“It does not do harm to the mystery to know a little about it.”

- Richard Feynman

“The brain is a world consisting of a number of unexplored continents and great stretches of unknown territory.”

- Santiago Ramón y Cajal

“Research is to see what everybody has seen and think what nobody has thought.”

- Albert Szent-Györgyi

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Foreword

Dear reader,

Before you lies the second issue of the 14th volume of the Journal of Neuroscience and Cognition. The theme of the journal is 'Mindblown: Hidden treasures in neuroscience'.

With this issue, the Editorial Board has shown that working online from home offices is not necessarily a bad thing. It is clear that the board is flexible and can easily adjust to new circumstances. Their inspiring group dynamic and creativity have not suffered from the inability to physically meet over the past months. In fact, I believe it might have even sparked more creativity, as the current issue is filled with upgraded and new sections. At the time of writing, we know that a face-to-face journal release party cannot be held due to the pandemic. Nevertheless, I am convinced that the board will come up with a way to present this issue to you in a festive and original manner.

As you start reading the sections, do keep an eye out on the short interviews with researchers working on mind-blowing topics, you might find some inspiration for your own research there. If you happen to be in a situation where your social life is still suffering from the effects of COVID-19, or if you just like to curl up in a chair with a book, be sure to check out the book section. The board has taken great care to construct a top 10 of fascinating books about neuroscience, which are bound to get you through fall and perhaps even winter. The COVID-19 related section is also a new addition in this issue, kicking off with a short piece on COVID-19 related research by yours truly. Judging from the latest updates, this section might be here to stay for a few volumes. With that in mind, do not forget to read the Ph.D. experience piece too, which this time focuses on an online Ph.D. defence. And if you want to read something truly mind-blowing, make sure to check out the newly incorporated section on Psychedelics!
Once again the Editorial Board has done a fantastic job in selecting interesting and inspiring content. I also would like to thank all the contributors for freeing up time in their schedule to write a piece for the journal or be interviewed. I hope that you, as readers of the journal, will feel just as inspired and amazed by it as the whole board did when creating this edition. After all, the one thing that unites all fields of science is that we are given the opportunity to have our minds blown!

Yours sincerely,
Anouk Keizer

Senior Supervisor Journal of Neuroscience and Cognition 2019 - 2020

Dear reader,

We are all interested in neuroscience - because of its complexity and multidisciplinarity, and because of the depth and breadth of knowledge it encapsulates. For this issue, we used a survey to ask you what you find most fascinating in this field – and your responses were as different and colourful as the subject itself. Replies spanned topics like social and affective neuroscience, synaptic plasticity, consciousness, and self-awareness[1]. Together with the other students from the Neuroscience and Cognition Journal, we want to share and celebrate some of this abundance of knowledge with you. This is what inspired us to create our second edition: 'Mindblown: hidden treasures in neuroscience'! This theme is important to us because we all want to follow different scientific paths, yet we are all united by our passion for neuroscience and the mindblowing revelations it offers about the human brain and mind. We think that reminding ourselves of how interesting neuroscience is keeps us motivated to work in this field, to follow correct research practices and make scientific findings available for open sharing and discussion. We believe that through this collaborative approach we can advance neuroscience as a discipline, and our theme symbolises this belief.

With this issue, we reveal some of the multiple facets of neuroscience with a variety of sections that will make your brains burn with desire to learn more! We start with interesting contributions by fellow students: Lucia, Joelle, Marta, Dirk, and Raquel. We then take you on a journey through the COVID-19 pandemic and the insights it teaches us about how humans behave with an article investigating human touch in times of social distancing by our Senior supervisor Anouk Keizer. But the mindblowing pieces don't end there! We further show you some impressive research conducted at Utrecht University and UMC Utrecht through a series of short articles by local researchers – from circular RNAs, through 7T MRI, to machine learning in patient diagnosis and care. In addition, we enriched the Methodology section by including the opinion of an expert researcher alongside those of two Master's students working on brain organoids. We then depict one aspect of the global pandemic reality and how it has affected academic life with a story on how defending a Ph.D. online feels like. We also surprise you with Career perspectives from a Research data management coordinator and a Senior medical writer. We truly believe that, as future members of the workplace, we would benefit from more information about the professions that are available to us outside academia. We hope that these career perspectives with a different twist compared to previous editions will be useful for you. They may even make you reconsider your career plans!

