up to 85% of Tregs. Moreover, we did not observe a negative effect on the phenotype of Tregs, as demonstrated by the expression of FOXP3, Helios, CTLA-4 and CCR4, nor on the TSDR methylation status. Importantly, mRNA-engineered Tregs were still able to induce *in vitro* suppression of effector T cells and produced anti-inflammatory, but not pro-inflammatory, cytokines when activated. In conclusion, our findings demonstrate that high numbers of stable and functional Tregs can be obtained with high purity and successfully engineered for gain of function, in a GMP-compliant manner. We envisage that this clinical-grade protocol will provide solid basis for future clinical application of mRNA-engineered Tregs.

Funding: This work was supported in part by Merit Award (I01 RX003201) from the Rehabilitation Research and Development Service, U.S. Department of Veterans Affairs; by the [Queen Elisabeth Medical Foundation](#) (Belgium); and the Concerted Research Action from the General Direction of Scientific Research of the French Community of Belgium (ARC17/22-083).

- Liston A, Humblet-Baron, S, Duffy D, Goris A (2021) ***Human immune diversity: from evolution to modernity.*** ***Nat Immunol.***; 22:1479-1489. IF 25.606

Abstract

The extreme diversity of the human immune system, forged and maintained throughout evolutionary history, provides a potent defense against opportunistic pathogens. At the same time, this immune variation is the substrate upon which a plethora of immune-associated diseases develop. Genetic analysis suggests that thousands of individually weak loci together drive up to half of the observed immune variation. Intense selection maintains this genetic diversity, even selecting for the introgressed Neanderthal or Denisovan alleles that have reintroduced variation lost during the out-of-Africa migration. Variations in age, sex, diet, environmental exposure, and microbiome each potentially explain the residual variation, with proof-of-concept studies demonstrating both plausible mechanisms and correlative associations. The confounding interaction of many of these variables currently makes it difficult

to assign definitive contributions. Here, we review the current state of play in the field, identify the key unknowns in the causality of immune variation, and identify the multidisciplinary pathways toward an improved understanding.

Acknowledgements: This project was supported by the European Union's Horizon 2020 research and innovation program under grant agreement No 874707 (EXIMIOUS). This work was also supported by the Biotechnology and Biological Sciences Research Council through Institute Strategic Program Grant funding BBS/E/B/000C0427 and BBS/E/B/000C0428. AG is supported by the Research Fund KU Leuven (C24/16/045), the Research Foundation-Flanders (FWO G.0734.15), the Belgian Charcot Foundation, the Queen Elisabeth Medical Foundation and the Horizon2020 'MultipleMS' consortium (grant EU RIA 733161). D. D. acknowledges support from the Laboratoire d'Excellence 'Milieu Intérieur', managed by the Agence Nationale de la Recherche, (ANR-10-LABX-69-01). S. H. B. is supported by KU Leuven BOFZAP start-up grant, VLAIO (Flanders Innovation & Entrepreneurship) within the project entitled PRISMA and by a grant from Stichting Alzheimer Onderzoek - Fondation Recherche Maladie Alzheimer (SAO-FMA).



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Universitaire onderzoeksprojecten
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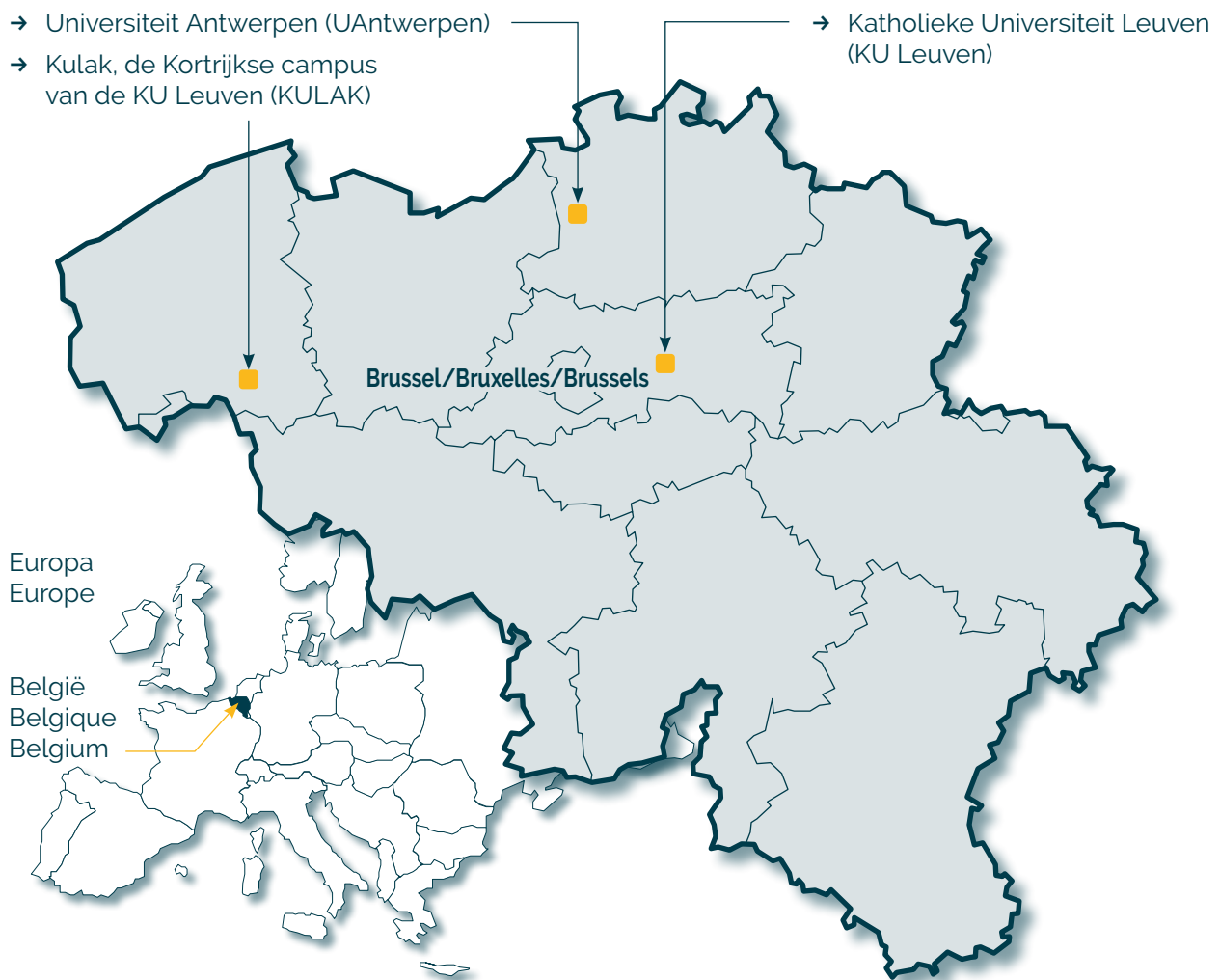
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Publicaties – Publications –
Publikationen – Publications

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www.kuleuven-kulak.be/irf/thrombosis

Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- De Meyer SF, Langhauser F, Haupteltshofer S, Kleinschnitz C, Casas AI, ***Thromboinflammation in brain ischemia: recent updates and future perspectives.*** **Stroke**, under review (invited review) (impact factor 7.91). – **Q1** 2022 May;53(5):1487-1499. <https://doi.org/10.1161/STROKEAHA.122.038733> **Stroke**. 2022;53:1487-1499

Abstract

Despite decades of promising preclinical validation and clinical translation, ischemic stroke still remains as one of the leading causes of death and disability worldwide. Within its complex pathophysiological signatures, thrombosis and inflammation, that is, thromboinflammation, are highly interconnected processes leading to cerebral vessel occlusion, inflammatory responses, and severe neuronal damage following the ischemic event. Hence, we here review the most recent updates on thromboinflammatory-dependent mediators relevant after stroke focusing on recent discoveries on platelet modulation, a potential regulation of the innate and adaptive immune system in thromboinflammation, utterly providing a thorough up-to-date overview of all therapeutic approaches currently undergoing clinical trial.

Keywords: inflammation; ischemic stroke; microglia; thromboinflammation; thrombosis

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 777111 (REPO- TRIAL). This reflects only the author's view, and the European Commission is not responsible for any use that may be made of the information it contains. Dr Casas was supported by the Deutsche Forschungsgemeinschaft (DFG) Walter Benjamin Program (ref. DFG CA 2642/1-1) and Förderprogramm der Corona-Stiftung im Stifterverband. Dr Kleinschnitz, Langhauser received funding from the DFG Forschungsgruppe FOR 2879 (ref. 428778684). Dr De Meyer received funding from Fonds voor Wetenschappelijk Onderzoek-Vlaanderen (FWO) (research grants G0A8613, G078517, 1509216 N and G0E7620N), the Katholieke Universiteit Leuven (OT/14/099, ISP/14/02L2 and PDM/20/147), the Queen Elisabeth Medical Foundation and by the European Union's Horizon 2020 Research and Innovation Program INSIST under grant agreement No. 777072.

- Staessens, S., Francois, O., Brinjikji, W., Doyle, K.M., Vanacker, P., Andersson, T., De Meyer, S.F. (2021).

Studying Stroke Thrombus Composition After Thrombectomy What Can We Learn? **Stroke**, 52 (11), 3718-3727. (Impact factor: 7.91) – Q 1 – 2021 Nov; 52(11): 3718-3727 DOI: [10.1161/STROKEAHA.121.034289](https://doi.org/10.1161/STROKEAHA.121.034289)

Abstract

The composition of ischemic stroke thrombi has gained an increasing amount of interest in recent years. The implementation of endovascular procedures in standard stroke care has granted researchers the unique opportunity to examine patient thrombus material. Increasing evidence indicates that stroke thrombi are complex and heterogenous, consisting of various biochemical (eg, fibrin, von Willebrand Factor, and neutrophil extracellular traps) and cellular (eg, red blood cells, platelets, leukocytes, and bacteria) components. This complex composition may explain therapeutic limitations and also offer novel insights in several aspects of stroke management. Better understanding of thrombus characteristics could, therefore, potentially lead to improvements in the management of patients with stroke. In this review, we provide a comprehensive overview of the lessons learned by examining stroke thrombus composition after endovascular thrombectomy and its potential relevance for thrombectomy success rates, thrombolysis, clinical outcomes, stroke etiology, and radiological imaging.