Further to this, we captured more of the thrill of neuroscience by incorporating a new section we are very proud of - it includes two commentaries on how psychedelics can be used for therapeutic purposes! A reminder that a good scientist is a scientist with an open mind! We finish off with a seminar impression, a book review and a list of captivating neuroscience books recommended by the Journal board. Of course, no issue is complete without the Mind the Brain section where the Chair of the board Emma shares with us about the work of the committee after the Symposium in May. We also get to read more about the winners in the categories of best presentation, abstract, and poster.

We truly hope that you will like this edition and that it will remind you why you like neuroscience so much – because it invigorates our brains, because it is exciting and full of mindblowing explorations! On behalf of the whole board, we thank everyone who contributed to our work this year – we wouldn't have delivered such great content without you! I personally also want to thank all the students who worked on the board for being brave to think outside the box. Despite the challenges of not meeting in person, we found great joy in our work and I think our two editions are a testament of our courage, dedication, and collaboration!

Wishing you the best of luck in your academic endeavours - stay motivated and stay fascinated!

Yours sincerely,
Ivana Kancheva

Editor in Chief Journal of Neuroscience and Cognition 2019 - 2020

[1] You can see the word-web containing your answers to our survey about what you find most fascinating in neuroscience at the back of the Journal.

'Advances in the development of therapeutic strategies for Juvenile CLN3 disease'

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Juvenile CLN3 disease is a rare neurodegenerative lysosomal storage disorder, caused by mutations in the CLN3 gene. The disease often presents in early childhood, with progressive vision loss, followed by seizures, motor and cognitive deficits, and inevitably ends with premature death. The pathology of CLN3 disease is characterised by accumulation of autofluorescent lipopigments in lysosomes, neuronal cell death and gliosis. Currently, there are no treatment strategies available to cure or slow down disease progression. Available therapies focus merely on mitigation and control of symptoms. Since the exact function of the affected CLN3 protein has not yet been fully uncovered, development of specific treatment strategies has proven extremely difficult. Nevertheless, substantial progress has been made in terms of treatment development and uncovering CLN3 functions, and the latter will be presented in this review.

Keywords: *Batten disease; Juvenile neuronal ceroid lipofuscinosis; CLN3; Translational research; Therapy*

Juvenile-onset neuronal ceroid lipofuscinosis (JNCL) is the most common form of a group of rare lysosomal storage diseases (LSDs) affecting the central nervous system, known collectively as the neuronal ceroid lipofuscinoses (NCLs), or Batten disease. The childhood forms of NCLs are inherited in an autosomal-recessive manner, while one rare adult variant can be inherited both in an autosomal-recessive and autosomal-dominant fashion (Wisniewski et al., 2001).

Before the identification of genes underlying the different types of NCLs, diagnosis was based upon clinical presentation, and distinguished from other lysosomal storage diseases by histopathological analysis. A common pathological characteristic of the NCLs is the accumulation of auto-fluorescent lipopigment, resembling ceroid or lipofuscin, which forms inclusions in the lysosomes of various cell types (Williams et al., 2006; Wisniewski et al., 2001). Based on electron microscopical evaluation of ultrastructural profiles of the storage material, the age of onset, and pattern of symptoms, NCLs were first classified into three main childhood forms: infantile, late infantile, and juvenile NCL (Williams et al., 2006). From 1995, the genetic basis for the NCLs was uncovered, and a new classification was proposed in 2012. To date, there are 13 distinct genes identified (CLN1-14, except CLN9), each corresponding to a different form of NCL (Mole & Cotman, 2015). This review will focus on the most common form, juvenile NCL, which is caused by mutations in the CLN3 gene and is now called juvenile CLN3 disease.

Diagnosis of juvenile CLN3 disease used to be supported by the presence of vacuolated lymphocytes upon ultrastructural examination of peripheral blood samples (Anderson et al., 2005), together with clinical symptoms. Since the recent advances in next generation sequencing, diagnosis is now confirmed by a CLN3 mutation analysis (Williams et al., 2006).