Keywords: fibrin; ischemic stroke; leukocytes; thrombectomy; von Willebrand Factor.

Funding: This work was supported by research grants to Dr De Meyer from the Fonds voor Wetenschappelijk Onderzoek—Vlaanderen (FWO; research grants G0A8613, G078517, 1509216N, and G0E7620N), the KU Leuven (OT/14/099, ISP/14/02L2, and PDM/20/147), the Queen Elisabeth Medical Foundation and by the European Union's Horizon 2020 Research and Innovation Program INSIST under grant agreement no. 777072. Dr Brinjikji received funding from the National Institutes of Health Grant 1R01NS105853-01. Dr Doyle received funding from Science Foundation Ireland, funding from Cerenovus, and funding from Sensome.

- Denorme, F., Martinod, K., Vandenbulcke, A., Denis, C.V., Lenting, P.J., Deckmyn, H., Vanhoorelbeke, K., De Meyer, S.F. (2021).

The von Willebrand factor A1 domain mediates thromboinflammation, aggravating ischemic stroke outcome in mice.

Haematologica, 106 (3), 819–828. (Impact factor: 9.94) –

Abstract

Von Willebrand factor (VWF) plays an important role in ischemic stroke. However, the exact mechanism by which VWF mediates progression of ischemic stroke brain damage is not completely understood. Using flow cytometric analysis of single cell suspensions prepared from brain tissue and immunohistochemistry, we investigated the potential inflammatory mechanisms by which VWF contributes to ischemic stroke brain damage in a mouse model of cerebral ischemia/reperfusion injury. Twenty-four hours after stroke, flow cytometric analysis of brain tissue revealed that overall white blood cell recruitment in the ipsilesional brain hemisphere of VWF knockout mice was two times lower than that in wildtype mice. More detailed analysis showed a specific reduction of proinflammatory monocytes, neutrophils and T cells in the ischemic brain of VWF knockout mice compared to wild-type mice. Interestingly, histological analysis revealed a substantial number of neutrophils and T cells still within the microcirculation of the stroke brain, potentially contributing to the no-reflow phenomenon. Specific therapeutic targeting of the VWF A1 domain in the wild-type mice resulted in reduced numbers of immune cells in the affected brain and protected mice from ischemic stroke brain damage. More specifically, recruitment of proinflammatory monocytes was reduced two-fold, neutrophil recruitment was reduced five-fold and T-cell recruitment was reduced two-fold in mice treated with a VWF A1-targeting nanobody compared to the recruitment in mice receiving a control nanobody. In conclusion, our data identify a potential role for VWF in the recruitment of proinflammatory monocytes, neutrophils and T cells to the ischemic brain through a mechanism that is mediated by its A1 domain.

Funding: This work was supported by Fonds voor Wetenschappelijk Onderzoek - Vlaanderen (research grants G.0A86.13, G.0785.17 and 1509216N to SFDM), by research grants from KU Leuven (OT/14/099 and ISP/14/02L2 to SFDM) and by a research grant from the Queen Elisabeth Medical Foundation (to SFDM). FD is a postdoctoral fellow of Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO, 12U7818N). KM was a H2020 Marie Skłodowska-Curie Actions fellow (under agreement number 747993, "VWF and NETs").

- Staessens, S., Francois, O., Desender, L., Vanacker, P., Dewaele, T., Sciote, R., Vanhoorelbeke, K., Andersson, T., De Meyer, S.F. (2021).

Detailed histological analysis of a thrombectomy-resistant ischemic stroke thrombus: a case report.

Thrombosis journal, 19 (1), Art.No. ARTN 11 (Impact factor: 5.50) –

Abstract

Background: Mechanical removal of a thrombus by thrombectomy can be quite challenging. For reasons that are not fully understood, some thrombi require multiple passes to achieve

successful recanalization, whereas other thrombi are efficiently removed in a single pass. Since first pass success is associated with better clinical outcome, it is important to better understand the nature of thrombectomy resistant thrombi. The aim of this study was therefore to characterize the cellular and molecular composition of a thrombus that was very hard to retrieve via mechanical thrombectomy.

Case presentation: In a patient that was admitted with a right middle cerebral artery M1-occlusion, 11 attempts using various thrombectomy devices and techniques were required for removal of the thrombus. This peculiar case provided a rare opportunity to perform an in-depth histopathological study of a difficult to retrieve thrombus. Thrombus material was histologically analyzed using hematoxylin and eosin, Martius Scarlet Blue stain (red blood cells and fibrin), Feulgen stain (DNA), von Kossa stain (calcifications) and immunohistochemical analysis of von Willebrand factor, platelets, leukocytes and neutrophil extracellular traps. Histological analysis revealed abnormally high amounts of extracellular DNA, leukocytes, von Willebrand factor and calcifications. Extracellular DNA stained positive for markers of leukocytes and NETs, suggesting that a significant portion of DNA is derived from neutrophil extracellular traps.

Conclusion: In this unique case of a nearly thrombectomy-resistant stroke thrombus, our study showed an atypical composition compared to the common structural features found in ischemic stroke thrombi. The core of the retrieved thrombus consisted of extracellular DNA that colocalized with von Willebrand factor and microcalcifications. These results support the hypothesis that von Willebrand factor, neutrophil extracellular traps and microcalcifications contribute to mechanical thrombectomy resistance. Such information is important to identify novel targets in order to optimize technical treatment protocols and techniques to increase first pass success rates.

Keywords: Histology; Ischemic stroke; NETs; Thrombectomy; Thrombus composition; von Willebrand factor.

Funding: This work was supported by research grants to S.F.D.M. from the Fonds voor Wetenschappelijk Onderzoek – Vlaanderen (FWO) (research grants G.0A86.13, G.0785.17 and 1509216N), the KU Leuven (OT/14/099 and ISP/14/02L2), the [Queen Elisabeth Medical Foundation](#) and by the European Union's Horizon 2020 Research and Innovation Program INSIST under grant agreement No 777072.

Publications in 2021 without acknowledging G.S.K.E. - Q.E.M.F – F.M.R.E.

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Evaluation for NeuroThrombectomy (EXCELLENT) Registry design and methods. Journal of neurointerventional surgery. (Impact factor: 5.84) –14(8)783-787

Published online 2021 Oct 13. doi: [10.1136/neurintsurg-2021-017671](https://doi.org/10.1136/neurintsurg-2021-017671)

Abstract

Background: Relationships between occlusive clot histopathology, baseline characteristics, imaging findings, revascularization rates, and clinical outcomes of stroke patients with large vessel occlusion (LVO) are not well understood. This study will assess the real-world experience on the efficacy and safety of using the EmboTrap device as the first approach in LVO patients and explore the associations between clot histological characteristics, imaging and clinical findings, revascularization rates, and clinical outcomes.

Methods: Prospective, global, multicenter, single-arm, imaging core laboratory, and clot analysis central laboratory observational registry. Adult patients (>18 years) with LVO, treated with EmboTrap as the first attempted device, will be eligible for study participation.

Results: Up to 1000 subjects at 50 international sites may be enrolled. Occlusive clots will be collected from at least 500 subjects. Independent central and imaging core laboratories will perform clot analysis and image adjudication. Statistical analysis will assess the association between imaging and clinical findings, clot characteristics, subject comorbidities, revascularization, and clinical outcomes. Study endpoints are functional independence (modified Rankin Scale score ≤ 2 at 90 days), expanded Thrombolysis In Cerebral Infarction (eTICI) score ≥ 2 b50 rate, first-pass effect, number of passes, embolization into new territory, symptomatic intracranial hemorrhage, and 90-day mortality.

Conclusions: The EXCELLENT registry will provide reproducible effectiveness and safety data of EmboTrap for its use for mechanical thrombectomy. Additionally, the study will characterize the blood clots retrieved during mechanical thrombectomy with respect to their composition and histopathological analysis and potential correlations with clinical and imaging findings.

Trial registration number: [NCT03685578](https://www.clinicaltrials.gov/ct2/show/study/NCT03685578).

Keywords: artery; stent; stroke; thrombectomy.

- Boodt, N., van Schauburg, P.R W S., Hund, H.M., Fereidoonzhad, B., McGarry, J.P., Akyildiz, A.C., van Es, A.C G M., De Meyer, S.F., Dippel, D.W J., Lingsma, H.F., van Beusekom, H.M M., van der Lugt, A., Gijzen, F.J H. (2021).

Mechanical Characterization of Thrombi Retrieved With Endovascular Thrombectomy in Patients With Acute Ischemic Stroke.

Stroke, 2021 Aug; 52(8): 2510–2517. 52 (8), 2510-2517. (Impact factor: 7.91) – Q1, PMID: 34078112

Abstract

Background and Purpose: Mechanical properties of thromboemboli play an important role in the efficacy of endovascular thrombectomy (EVT) for acute ischemic stroke. However, very limited data on mechanical properties of human stroke thrombi are available. We aimed to mechanically characterize thrombi retrieved with EVT, and to assess the relationship between thrombus composition and thrombus stiffness.

Methods: Forty-one thrombi from 19 patients with acute stroke who underwent EVT between July and October 2019 were mechanically analyzed, directly after EVT. We performed

unconfined compression experiments and determined tangent modulus at 75% strain (E_{t75}) as a measure for thrombus stiffness. Thrombi were histologically analyzed for fibrin/platelets, erythrocytes, leukocytes, and platelets, and we assessed the relationship between histological components and E_{t75} with univariable and multivariable linear mixed regression.

Results: Median E_{t75} was 560 (interquartile range, 393–1161) kPa. In the multivariable analysis, fibrin/platelets were associated with increased E_{t75} ($\alpha\beta$, 9 [95% CI, 5 to 13]) kPa, erythrocytes were associated with decreased E_{t75} ($\alpha\beta$, -9 [95% CI, -5 to -13]) kPa. We found no association between leukocytes and E_{t75} . High platelet values were strongly associated with increased E_{t75} ($\alpha\beta$, 56 [95% CI, 38–73]).

Conclusions: Fibrin/platelet content of thrombi retrieved with EVT for acute ischemic stroke is strongly associated with increased thrombus stiffness. For thrombi with high platelet values, there was a very strong relationship with thrombus stiffness. Our data provide a basis for future research on the development of next-generation EVT devices tailored to thrombus composition.

Keywords: acute stroke, fibrin, leukocytes, medical center, thrombectomy

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- Iwata, R., Casimir, P., Erkol, E., Boubakar, L., Planque, M., Ditkowska, M., Vints, K., Gaspariunaite, V., Bird, M., Corthout, N., Vermeersch, P., Davie, K., Gounko, V., Aerts, S., Ghesquière, B., Fendt, S-M, Vanderhaeghen

Species-specific mitochondria dynamics and metabolism regulate the timing of neuronal development.

P. Science, in review. Biorxiv 2021 <https://doi.org/10.1101/2021.12.27.474246>. –

- Libé-Philippot B, Vanderhaeghen

Cellular and molecular mechanisms linking human cortical development and evolution.

P. Annu. Rev. Genetics. 2021. 55:555-581. – IF: 16.83; **Q1**). 2021. PMID: 34535062

Abstract

The cerebral cortex is at the core of brain functions that are thought to be particularly developed in the human species. Human cortex specificities stem from divergent features of corticogenesis, leading to increased cortical size and complexity. Underlying cellular mechanisms include prolonged patterns of neuronal generation and maturation, as well as the amplification of specific types of stem/progenitor cells. While the gene regulatory networks of corticogenesis appear to be largely conserved among all mammals including humans, they have evolved in primates, particularly in the human species, through the emergence of rapidly divergent transcriptional regulatory elements, as well as recently duplicated novel genes. These human-specific molecular features together control key cellular milestones of human corticogenesis and are often affected in neurodevelopmental disorders, thus linking human neural development, evolution, and diseases.

Keywords: cerebral cortex development; heterochrony; hominid-specific gene; human brain evolution; neoteny; neurogenesis.

Acknowledgements: We wish to apologize to the authors whose work could not be discussed due to space constraints. Work from the P.V. lab discussed here was funded by European Research Council (ERC) Advanced Grants (GENDEVOCORTEX and NEUROTEMPO), the Fondation ROGER DE SPOELBERCH, the Generet Fund, the Fondation JED, the EOS Programme, the Belgian Queen Elisabeth Medical Foundation, the Belgian Flemish Research Council (FWO) and Fund for Scientific Research (FRS-FNRS), the AXA Research Fund, and the Fondation ULB.

- Iwata R, Vanderhaeghen

Regulatory Roles of Mitochondria and Metabolism in Neurogenesis.

P. Curr Opin Neurobiol. 2021; 69:231-240. – (IF: 6.63; **Q1**). 2021 Aug;69:231-240. doi: 10.1016/j.conb.2021.05.003. Epub 2021 Jun 23.

Abstract

Neural stem cells (NSCs) undergo massive molecular and cellular changes during neuronal differentiation. These include mitochondria and metabolism remodelling, which were thought to be mostly permissive cues, but recent work indicates that they are causally linked to neurogenesis. Striking remodelling of mitochondria occurs right after mitosis of NSCs, which influences the postmitotic daughter cells towards self-renewal or differentiation. The transitioning to neuronal fate requires metabolic rewiring including increased oxidative phosphorylation activity, which drives transcriptional and epigenetic effects to influence cell fate. Mitochondria metabolic pathways also contribute in an essential way to the regulation of NSC proliferation and self-renewal. The influence of mitochondria and metabolism on

neurogenesis is conserved from fly to human systems, but also displays striking differences linked to cell context or species. These new findings have important implications for our understanding of neurodevelopmental diseases and possibly human brain evolution.

Acknowledgements: The authors wish to apologise to the authors whose work could not be discussed because of space constraints. Work from the P.V. laboratory described here was funded by the European Research Council (ERC Adv Grant), the Fondation Roger de Spoelberch, the Belgian FWO and FRS/FNRS, the AXA Research Fund, the Belgian Queen Elisabeth Medical Foundation and the Fondation ULB. R.I. was a postdoctoral fellow of the Belgian FRS/FNRS.

- Bonnefont J, Vanderhaeghen

Neuronal fate acquisition and specification : time for a change.

P. Curr Opin Neurobiol. 2021;66:195-20. (IF: 6.63; Q1) 2021 Feb;66:195-204.

Abstract

During embryonic development, neural stem/progenitor cells generate hundreds of different cell types through the combination of intrinsic and extrinsic cues. Recent data obtained in mouse and human cortical neurogenesis provide novel views about this interplay and how it evolves with time, whether during irreversible cell fate transitions that neural stem cells undergo to become neurons, or through gradual temporal changes of competence that lead to increased neuronal diversity from a common stem cell pool. In each case the temporal changes result from a dynamic balance between intracellular states and extracellular signalling factors. The underlying mechanisms are mostly conserved across species, but some display unique features in human corticogenesis, thereby linking temporal features of neurogenesis and human brain evolution.

Acknowledgements: We wish to apologise to the authors whose work could not be discussed due to space constraints. Work from the authors described here was funded by the European Research Council (ERC Adv Grant GENDEVOCORTEX), the EOS Programme, the Fondation ROGER DE SPOELBERCH, the Belgian FWO, the Belgian FRS/FNRS, the WELBIO Programme, the AXA Research Fund, the Belgian Queen Elisabeth Medical Foundation, and the Fondation ULB.

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- M. Vanneste, M. Mulier, A.C.N. Freitas, N. Van Ranst, A. Kerstens, T. Voets, W. Everaerts. ***TRPM3 Is Expressed in Afferent Bladder Neurons and Is Upregulated during Bladder Inflammation.*** *Int J Mol Sci.* (2021) – (IF: 4.56; **Q1**). 2021. PMID: 35008533

Abstract

The cation channel TRPM3 is activated by heat and the neurosteroid pregnenolone sulfate. TRPM3 is expressed on sensory neurons innervating the skin, where together with TRPV1 and TRPA1, it functions as one of three redundant sensors of acute heat. Moreover, functional upregulation of TRPM3 during inflammation contributes to heat hyperalgesia. The role of TRPM3 in sensory neurons innervating internal organs such as the bladder is currently unclear. Here, using retrograde labeling and single-molecule fluorescent RNA in situ hybridization, we demonstrate expression of mRNA encoding TRPM3 in a large subset of dorsal root ganglion (DRG) neurons innervating the mouse bladder, and confirm TRPM3 channel functionality in these neurons using Fura-2-based calcium imaging. After induction of cystitis by injection of cyclophosphamide, we observed a robust increase of the functional responses to agonists of TRPM3, TRPV1, and TRPA1 in bladder-innervating DRG neurons. Cystometry and voided spot analysis in control and cyclophosphamide-treated animals did not reveal differences between wild type and TRPM3-deficient mice, indicating that TRPM3 is not critical for normal voiding. We conclude that TRPM3 is functionally expressed in a large proportion of sensory bladder afferent, but its role in bladder sensation remains to be established.

Keywords: TRPM3; bladder inflammation; bladder pain syndrome; urinary bladder.

Funding: This work was supported by the Research Council of the KU Leuven (C2-TRP; to T.V. and W.E.), the Flemish Research Organization (FWO; GoB7620N to T.V.), a grant from the Queen Elisabeth Foundation for Neurosciences (to T.V.), and an unrestricted grant from the VIB (to T.V.). W.E. is a senior clinical researcher and M.M. a junior postdoctoral researcher of FWO Flanders.

- K. Held, V.D. Aloï, A.C.N. Freitas, A. Janssens, A. Segal, J. Przibilla, S.E. Philipp, Y.T. Wang, T. Voets, J. Vriens, Pharmacological properties of TRPM3 isoforms are determined by the length of the pore loop, ***Br J Pharmacol*** – (IF: 8.74; **Q1**) 2022 Jul;179(14):3560–3575. doi: 10.1111/bph.15223.

Abstract

Background and purpose: Transient receptor potential melastatin 3 (TRPM3) is a non-selective cation channel that plays a pivotal role in the peripheral nervous system as a transducer of painful heat signals. Alternative splicing gives rise to several TRPM3 variants. The functional consequences of these splice isoforms are poorly understood. Here, the pharmacological properties of TRPM3 variants arising from alternative splicing in the pore-forming region were compared.

Experimental approach: Calcium microfluorimetry and patch clamp recordings were used to compare the properties of heterologously expressed TRPM3 α 1 (long pore variant) and TRPM3 α 2- α 6 (short pore variants). Furthermore, site-directed mutagenesis was done to investigate the influence of the length of the pore loop on the channel function.

Key results: All short pore loop TRPM3 α variants (TRPM3 α 2- α 6) were activated by the neurosteroid pregnenolone sulphate (PS) and by nifedipine, whereas the long pore loop variant TRPM3 α 1 was insensitive to either compound. In contrast, TRPM3 α 1 was robustly activated by clotrimazole, a compound that does not directly activate the short pore variants but potentiates their responses to PS. Clotrimazole-activated TRPM3 α 1 currents were largely insensitive to

established TRPM3 α 2 antagonists and were only partially inhibited upon activation of the μ opioid receptor. Finally, by creating a set of mutant channels with pore loops of intermediate length, we showed that the length of the pore loop dictates differential channel activation by PS and clotrimazole.

Conclusion and implications: Alternative splicing in the pore-forming region of TRPM3 defines the channel's pharmacological properties, which depend critically on the length of the pore-forming loop.