Brain atrophy caused by prolonged loss of nerve cells and their subsequent processes includes thinning of gyri, gaping sulci and thickening of the leptomeninges (Radke et al., 2015). Atrophy usually becomes visible on MRI scans around 14 years of age, and may appear to be more severe in occipital lobes compared to frontal lobes (Radke et al., 2015; Williams et al., 2006). Starting from the age of 11, children may already start showing an abnormally low signal intensity in the thalamus, and a higher signal intensity in periventricular white matter. However, since the disease often presents at a younger age, and MRI scans look normal in children under 10 years old, MRI is not a useful tool for diagnosis (Williams et al., 2006).

Despite the availability of some treatment strategies focused on mitigation and control of symptoms, almost all NCLs ultimately lead to premature death. Currently, there is no curative treatment for JNCL, making the need for discovery of novel therapeutic targets extremely high. This review will summarise what is known about JNCL pathology and the role of CLN3 herein, as well as give an overview of the advances that have been made regarding the identification of therapeutic targets and the research models that are used for this purpose.

1. THE PATHOLOGICAL AND CLINICAL CHARACTERISTICS OF JUVENILE NCL DISEASE

The pathology of the NCLs is defined by two major features, namely the abnormal accumulation of autofluorescent lipopigments and neuronal degeneration (Williams et al., 2006). While the lipopigments are of substantial concern in the diagnosis of distinct NCLs, neuronal degeneration is most likely responsible for the clinical symptoms, progression of the disease and

premature death (Williams et al., 2006).
The onset of juvenile CLN3 disease usually starts between the fourth and tenth year of life with quickly progressing visual impairment and intractable seizures. At a later age, patients experience cognitive decline and motor deterioration, ultimately followed by premature death in the second or third decade of life (reviewed by Wisniewski et al., 2001; Williams et al., 2006). The clinical features of JNCL will be further elucidated and discussed in the sections below.
It should be noted, however, that there is great variability in the clinical course, both within and between families. The course of the disease can roughly be divided into two categories: classical/typical and delayed-classical or atypical. Like classical JNCL, the delayed-classical form also presents with quickly progressing visual failure at a young age, but other symptoms may take longer to develop. In some cases, patients can even stay free of other symptoms for many years (Kuper et al., 2017). Recently, with the advances of next-generation sequencing, individuals have been identified that experience visual loss but no other classical JNCL symptoms, even until middle-age. These cases indicate that there is a wide spectrum of symptoms caused by different mutations in CLN3, which do not necessarily lead to juvenile CLN3 disease (Chen et al., 2019; Ku et al., 2017; Wang et al., 2014).

1.1 Lysosomal accumulation of storage material

The lipopigments in JNCL patients display characteristic ultrastructural profiles, known as fingerprint profiles (FP; Figure 1). Although the complete composition of these inclusions is unknown due to its heterogeneity (reviewed by Seehafer and Pearce, 2006), several components have been identified by immunohistochemistry studies, including primarily subunit c of the mitochondrial ATP synthase (SCMAS) (Hall et al., 1991) and small amounts of sphingolipid activator proteins (SAPs) (Tyynelä et al., 1995). Other identified contents of the storage material include alcohol-insoluble lipids, dolichols, and metals such as iron (Kida et al., 2000).
Furthermore, amyloid-bèta peptide (Aβ), which has been associated with disturbance of mitochondrial function and plaque formation in Alzheimer's disease (Du et al., 2010), may also be found in the inclusions (Kitaguchi et al., 1990; Wisniewski, Kida, et al., 1990; Wisniewski, Maslinska, et al., 1990).
Ceroid deposition is considered to be at the base of severe cell dysfunction and neuronal death, but is also proposed to start a neurologic auto-immune response against many proteins found in the central nervous system (CNS), resulting in the activation of astrocytes and microglia (Pontikis et al., 2005). This glial activation typically occurs before neuronal loss, and the study of Parviainen et al. (2017) has shown that CLN3 deficient glial cells can harm and negatively impact survival of healthy, as well as mutant neurons in a CLN3 knock-in mouse model.
Even though it has not yet been confirmed that ceroid deposition is at the basis of an autoimmune response, several studies have shown that JNCL patients and CLN3 knock-out mice present with elevated levels of autoimmune antibodies against several CNS proteins, including GAD65 and α-fetoprotein (Hersrud et al., 2016; Seehafer et al., 2011). The presence of autoantibodies against these CNS proteins indicates that there may be an autoimmune component to this disease.