Linked articles: This article is part of a themed issue on Structure Guided Pharmacology of Membrane Proteins (BJP 75th Anniversary). To view the other articles in this section visit <http://onlinelibrary.wiley.com/doi/10.1111/bph.v179.14/issuetoc>.

Keywords: Splice variants TRP channels; TRPM3; nociception.

Acknowledgements: We thank all the members of the Laboratory of Ion Channel Research and the Laboratory of Endometrium, Endometriosis and Reproduction at the KU Leuven for their helpful discussions and comments. This project has received funding from the Belgian Federal Government (IUAP P7/13 to T.V.), the Research Foundation–Flanders (G.0565.07 and G.0825.11 to T.V. and J.V.; G.084515N and G.0B1819N to J.V.), the Research Council of the KU Leuven (C1-TRPLe to T.V.), the Queen Elisabeth Medical Foundation for Neurosciences (to T.V.), the Belgian Foundation Against Cancer (to J.V. and T.V.) and the Saarland University (HOMFOR) (to S.E.P.). The manuscript was published with support of the University Foundation from Belgium (WA-0292 to K.H.). K.H. is a "postdoctoral fellow" of the Research Foundation–Flanders, Belgium and a research trainee of the Michael Smith Foundation for Health Research/Vancouver Coastal Health Research Institute/VGH and UBC Hospital Foundation.

- B. Kelemen, S. Pinto, N. Kim, E. Lisztes, M. Hanyicska, A. Vladar, A. Olah, Z. Penzes, B. Shu, J. Vriens, T. Biro, T. Rohacs, T. Voets*, B.I. Toth,

The TRPM3 ion channel mediates nociception but not itch evoked by endogenous pruritogenic mediators,

***Biochem Pharmacol* 183** (2021) 114310. – (IF: 5.86; Q1). 2021. PMID: 33130130

Abstract

During the molecular transduction of itch, the stimulation of pruriceptors on sensory fibers leads to the activation or sensitization of ion channels, which results in a consequent depolarization of the neurons. These ion channels mostly belong to the transient receptor potential (TRP) channels, which are involved in nociception and thermosensation. In particular, TRPV1 and TRPA1 were described in the transduction of both thermal nociception as well as histaminergic and non-histaminergic itch. The thermosensitive TRPM3 plays an indispensable role in heat nociception together with TRPV1 and TRPA1. However, the role of TRPM3 in the development of pruritus has not been studied yet. Therefore, in this study we aimed at investigating the potential role of TRPM3 in the transduction of pruritus and pain by investigating itch- and nociception-related behavior of Trpm3^{+/+} and Trpm3^{-/-} mice, and by studying the activation of somatosensory neurons isolated from trigeminal ganglia upon application of algogenic and pruritogenic substances. Activators of TRPM3 evoked only nocifensive responses, but not itch in Trpm3^{+/+} animals, and these nocifensive responses were abolished in the Trpm3^{-/-} strain. Histamine and endogenous non-histaminergic pruritogens induced itch in both Trpm3^{+/+} and Trpm3^{-/-} mice to a similar extent. Genetic deletion or pharmacological blockade diminished TRPM3 mediated Ca²⁺ responses of sensory neurons, but did not affect responses evoked by pruritogenic substances. Our results demonstrate that, in contrast to other thermosensitive TRP channels, TRPM3 selectively mediates nociception, but not itch sensation, and suggest that TRPM3 is a promising candidate to selectively target pain sensation.

Keywords: Cheek model; Endogenous pruritogens; Itch; Nociception; TRP channels; TRPM3.

Acknowledgements: The presented work was supported by research grants of the National Research, Development and Innovation Office (K_120187, PD_121360, PD-134791, FK_125055, GINOP-2.3.2-15-2016-00015, EFOP-3.6.3-VEKOP-16-2017-00009, EFOP-3.6.1-16-2016-00022). The work of AO, and BIT was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. B. I. T. was also supported by the New National Excellence Program of the Ministry for Innovation and Technology (ÚNKP-20-5-DE-422). The work of NK and TR was supported by NIH grants NS055159, GM093290 and GM131048 to T.R. Research in the lab of T.V. is supported by grants from the VIB, the KU Leuven Research Council, the Research Foundation-Flanders (FWO GoB7620N to T.V. and Go84515N and GoB1819N to J.V.), the Belgian Foundation Against Cancer and the [Queen Elisabeth Medical Foundation](#) for Neurosciences.



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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

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KCNQ2 R144 variants cause ID with language impairment and autistic features through a gain-of-function mechanism

EBioMedicine: Submitted to EbioMedicine in 2021:

(IF: [8.14](#); **Q1**) 2022 Jul;81:104130. doi: 10.1016/j.ebiom.2022.104130.

Abstract

Background: Prior studies have revealed remarkable phenotypic heterogeneity in KCNQ2-related disorders, correlated with effects on biophysical features of heterologously expressed channels. Here, we assessed phenotypes and functional properties associated with KCNQ2 missense variants R144W, R144Q, and R144G. We also explored *in vitro* blockade of channels carrying R144Q mutant subunits by amitriptyline.

Methods: Patients were identified using the RIKEE database and through clinical collaborators. Phenotypes were collected by a standardized questionnaire. Functional and pharmacological properties of variant subunits were analyzed by whole-cell patch-clamp recordings.

Findings: Detailed clinical information on fifteen patients (14 novel and 1 previously published) was analyzed. All patients had developmental delay with prominent language impairment. R144Q patients were more severely affected than R144W patients. Infantile to childhood onset epilepsy occurred in 40%, while 67% of sleep-EEGs showed sleep-activated epileptiform activity. Ten patients (67%) showed autistic features. Activation gating of homomeric Kv7.2 R144W/Q/G channels was left-shifted, suggesting gain-of-function effects. Amitriptyline blocked channels containing Kv7.2 and Kv7.2 R144Q subunits.

Interpretation: Patients carrying KCNQ2 R144 gain-of-function variants have developmental delay with prominent language impairment, autistic features, often accompanied by infantile-to childhood-onset epilepsy and EEG sleep-activated epileptiform activity. The absence of neonatal seizures is a robust and important clinical differentiator between KCNQ2 gain-of-function and loss-of-function variants. The Kv7.2/7.3 channel blocker amitriptyline might represent a targeted treatment.

Funding: Supported by FWO, GSKE, KCNQ2-Cure, Jack Pribaz Foundation, European Joint Programme on Rare Disease 2020, the Italian Ministry for University and Research, the Italian Ministry of Health, the European Commission, the University of Antwerp, NINDS, and Chalk Family Foundation.

Keywords: Amitriptyline; Autism; Developmental and epileptic encephalopathy; Gain-of-function; KCNQ2.

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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Jonas Van Lent, Peter Verstraelen, Bob Asselbergh, Elias Adriaenssens, Ligia Mateiu, Christophe Verbist, Vicky De Winter, Kristel Eggermont, Ludo Van Den Bosch, Winnok H. De Vos and Vincent Timmerman,

Induced pluripotent stem cell-derived motor neurons of CMT type 2 patients reveal progressive mitochondrial dysfunction

Brain. 2021 Sep 4;144(8):2471-2485. doi: 10.1093/brain/awab226.

Abstract

Axonal Charcot-Marie-Tooth neuropathies (CMT type 2) are caused by inherited mutations in various genes functioning in different pathways. The types of genes and multiplicity of mutations reflect the clinical and genetic heterogeneity in CMT2 disease, which complicates its diagnosis and has inhibited the development of therapies. Here, we used CMT2 patient-derived pluripotent stem cells (iPSCs) to identify common hallmarks of axonal degeneration shared by different CMT2 subtypes. We compared the cellular phenotypes of neurons differentiated from CMT2 patient iPSCs with those from healthy controls and a CRISPR/Cas9-corrected isogenic line. Our results demonstrated neurite network alterations along with extracellular electrophysiological abnormalities in the differentiated motor neurons. Progressive deficits in mitochondrial and lysosomal trafficking, as well as in mitochondrial morphology, were observed in all CMT2 patient lines. Differentiation of the same CMT2 iPSC lines into peripheral sensory neurons only gave rise to cellular phenotypes in subtypes with sensory involvement, supporting the notion that some gene mutations predominantly affect motor neurons. We revealed a common mitochondrial dysfunction in CMT2-derived motor neurons, supported by alterations in the expression pattern and oxidative phosphorylation, which could be recapitulated in the sciatic nerve tissue of a symptomatic mouse model. Inhibition of a dual leucine zipper kinase could partially ameliorate the mitochondrial disease phenotypes in CMT2 subtypes. Altogether, our data reveal shared cellular phenotypes across different CMT2 subtypes and suggests that targeting such common pathomechanisms could allow the development of a uniform treatment for CMT2.

Funding: This work was supported in part by the University of Antwerp (DOC-PRO4 PhD fellowship to J.V.L., TOP-BOF research grant N38694 to V.T., BOF research grant N41739 and VLAIO grant HBC.2016.0534 to W.D.V.), the Fund for Scientific Research (FWO Flanders research grant N G041416N to V.T., grant NG017618N to W.D.V, and FWO postdoc fellowship to EA), the "Association Belge contre les Maladies Neuromusculaires" (ABMM grants to E.A. and V.T.), the Queen Elisabeth Medical Foundation (GSKE grant to V.T.), the American Muscular Dystrophy Association (MDA research grant N577497 to V.T.), and the EU projects NEUROMICS (FP7 under grant agreement number N2012-305121) and Solve-RD (Horizon 2020 under grant agreement N779257). The UltraView ERS confocal spinning disk microscope and Tecnai G2 Spirit Bio Twin Electron Microscope was supported by FWO-HERCULES large infrastructure grants N 23714 and N 25340. The Seahorse XF HS Mini Analyzer was supported by the University of Antwerp Basic Research Infrastructure grant N 41438. V.T. and W.D.V. are members of the mNeuro Center of Excellence at the University of



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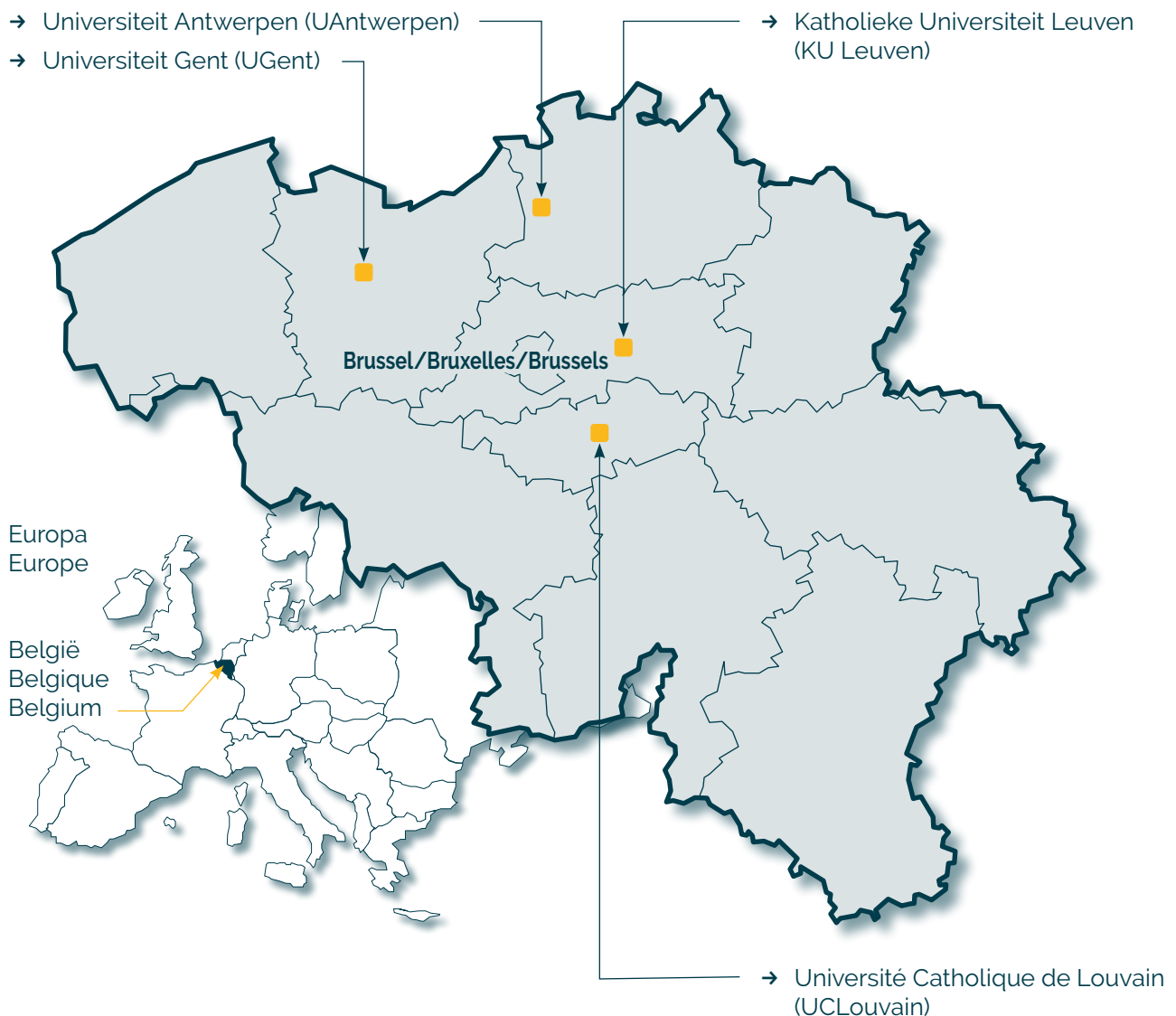
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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Gadeyne S, Mertens A, Carrette E, Van den Bossche F, Boon P, Raedt R, Vonck K (2021). ***Transcutaneous auricular vagus nerve stimulation cannot modulate the P3b event-related potential in healthy volunteers.*** Clin Neurophysiol. 2021 Dec 29;135:22-29. doi: 10.1016/j.clinph.2021.11.079. Epub ahead of print. PMID: 35007840. –

Abstract

Objective: The release of cortical norepinephrine is one of the possible mechanisms of action of vagus nerve stimulation (VNS), a neuromodulatory treatment currently under investigation for cognitive impairment. Transcutaneous auricular VNS (taVNS) may be able to activate vagal nerve branches ending in the brainstem's locus coeruleus (LC) non-invasively. The aim was to investigate if acute taVNS can modulate the P3b, a cognitive event-related potential (ERP) reflecting noradrenergic brain activation under control of the LC.

Methods: Thirty-nine healthy volunteers performed an auditory oddball task during no stimulation, sham stimulation and taVNS in a randomized order. P3b amplitude, latency and behavioral outcome parameters were compared between conditions using linear mixed models.

Results: P3b amplitude and latency during taVNS did not differ significantly from sham or control. Reaction time shortened and P3b latency prolonged with repetition of the oddball task.

Conclusions: We were unable to modulate cognitive ERPs by means of acute taVNS in a large group of healthy volunteers. Significance: Targeting vagal nerve fibres via a transcutaneous approach did not alter the P3b in healthy participants. The stimulation parameters used and transient delivery of taVNS might be insufficient to adequately modulate the LC. Also, a disbalanced locus coeruleus - norepinephrine system in patients may be more prone for improvement.

Keywords: Transcutaneous auricular vagus nerve stimulation P3b, Event-related potentials, Locus coeruleus Norepinephrine Healthy volunteers

Acknowledgements: Thanks to Eva Claeys and Freek Van den Bossche for work on the data collection and analysis and Stefanie De Buyser for the advice on the statistical analysis. Ann Mertens is supported by an "Aspirant" grant of the "Fonds voor Wetenschappelijk Onderzoek" (FWO) Flanders. Evelien Carrette is supported by a research grant of Ghent University Hospital and Geneeskundige Stichting Koningin Elisabeth (G.S.K.E.). Paul Boon is supported by grants of the FWO Flanders, BOF-UGent, Ghent University Hospital, and E-Epilepsy (EU). Robrecht Raedt is supported by grants of the FWO Flanders and BOF-UGent special research fund. Kristl Vonck has been funded by the BOF-UGent special research.

- Mertens A, Carrette S, Klooster D, Lescrauwaet E, Delbeke J, Wadman WJ, Carrette E, Raedt R, Boon P, Vonck K (2021). ***Investigating the Effect of Transcutaneous Auricular Vagus Nerve Stimulation on Cortical Excitability in Healthy Males.*** Neuromodulation. 2021 Jul 20. doi: 10.1111/ner.13488. Epub ahead of print. PMID: 34288274. – Poppa T,

Abstract

Objectives: As a potential treatment for epilepsy, transcutaneous auricular vagus nerve stimulation (taVNS) has yielded inconsistent results. Combining transcranial magnetic stimulation with electromyography (TMS-EMG) and electroencephalography (TMS-EEG) can be used to investigate the effect of interventions on cortical excitability by evaluating changes

in motor evoked potentials (MEPs) and TMS-evoked potentials (TEPs). The goal of this study is to objectively evaluate the effect of taVNS on cortical excitability with TMS-EMG and TMS-EEG. These findings are expected to provide insight in the mechanism of action and help identify more optimal stimulation paradigms.

Materials and methods: In this prospective single-blind cross-over study, 15 healthy male subjects underwent active and sham taVNS for 60 min, using a maximum tolerated stimulation current. Single and paired pulse TMS was delivered over the right-sided motor hotspot to evaluate MEPs and TEPs before and after the intervention. MEP statistical analysis was conducted with a two-way repeated measures ANOVA. TEPs were analyzed with a cluster-based permutation analysis. Linear regression analysis was implemented to investigate an association with stimulation current.

Results: MEP and TEP measurements were not affected by taVNS in this study. An association was found between taVNS stimulation current and MEP outcome measures indicating a decrease in cortical excitability in participants who tolerated higher taVNS currents. A subanalysis of participants ($n = 8$) who tolerated a taVNS current ≥ 2.5 mA showed a significant increase in the resting motor threshold, decrease in MEP amplitude and modulation of the P60 and P180 TEP components.

Conclusions: taVNS did not affect cortical excitability measurements in the overall population in this study. However, taVNS has the potential to modulate specific markers of cortical excitability in participants who tolerate higher stimulation levels. These findings indicate the need for adequate stimulation protocols based on the recording of objective outcome parameters.

Keywords: Cortical excitability; TMS-evoked potential; motor evoked potential; transcranial magnetic stimulation; transcutaneous vagus nerve stimulation.

Grant support

Source(s) of financial support: Ann Mertens is supported by an "Aspirant" grant of the "Fonds voor Wetenschappelijk Onderzoek" (FWO) Flanders. Debby Klooster is supported by a "Junior Postdoc" grant of the FWO Flanders. Evelien Carrette is supported by a research grant of Ghent University Hospital and Geneeskundige Stichting Koningin Elisabeth (G.S.K.E.). Robrecht Raedt is supported by grants of the FWO Flanders and BOF-UGent special research fund. Kristl Vonck has been funded by the BOF-UGent special research fund. Paul Boon is supported by grants of the FWO Flanders, BOF-UGent, Ghent University Hospital, and E-Epilepsy (EU).

- Benschop L, Horczak P, Vanderhasselt MA, Carrette E, Bechara A, Baeken C, Vonck K.

Auricular transcutaneous vagus nerve stimulation modulates the heart-evoked potential.

Brain Stimul. 2021 Dec 18;15(1):260- 269. doi: 10.1016/j.brs.2021.12.004. Epub ahead of print. PMID: 34933143.

Abstract

Background: There is active interest in biomarker discovery for transcutaneous auricular vagus nerve stimulation (taVNS). However, greater understanding of the neurobiological mechanisms is needed to identify candidate markers. Accumulating evidence suggests that taVNS influences activity in solitary and parabrachial nuclei, the primary brainstem relays for the transmission of visceral sensory afferents to the insula. The insula mediates interoception, which concerns the representation and regulation of homeostatic bodily states. Consequently, interoceptive pathways may be relevant to taVNS mechanisms of action.