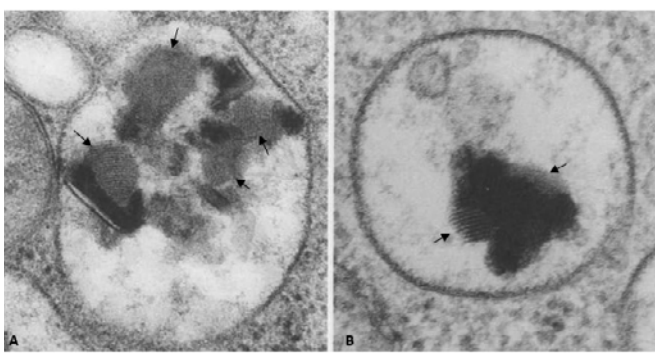

Figure 1. *Electron microscopic image of Fingerprint profiles found within membrane-bound lysosomal vacuoles of homozygous Juvenile CLN3 disease patients, indicated by arrows. Magnification 73,500x (A) and 225,000x (B). Adapted from Wisniewski et al. (2001).*

1.2 Visual impairment

Rapidly progressing visual loss is often the only symptom during the first two-to-four years after disease onset, and in most cases results in blindness within this period. Ophthalmological studies have demonstrated that despite the visual loss, the eyes generally look normal. Especially during the early stages of the disease, retinal changes may be very subtle, and can easily be overlooked. Optic nerve atrophy and retinal degradation, with a uniform decrease in retinal thickness are the most prominent features of ocular changes in JNCL patients (reviewed by Ouseph, Kleinman and Wang, 2016).

1.3 Impact on the central nervous system

As the disease gradually develops, patients generally start to experience seizures a few years after the onset of visual loss, usually between the ages of 5 and 18. The most common type are generalised tonic-clonic seizures, but some children may also experience myoclonic or complex-partial seizures. As they grow older, seizure frequency and severity tends to increase (Aberg et al., 2000; Williams et al., 2006).
Already from a young age, CLN3 patients experience slowly progressing cognitive decline, affecting short-term memory and comprehension, while ultimately resulting in dementia around their mid-teens (Bennett & Rakheja, 2013; Williams et al., 2006). Motor dysfunction and

muscle weakness result in a characteristic compulsive speech pattern, with severe dysarthria. Additionally, about half the patients experience extrapyramidal signs leading to impaired ability to move around, from early adolescence. In the rest of the patients these symptoms occur at a later age. Symptoms are Parkinson-like and can include impaired balance, rigidity, a stooped posture, hypokinesia, shuffling gait, and a mild, inconsistent tremor (reviewed by Williams et al., 2006).
Starting in their early twenties, patients might get a number of psychiatric symptoms, including social, thought and attention problems, aggressive behaviour or somatic complaints. Moreover, some patients suffer from depression and sleeping problems (Bennett & Rakheja, 2013).

1.4 Cardiac involvement

Recently, it has become more and more obvious that storage material accumulation not only affects the central nervous system, but also other organ systems, such as the heart. About half of the JNCL patients experience cardiac problems, including ventricular hypertrophy and deficits in the conduction system (Ofman et al., 2001; Østergaard et al., 2011). Possibly, cardiac defects are caused by deposition of storage material in hypertrophied myocardiocytes of the atrioventricular conduction system (Ofman et al., 2001).
These severe deficits in the cardiac system show that sole treatment of the brain will likely be insufficient, as distinct organs are also implicated by the disease (Geraets et al., 2019).