Hypotheses: We hypothesized that taVNS would modulate an EEG-derived marker of interoceptive processing known as the heart-evoked potential (HEP). We also hypothesized that taVNS-induced HEP effects would be localizable to the insula.

Methods: Using a within-subject, sham-controlled design, we recorded EEG and ECG concurrent to taVNS in 43 healthy adults. Using ECG and EEG data, we extracted HEPs. Estimation of the cortical sources of the taVNS-dependent HEP responses observed at the scalp were computed

using the Boundary Element Method and weighted Minimum Norm Estimation. Statistics were calculated using cluster-based permutation methods.

Results: taVNS altered HEP amplitudes at frontocentral and centroparietal electrode sites at various latencies. The taVNS-dependent HEP effect was localized to the insula, operculum, somatosensory cortex, and orbital and ventromedial prefrontal regions.

Conclusion: The results support the hypothesis that taVNS can access the insula as well as functionally and anatomically connected brain regions. HEPs may serve as an objective, non-invasive outcome parameter for the cortical effects of taVNS.

Keywords: Auricular; Central autonomic network; Electroencephalography; Heart-evoked potential; Insula; Interoception; Source-localization; Transcutaneous; Vagus nerve stimulation.

Funding This study was supported by a Graduate Research Fellowship from the National Science Foundation (DGE-1418060) and a Belgian American Educational Foundation (BAEF) fellowship to TP. KV and MAV are funded by special research grants from Ghent University. KV is also funded by a grant from Het Fonds Wetenschappelijk Onderzoek – Vlaanderen (FWO) (WOG-tVNS group) . EC is funded by a [Geneeskundige Stichting Koningin Elisabeth \(GSKE\)](#) fellowship. The funders had no influence on the study design; in the collection, analysis, or interpretation of data; in the writing of the report; or the decision to submit the article for publication.

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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Acharya AR, Larsen LE, Van Lysebettens W, Wadman WJ, Delbeke J, Vonck K, Meurs A, Boon P, Raedt R.

Attenuation of hippocampal evoked potentials in-vivo by activation of GtACR2, an optogenetic chloride-channel.

Frontiers in Neuroscience. 29 March 2021 <https://doi.org/10.3389/fnins.2021.653844>. Peer Reviewed, Impact factor (2019, most recent) = 3.707, Category: Neurosciences, Rank 96/271, Q2
In this paper, we showed the utility of the GtACR2 opsin, a blue light gated chloride channel, for inhibition of evoked hippocampal activity. Opsins, such as GtACR2 is a powerful alternative to photopharmacology for closed-loop applications.

Abstract

Aim: GtACR2, a light-activated chloride channel, is an attractive tool for neural inhibition as it can shunt membrane depolarizations. In this study, we assessed the effect of activating GtACR2 on *in vivo* hippocampal CA1 activity evoked by Schaffer collateral (SC) stimulation.

Methods: Adult male Wistar rats were unilaterally injected with 0.5 μ L of adeno associated viral vector for induction of GtACR2-mCherry ($n = 10$, GtACR2 group) or mCherry ($n = 4$, Sham group) expression in CA1 pyramidal neurons of the hippocampus. Three weeks later, evoked potentials (EPs) were recorded from the CA1 subfield placing an optrode (bipolar recording electrode attached to an optic fiber) at the injection site and a stimulation electrode targeting SCs. Effects of illumination parameters required to activate GtACR2 such as light power densities (LPDs), illumination delays, and light-pulse durations were tested on CA1 EP parameters [population spike (PS) amplitude and field excitatory postsynaptic potential (fEPSP) slope].

Results In the GtACR2 group, delivery of a 10 ms light-pulse induced a negative deflection in the local field potential which increased with increasing LPD. When combined with electrical stimulation of the SCs, light-induced activation of GtACR2 had potent inhibitory effects on CA1 EPs. An LPD of 160 mW/mm² was sufficient to obtain maximal inhibition CA1 EPs. To quantify the duration of the inhibitory effect, a 10 ms light-pulse of 160 mW/mm² was delivered at increasing delays before the CA1 EPs. Inhibition of EPs was found to last up to 9 ms after the cessation of the light-pulse. Increasing light-pulse durations beyond 10 ms did not result in larger inhibitory effects.

Conclusion: Precisely timed activation of GtACR2 potently blocks evoked activity of CA1 neurons. The strength of inhibition depends on LPD, lasts up to 9 ms after a light-pulse of 10 ms, and is independent of the duration of the light-pulse given.

Keywords: CA1 inhibition; GtACR2; chloride channel; evoked potential; hippocampus; optogenetics.

Funding. AA was supported by a Ph.D. grant from the Ghent Institute for Neuroscience. LL was supported by the Fund for Scientific Research Flanders (FWO) and the Queen Elisabeth Medical Foundation (GSKE). PB, KV, AM, and RR were supported by FWO and the Special Research Funds (BOF) of Ghent University

- Acharya AR, Vandekerckhove B, Larsen LE, Delbeke J, Wadman WJ, Vonck K, Carette E, Meurs A, Vanfleteren J, Boon P, Missinne J, Raedt R.

In vivo blue light illumination for optogenetic inhibition: effect on local temperature and excitability of the rat hippocampus.

Journal of Neural Engineering. 2021 Dec 24;18(6) doi: 10.1088/1741-2552/ac3ef4. Peer Reviewed, Impact factor (2020, most recent) = 5.379, Category: Engineering, Biomedical, Rank 20/89, Q1

In this paper, effects of blue light on local temperature was explored through modeling and simulations and subsequently validated with experimental recordings from the rat brain. The models constructed and validated in this paper will be of great value in the in vitro to in vivo translation of our photopharmacology work.

Abstract

Objective: The blue light-activated inhibitory opsin, stGtACR2, is gaining prominence as a neuromodulatory tool due its ability to shunt-inhibit neurons and is being frequently used *in vivo* experimentation. However, experiments involving stGtACR2 use longer durations of blue light pulses, which inadvertently heat up the local brain tissue and confound experimental results. Therefore, the heating effects of illumination parameters used for *in vivo* optogenetic inhibition must be evaluated.

Approach: To assess blue light (473 nm)-induced heating of the brain, we used a computational model as well as direct temperature measurements using a fiber Bragg grating (FBG). The effects of different light power densities (LPDs) and pulse durations on evoked potentials (EP) recorded from dentate gyrus were assessed. For opsin-negative rats, LPDs between 127 and 636 mW mm⁻² and pulse durations between 20 and 5120 ms were tested while for stGtACR2 expressing rats, LPD of 127 mW mm⁻² and pulse durations between 20 and 640 ms were tested.

Main results: Increasing LPDs and pulse durations logarithmically increased the peak temperature and significantly decreased the population spike (PS) amplitude and latencies of EPs. For a pulse duration of 5120 ms, the tissue temperature increased by 0.6 °C-3.4 °C. All tested LPDs decreased the PS amplitude in opsin-negative rats, but 127 mW mm⁻² had comparatively minimal effects and a significant effect of increasing light pulse duration was seen from 320 ms and beyond. This corresponded with an average temperature increase of 0.2 °C-1.1 °C at the recorded site. Compared to opsin-negative rats, illumination in stGtACR2-expressing rats resulted in much greater inhibition of EPs.

Significance: Our study demonstrates that light-induced heating of the brain can be accurately measured *in vivo* using FBG sensors. Such light-induced heating alone can affect neuronal excitability. Useful neuromodulation by the activation of stGtACR2 is still possible while minimizing thermal effects.

Keywords: GtACR2; evoked potentials; heating; inhibition; optogenetics; temperature.



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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Vrancx C, Vadukul DM, Suelves N, Contino S, D'Auria L, Perrin F, van Pesch V, Hanseeuw B, Quinton L, Kienlen-Campard P. (2021).

Mechanism of Cellular Formation and In Vivo Seeding Effects of Hexameric β -Amyloid Assemblies.

Molecular Neurobiology. – (IF: 5.59; **Q1**) 2021 Dec;58(12):6647-6669.

doi: 10.1007/s12035-021-02567-8.

Abstract

The β -amyloid peptide ($A\beta$) is found as amyloid fibrils in senile plaques, a typical hallmark of Alzheimer's disease (AD). However, intermediate soluble oligomers of $A\beta$ are now recognized as initiators of the pathogenic cascade leading to AD. Studies using recombinant $A\beta$ have shown that hexameric $A\beta$ in particular acts as a critical nucleus for $A\beta$ self-assembly. We recently isolated hexameric $A\beta$ assemblies from a cellular model, and demonstrated their ability to enhance $A\beta$ aggregation *in vitro*. Here, we report the presence of similar hexameric-like $A\beta$ assemblies across several cellular models, including neuronal-like cell lines. In order to better understand how they are produced in a cellular context, we investigated the role of presenilin-1 (PS1) and presenilin-2 (PS2) in their formation. PS1 and PS2 are the catalytic subunits of the γ -secretase complex that generates $A\beta$. Using CRISPR-Cas9 to knockdown each of the two presenilins in neuronal-like cell lines, we observed a direct link between the PS2-dependent processing pathway and the release of hexameric-like $A\beta$ assemblies in extracellular vesicles. Further, we assessed the contribution of hexameric $A\beta$ to the development of amyloid pathology. We report the early presence of hexameric-like $A\beta$ assemblies in both transgenic mice brains exhibiting human $A\beta$ pathology and in the cerebrospinal fluid of AD patients, suggesting hexameric $A\beta$ as a potential early AD biomarker. Finally, cell-derived hexameric $A\beta$ was found to seed other human $A\beta$ forms, resulting in the aggravation of amyloid deposition *in vivo* and neuronal toxicity *in vitro*.

Keywords: Alzheimer's disease; $A\beta$ oligomers; FAD model; Hexameric $A\beta$; Presenilins; Seeding.

Funding: This work was supported by a grant of the Belgian F.N.R.S FRIA (Fonds National pour la Recherche Scientifique) and a grant of UCLouvain Fonds du Patrimoine to CV. Funding to PKC is acknowledged from SAO-FRA Alzheimer Research Foundation, Fondation Louvain and Queen Elisabeth Medical Foundation (FMRE to PKC and LQ). The work was supported by funds from FNRS Grant PDRT.0177.18 to PKC and LQ.

- Hanseeuw, B. J., Malotau, V., Dricot, L., Quenon, L., Sznajer, Y., Cerman, J., Woodard, J. L., Buckley, C., Farrar, G., Ivanoiu, A., & Lhommel, R. (2021).

Defining a Centiloid scale threshold predicting long-term progression to dementia in patients attending the memory clinic : An [18F] flutemetamol amyloid PET study.

European Journal of Nuclear Medicine and Molecular Imaging. – (IF: 9.24; **Q1**) 2021 Jan;48(1):302-310. doi: 10.1007/s00259-020-04942-4. Epub 2020 Jun 29.

Abstract

Purpose: To evaluate cerebral amyloid- β ($A\beta$) pathology in older adults with cognitive complaints, visual assessment of PET images is approved as the routine method for image interpretation. In research studies however, $A\beta$ -PET semi-quantitative measures are associated with greater risk of progression to dementia; but until recently, these measures lacked standardization. Therefore, the Centiloid scale, providing standardized $A\beta$ -PET semi-quantitation, was recently validated. We aimed to determine the predictive values of visual assessments and Centiloids

in non-demented patients, using long-term progression to dementia as our standard of truth.
Methods: One hundred sixty non-demented participants (age, 54-86) were enrolled in a monocentric [¹⁸F] flutemetamol Aβ-PET study. Flutemetamol images were interpreted visually following the manufacturers recommendations. SUVR values were converted to the Centiloid scale using the GAAIN guidelines. Ninety-eight persons were followed until dementia diagnosis or were clinically stable for a median of 6 years (min = 4.0; max = 8.0). Twenty-five patients with short follow-up (median = 2.0 years; min = 0.8; max = 3.9) and 37 patients with no follow-up were excluded. We computed ROC curves predicting subsequent dementia using baseline PET data and calculated negative (NPV) and positive (PPV) predictive values.

Results: In the 98 participants with long follow-up, Centiloid = 26 provided the highest overall predictive value = 87% (NPV = 85%, PPV = 88%). Visual assessment corresponded to Centiloid = 40, which predicted dementia with an overall predictive value = 86% (NPV = 81%, PPV = 92%). Inclusion of the 25 patients who only had a 2-year follow-up decreased the PPV = 67% (NPV = 88%), reflecting the many positive cases that did not progress to dementia after short follow-ups.

Conclusion: A Centiloid threshold = 26 optimally predicts progression to dementia 6 years after PET. Visual assessment provides similar predictive value, with higher specificity and lower sensitivity.

Trial registration: Eudra-CT number: 2011-001756-12.

Keywords: AD dementia; Amyloid PET; Centiloids; Diagnostic accuracy; Mild cognitive impairment.

Information: Are data acquired from 2012-2018, analyzed in 2019, which served as preliminary data during the QEMF project submitted in 2019. The project supported by the QEMF is really a continuation of this work. *No funding QEMF*

- Mormont, E., Bier, J.-C., Bruffaerts, R., Cras, P., De Deyn, P., Deryck, O., Engelborghs, S., Petrovic, M., Picard, G., Segers, K., Thiery, E., Versijpt, J., & Hanseeuw, B. (2020).

Practices and opinions about disclosure of the diagnosis of Alzheimer's disease to patients with MCI or dementia : A survey among Belgian medical experts in the field of dementia.

Acta Neurologica Belgica. – (IF: 2.4; **Q4**) 2020 Oct;120(5):1157-1163.doi: 10.1007/s13760-020-01448-6.

Abstract

Previous surveys revealed that only a minority of clinicians routinely disclosed the diagnosis of Alzheimer's disease (AD) to their patients. Many health professionals fear that the disclosure could be harmful to the patient. Recent advances in the development of biomarkers and new diagnostic criteria allow for an earlier diagnosis of AD at the mild cognitive impairment (MCI) stage. The Belgian Dementia Council, a group of Belgian experts in the field of dementia, performed a survey among its 44 members about their opinions and practices regarding disclosure of the diagnosis of AD, including MCI due to AD, and its consequences. Twenty-six respondents declared that they often or always disclose the diagnosis of AD to patients with dementia and to patients with MCI when AD CSF biomarkers are abnormal. The majority observed that the disclosure of AD is rarely or never harmful to the patients. Their patients and their caregivers rarely or never demonstrated animosity towards the clinicians following disclosure of the diagnosis of AD. These results should reassure clinicians about the safety of AD diagnosis disclosure in most cases whether the patient is at the MCI or the dementia stage.

Keywords: Alzheimer disease; Diagnosis; Disclosure; Mild cognitive impairment.

Information: Are data acquired from 2012-2018, analyzed in 2019, which served as preliminary data during the QEMF project submitted in 2019. The project supported by the QEMF is really a continuation of this work. *No funding QEMF.*

- Hanseeuw, B. J., Scott, M. R., Sikkes, S. A. M., Properzi, M., Gatchel, J. R., Salmon, E., Marshall, G. A., & Vannini, P. (2020).

Evolution of anosognosia in alzheimer's disease and its relationship to amyloid.

Annals of Neurology, 87(2), 267-280. – IF: [10.42](#); **Q1** 2020 Feb;87(2):267-280.

doi: 10.1002/ana.25649. Epub 2019 Dec 5.

Abstract

Objective: Unawareness, or anosognosia, of memory deficits is a challenging manifestation of Alzheimer's disease (AD) that adversely affects a patient's safety and decision-making. However, there is a lack of consensus regarding the presence, as well as the evolution, of altered awareness of memory function across the preclinical and prodromal stages of AD. Here, we aimed to characterize change in awareness of memory abilities and its relationship to beta-amyloid (A β) burden in a large cohort (N = 1,070) of individuals across the disease spectrum.

Methods: Memory awareness was longitudinally assessed (average number of visits = 4.3) and operationalized using the discrepancy between mean participant and partner report on the Everyday Cognition scale (memory domain). A β deposition was measured at baseline using [18F]florbetapir positron emission tomographic imaging.

Results: A β predicted longitudinal changes in memory awareness, such that awareness decreased faster in participants with increased A β burden. A β and clinical group interacted to predict change in memory awareness, demonstrating the strongest effect in dementia participants, but could also be found in the cognitively normal (CN) participants. In a subset of CN participants who progressed to mild cognitive impairment (MCI), heightened memory awareness was observed up to 1.6 years before MCI diagnosis, with memory awareness declining until the time of progression to MCI (-0.08 discrepant-points/yr). In a subset of MCI participants who progressed to dementia, awareness was low initially and continued to decline (-0.23 discrepant-points/yr), reaching anosognosia 3.2 years before dementia onset.

Interpretation: A β burden is associated with a progressive decrease in self-awareness of memory deficits, reaching anosognosia approximately 3 years before dementia diagnosis. ANN NEUROL 2020;87:267-280.

Information: Is a work from large number of Belgian hospitals that Prof. Bernard Hanseeuw had the opportunity as secretary of the Belgian dementia council. This work is the result of the coordination of a beneficiary of the QEMF. No funding QEMF.

- Hanseeuw, B. J., Betensky, R. A., Jacobs, H. I. L., Schultz, A. P., Sepulcre, J., Becker, J. A., Cosio, D. M. O., Farrell, M., Quiroz, Y. T., Mormino, E. C., Buckley, R. F., Papp, K. V., Amariglio, R. A., Dewachter, I., Ivanoiu, A., Huijbers, W., Hedden, T., Marshall, G. A., Chhatwal, J. P., ... Johnson, K. (2019).

Association of Amyloid and Tau With Cognition in Preclinical Alzheimer Disease : A Longitudinal Study.

JAMA Neurology, 76(8), 915. ◊ (IF: [18.3](#); **Q1**). 2019. PMID: 31157827

The last publication received the FMRE CBC Prize in 2021.

Abstract

Importance: Positron emission tomography (PET) imaging now allows in vivo visualization of both neuropathologic hallmarks of Alzheimer disease (AD): amyloid- β (A β) plaques and tau neurofibrillary tangles. Observing their progressive accumulation in the brains of clinically normal older adults is critically important to understand the pathophysiologic cascade leading to AD and to inform the choice of outcome measures in prevention trials.

Objective: To assess the associations among A β , tau, and cognition, measured during different observation periods for 7 years.

Design, setting, and participants: Prospective cohort study conducted between 2010 and 2017 at the Harvard Aging Brain Study, Boston, Massachusetts. The study enrolled 279 clinically

normal participants. An additional 90 individuals were approached but declined the study or did not meet the inclusion criteria. In this report, we analyzed data from 60 participants who had multiple A β and tau PET observations available on October 31, 2017.

Main outcomes and measures: A median of 3 Pittsburgh compound B-PET (A β , 2010-2017) and 2 flortaucipir-PET (tau, 2013-2017) images were collected. We used initial PET and slope data, assessing the rates of change in A β and tau, to measure cognitive changes. Cognition was evaluated annually using the Preclinical Alzheimer Cognitive Composite (2010-2017). Annual consensus meetings evaluated progression to mild cognitive impairment.

Results: Of the 60 participants, 35 were women (58%) and 25 were men (42%); median age at inclusion was 73 years (range, 65-85 years). Seventeen participants (28%) exhibited an initial high A β burden. An antecedent rise in A β was associated with subsequent changes in tau (1.07 flortaucipir standardized uptake value ratios [SUVR]/PiB-SUVR; 95% CI, 0.13-3.46; P = .02). Tau changes were associated with cognitive changes (-3.28 z scores/SUVR; 95% CI, -6.67 to -0.91; P = .001), covarying baseline A β and tau. Tau changes were greater in the participants who progressed to mild cognitive impairment (n = 6) than in those who did not (n = 11; 0.05 SUVR per year; 95% CI, 0.03-0.07; P = .001). A serial mediation model demonstrated that the association between initial A β and final cognition, measured 7 years later, was mediated by successive changes in A β and tau.