2. THE CLN3 PROTEIN AND ITS ROLE IN JNCL

Juvenile NCL is caused by mutations in the ceroid-lipofuscinosis, neuronal 3 (CLN3) gene, which is located on chromosome 16p11.2-12.1 (Lerner et al., 1995) and is highly conserved across many eukaryotic species, including mice and yeast. There are different isoforms reported, each consisting of 13 to 16 exons, though there have also been shorter isoforms reported (reviewed by Haslett et al., 2019). The main CLN3 isoform encodes a 438 amino acid lysosomal/late-endosomal transmembrane protein, with an early predicted structure of six transmembrane domains and one predicted amphipathic helix in the third luminal loop (Figure 2). In this predicted structure, both the N- and C-terminus are located in the cytosol (Nugent et al., 2008; Ratajczak et al., 2014).
However, some early experimental studies have supported a structure for CLN3 whereby the N-terminal region is located inside the lumen of the organelle, while the C-terminal region is located in the cytosol (Mao et al., 2003). Due to lack of proper biochemical tools to isolate CLN3, identification of its exact structure has proven difficult. The high-resolution methods necessary for a detailed and accurate characterisation of the CLN3 structure, such as crystallography, X-ray, or 3D cryo-electron microscopy, require the isolation of high amounts of properly folded CLN3 protein. Nevertheless, some have been able to experimentally investigate membrane topology of CLN3 (Kyttälä et al., 2004; Nugent et al., 2008; Ratajczak et al., 2014). For example, the group of Ratajczak and colleagues (2014) has done so by measuring Förster Resonance Energy Transfer (FRET) between a fluorophore donor-acceptor pair inserted within the same CLN3 molecule.
In brief, it was assumed that localisation of both the acceptor and donor on the same side of the membrane would result in a measurable FRET signal, while localisation on opposing sides of the membrane would inhibit FRET. The data obtained from FRET was used in a TOPCONS membrane prediction algorithm, together with other available experimental data, to create a novel computational model of CLN3 topology. This new model is mostly in compliance with previous predictions regarding the CLN3 structure, and only differs in the precise location of the transmembrane domains and lengths of the cytosolic and intraluminal loops.
Moreover, results from this same study suggest that the C-terminus of truncated CLN3 protein is located in the cytosol (Figure 2), whereas other studies based on bioinformatic predictions have often suggested it to be located in the lumen. These differences in location of the C-terminus may have interesting consequences regarding the understanding of the function of this truncated protein, and thereby its role in juvenile CLN3 disease (Ratajczak et al., 2014).

2.1 Localisation and function of CLN3

The CLN3 protein, at least when overexpressed, has primarily been found in endosomes and lysosomes (Kyttälä et al., 2004; Storch et al., 2007), and expression studies have shown that the CLN3 protein is ubiquitously expressed in various human and mouse tissues. Immuno-electron microscopy studies have confirmed CLN3 localisation in late-endosomal, and lysosomal structures (Järvelä et al., 1999). Additionally, CLN3 was also found to be localised in the Golgi apparatus and trans-Golgi network, and some particles were even found to be located at the plasma membrane and mitochondria. These findings suggest intracellular trafficking of CLN3 through the endoplasmic reticulum and Golgi compartments, possibly via recycling through the plasma membrane while it is transported to the lysosome (Järvelä et al., 1999). Nevertheless, a detailed intracellular trafficking route and expression pattern have not yet been discovered. This is mainly due to the lack of specific antibodies against CLN3 and its low, endogenous expression levels (Cárcel-Trullols et al., 2015).
Moreover, despite many years of research, the exact