Conclusions and relevance: We identified sequential changes in normal older adults, from A β to tau to cognition, after which the participants with high A β with greater tau increase met clinical criteria for mild cognitive impairment. These findings highlight the importance of repeated tau-PET observations to track disease progression and the importance of repeated amyloid-PET observations to detect the earliest AD pathologic changes.

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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- van den Broeke EN, Urdí M, Mouraux A, Biurrún Manresa JA, Torta DME.

high-frequency electrical stimulation of cutaneous nociceptors differentially affects pain perception elicited by homotopic and heterotopic electrical stimuli.

J Neurophysiol. 2021; 126(4): 1038-1044. doi: 10.1152/jn.00289.202 – (IF: 2.71; **Q3**)2021 Oct 1;126(4):1038-1044. doi: 10.1152/jn.00289.2021.

Abstract

Animal studies have shown that high-frequency electrical stimulation (HFS) of peripheral C-fiber nociceptors induces both homosynaptic and heterosynaptic long-term potentiation (LTP) within spinal nociceptive pathways. In humans, when HFS is applied onto the skin to activate nociceptors, single electrical stimuli are perceived more intense at the HFS site compared with a control site, a finding that was interpreted as a perceptual correlate of homosynaptic LTP. The present study aimed to investigate if after HFS the pain elicited by electrical stimuli delivered at the skin next to the HFS site is perceived as more intense compared with the pain at a control site (contralateral arm). To test this, HFS was applied to one of the two ventral forearms of 24 healthy participants. Before and after HFS, single electrical stimuli were delivered through the HFS electrode, through an identical electrode next to the HFS electrode and an identical electrode at the contralateral arm. After HFS, the pain elicited by the single electrical stimuli was reduced at all three sites, with the largest reduction at the HFS site. Nevertheless, electrical stimuli delivered to the skin next to the HFS site were perceived as more intense than control stimuli. This result indicates that higher pain ratings to electrical stimuli after HFS at the HFS site cannot solely be interpreted as a perceptual correlate of homosynaptic changes. Furthermore, we show for the first time, in humans, that HFS can reduce pain elicited by single electrical stimuli delivered through the same electrode.

New & noteworthy: High-frequency electrical stimulation (HFS) of cutaneous nociceptors can reduce pain perception to single electrical stimuli delivered through the same electrode. Moreover, single electrical stimuli delivered to the skin next to the site at which HFS was applied are perceived as more intense compared with that at the contralateral control site, indicating the presence of heterosynaptic effects for electrical stimuli.

Keywords: high-frequency stimulation; hyperalgesia; long-term potentiation; nociception

Funding: ENvdB is supported by the Fonds de Recherche Clinique (FRC) provided by the Université catholique de Louvain and the Young Belgian Researcher grant for Neurosciences provided by the Queen Elisabeth Medical Foundation, Belgium. DT is supported by the KU Leuven starting grant. MU is supported by an Erasmus Plus Scholarship from the University of Barcelona.

- van den Broeke EN, Vanmaele T, Mouraux A, Stouffs A, Biurrún-Manresa J. and Torta DM.

Perceptual correlates of homosynaptic long-term potentiation in human nociceptive pathways: a replication study

R. Soc. Open. Sci. 2021; 8200830. <http://doi.org/10.1098/rsos.200830>.

(IF: 2.96; **Q2**) 2021 Jan 20;8(1):200830. doi: 10.1098/rsos.200830.

Abstract

Animal studies have shown that high-frequency stimulation (HFS) of peripheral C-fibres induces long-term potentiation (LTP) within spinal nociceptive pathways. The aim of this replication study was to assess if a perceptual correlate of LTP can be observed in humans. In 20 healthy volunteers, we applied HFS to the left or right volar forearm. Before and after applying HFS, we

delivered single electrical test stimuli through the HFS electrode while a second electrode at the contra-lateral arm served as a control condition. Moreover, to test the efficacy of the HFS protocol, we quantified changes in mechanical pinprick sensitivity before and after HFS of the skin surrounding both electrodes. The perceived intensity was collected for both electrical and mechanical stimuli. After HFS, the perceived pain intensity elicited by the mechanical pinprick stimuli applied on the skin surrounding the HFS-treated site was significantly higher compared to control site (heterotopic effect). Furthermore, we found a higher perceived pain intensity for single electrical stimuli delivered to the HFS-treated site compared to the control site (homotopic effect). Whether the homotopic effect reflects a perceptual correlate of homosynaptic LTP remains to be elucidated.

Keywords: high-frequency stimulation; homotopic hyperalgesia; long-term potentiation.

Funding: E.N.v.d.B. is supported by the Fonds de Recherche Clinique (Clinique Universitaire St Luc) and by the [Queen Elisabeth Medical Foundation](#) for Neuroscience (Young Researchers grant); D.M.T. is supported by a BOFZAP Starting Grant (KU Leuven), by the 'Asthenes' Methusalem grant by the Flemish Government, Belgium and by a Research Grant (Junior Project) by the FWO.



Geneeskundige Stichting Koningin Elisabeth
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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Bertels H., Ortiz-Vicente G., El Kanbi K., Takeoka A.
Flexible neurotransmitter phenotype of spinal excitatory interneurons defines locomotor ability after spinal cord injury. In revision at
Nature Neuroscience

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Publications in 2021 with acknowledging G.S.K.E. – Q.E.M.F – F.M.R.E.

- Uytterhoeven, V. et al. (2021)

Increased HSC70-4/HSPA8 regulated autophagy reduces Tau-mediated synaptic dysfunction

Background: Tau tangles are present in the brains of Alzheimer diseased (AD) patients and strategies that remove Tau oligomers and Tau tangles are tested in clinical trials. These anti-Tau drugs may keep misfolded Tau from spreading and damaging additional neurons, but they may be inefficient at targeting soluble Tau early in the disease before tangles are formed and thus be relatively late to spare cognitive defects. Our research is therefore refreshing as it may tackle early Tau induced dysfunction. We have shown in *Drosophila* that the overexpression of the chaperone Hsc70-4 promotes endosomal microautophagy at presynapses and regulates the turnover of synaptic proteins harboring pentapeptide motifs, biochemically related to the KFERQ sequence (Uytterhoeven, 2015). Intriguingly, Tau harbors two such motifs.

Method: We tested if increasing endosomal microautophagy by overexpressing Hsc70-4 has an effect on Tau-mediated synaptic defects .

Result: Indeed, increasing microautophagy reduces presynaptic Tau levels and presynaptic vesicle sequestrations at presynapses, two phenotypes previously shown by our lab to appear after expressing pathogenic Tau in the fly nervous system (Zhou, 2017 and McInnes, 2018). In addition, via lentiviral-mediated overexpression of HSPA8, the human homologue of Hsc70-4, we are able to increase HSPA8 regulated autophagy in human neurons.

Conclusion: Together, our work provides important molecular insight into the contribution of Hsc70-4/HSPA8 regulated autophagy on AD disease onset and progression and may contribute to the development of methods and treatments to alleviate the defects in dementia.

Funding: These are publications of abstracts of my GSKE supported conference work. No financial support is mentioned on such abstracts. This is determined by the organization of the congress.

I have mentioned these publications to show that there is progress in my work and that I present it internationally.

The pathogenic mutation in Tau defines the route of Tau accumulation at presynapses.

ALZ online. (conference abstract publication) (<https://alz.confex.com/alz/2021/meetingapp.cgi/Paper/53728>)

Abstract

Background: Tau's primary localization is in the axons of neurons. At axons, Tau binds microtubuli and stabilizes them. Many of the pathogenic mutations interfere with binding of Tau to the microtubuli resulting in the relocation of Tau to different neuronal compartments, including the presynapse. Our lab has shown that Tau interact with synaptic vesicles via the synaptic vesicle protein, Synaptogyrin-3 and excessive Tau levels at presynapses results in the sequestration of synaptic vesicles compromising neurotransmission in Tau mutant fly and primary mouse neurons (Zhou, Nat. Commun. 2017; McInnes, Neuron, 2018). In addition, lowering Synaptogyrin-3 expression in TauP301L mutant mice reduces Tau-synaptic vesicle interactions and rescues Tau-mediated memory defects and synapse loss in these mice (Largo-Barrientos, Neuron, 2021).

Method: Although, the interaction of Tau with synaptic vesicles is reduced with lowering Synaptogyrin-3 expression, Tau continues to accumulate at presynapses. To reduce Tau levels and keep the presynapse healthy over time, we increased endosomal microautophagy by the expression of Hsc70-4 (Uytterhoeven, Neuron, 2015) in Tau mutant fly neurons . **Result:** Despite,

the overall reduced activity of degradation pathways in pathogenic conditions, we were able to reduce presynaptic TauP301L levels and restore reduced synaptic vesicle mobility and neurotransmission by increasing endosomal microautophagy. However, increasing endosomal microautophagy is not effective in lowering pathogenic TauV337M. Using a fluorescent timer attached to Tau, we show that older Tau is present in TauV337M compared to TauP301L mutant presynapses indicating that the turnover of TauV337M is hindered. On the contrary, with a fluorescence recovery after photobleaching assay we show that TauP301L is more mobile in axons compared to TauV337M indicating that TauP301L detaches more easily from the microtubuli.

Conclusion: Together, these data show that both the detachment of Tau from microtubuli and defective turnover of Tau at presynapses contribute to the accumulation of Tau at presynapses and depending on the pathogenic mutation one of the two pathways plays a more prominent role in the accumulation of Tau at presynapses. Hence, these observations have further implications in the development of new therapies acting on Tau. Alzheimer's Dement. 2021;17(Suppl. 3):e053728. © 2021 the A

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