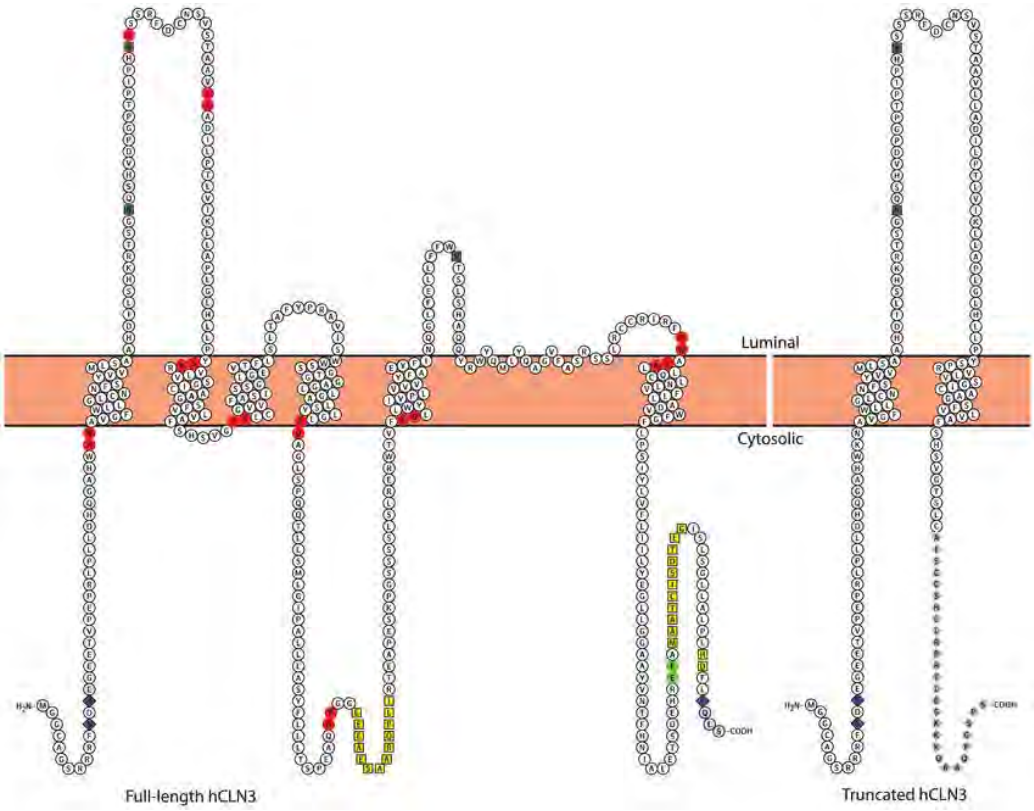

Figure 2. *A schematic overview of the proposed structure for the CLN3 protein, consisting of 6 transmembrane domains and one proposed amphipathic helix in the third luminal loop. On the right side, the predicted structure of the most common form of truncated CLN3 is shown. Grey diamond residues indicate novel residues. Adapted from Ratajczak et al. (2014).*

function of CLN3 remains unknown. Considering its supposed cellular localisation, CLN3 is proposed to play a role in processes like endocytosis and endocytic trafficking (Cárcel-Trullols et al., 2015; Timm et al., 2018), but the exact function of CLN3 in endosomal and lysosomal compartments remains elusive as its interactions in these compartments are poorly understood (Uusi-Rauva et al., 2012).

Other studies have also associated the CLN3 with roles in other cellular processes, such as autophagy (Cao et al., 2006), lysosomal transport, apoptosis and cell cycle control. Additionally, CLN3 has often been associated with intracellular membrane trafficking, and several studies have shown that failure of these processes may contribute to the onset of JNCL, including implications in endocytosis (Luiro et al., 2004) and in fast axonal transport of optic nerves (Weimer et al., 2006) in a mouse model of JNCL.

Early in silico predictions of CLN3 homologues have suggested a distinct but significant sequence similarity between CLN3 and members of the SLC29 family of equilibrative nucleoside transporters. However, more recent algorithms have put forward that most of the CLN3 protein has a domain structure consistent with members of the major facilitator superfamily (MFS) of membrane transporters (reviewed by Cotman & Staropoli, 2012; Haslett et al., 2019). The structural similarities between the CLN3 protein and the MFS superfamily may give rise to new insights in what the CLN3 function might entail.

When comparing results from several studies, it seems that CLN3 is involved in many cellular processes (Cárcel-Trullols et al., 2015), suggesting it is working at the intersection of several molecular pathways, and that many defective pathways can contribute to the disease mechanism. Nonetheless, it remains unclear what the original function of CLN3 is, and which processes are affected as a secondary effect resulting from its primary function.

2.2 *CLN3 mutations*

To date, there are more than 70 JNCL-associated mutations for CLN3 described, including deletions, insertions, missense, nonsense, and splice-site mutations. All mutations are listed in the NCL mutation database (Mole & Gardner, 1998). The most common CLN3 mutation is an intragenic 966-base pair (1 kb) deletion spanning exons 7 and 8, which is found in about 80% of disease chromosomes. Most patients are

homozygous for this mutation, but some are compound heterozygous (Lerner et al., 1995).
In many cases, this 1kb deletion results in a frameshift of the coding sequence after the first 153 amino acids, generating 28 novel amino acids and an early stop codon. However, there have also been some cases reported where after exon skipping, resulting in a loss of amino acids 154 to 263, the mRNA comes back into frame at the novel splice site (Kitzmüller et al., 2008). It is unclear whether these mutated versions of CLN3 are expressed, but this is certainly likely, since knockdown of this truncated protein shows an effect on lysosome size.
The study of Chan and colleagues (2008) shows that expression of a partial CLN3 protein is highly unlikely du to a lack of appropriate translation initiation, in a mouse model mimicking a null-mutation of CLN3. They argue that the truncated CLN3 mRNA transcript is likely targeted for degradation by the nonsense-mediated decay pathway, since lower amounts of CLN3 transcripts were found in JNCL patients. On the other hand, the study of Kitzmüller et al. (2008) has demonstrated that truncated and mutant versions of CLN3 may still have some original, or perhaps even novel functions.
Since the deleted region in the common 1kb deletion is the most conserved domain of CLN3 in both sequence and length, it is suggested that this region may be of pivotal importance for its structure or functionality (Kitzmüller et al., 2008).

3. RESEARCH MODELS FOR JNCL

For the investigation of CLN3 function and localisation, as well as for the development of therapeutic strategies, different model organisms have been used. Sequence comparison has shown that CLN3 is highly conserved across eukaryotic species, including the fission yeast Schizosaccharomyces pombe (S. pombe), mice, and the nematode Caenorhabditis elegans (C. elegans). Although a wide range of natural mutant animal species display NCL characteristics, none of them are caused by mutations in the CLN3 gene, which is necessary for an ideal JNCL model. Therefore, to study the function of natural and mutated CLN3 proteins, different knock-out animal models have been developed. In this section, the different research models used within JNCL research will be described.

3.1 Neuronal cell model

For evaluating the pathophysiology of rare diseases, such as JNCL, it is optimal to use disease-specific mammalian cell types. The generation of disease-specific cell lines can be accomplished by reprogramming patient skin fibroblasts to induced pluripotent stem cells (iPSCs), which can then be differentiated into a neuronally committed lineage, such as neuronal progenitor cells (Burnight et al., 2018; Chandrachud et al., 2015).
However, it can be challenging to select appropriate control lines for phenotypic comparison, due to normal genetic variation that is present within the human population. With age- and gender-matched controls, phenotypic behaviour cannot always specifically be assigned to the mutated loci. One solution for this problem can be the generation of isogenic controls. With this approach, patient iPSCs are corrected before differentiation, so that control lines only differ at the disease-causing loci. Another approach to do this could be to start with control stem cell lines and introduce different mutations to these cells. This way, the mutated loci would likewise be the only differences. The study of Burnight and colleagues (2018) demonstrated that genome correction using CRISPR-Cas9 could successfully restore CLN3 transcription in iPSCs derived from two different JNCL patients with the common 1kb deletion, one patient harbouring a homozygous deletion, while the other patient was compound heterozygous.

3.2 The Schizosaccharomyces pombe btn1 model

The fission yeast Schizosaccharomyces pombe (S. pombe) has a single orthologue for CLN3, namely btn1, which encodes a 396 amino acid transmembrane protein. Protein alignments have shown that the Btn1 protein is 30% identical and 48% similar to human CLN3 (Figure 3; Gachet et al., 2005). Btn1 was shown to localise to vacuoles and pre-vacuolar compartments, which are the yeast equivalent to human lysosomes and late endosomes respectively.
Btn1 was shown to be involved in vacuole homeostasis, since deletion of btn1 (btn1Δ) resulted in enlarged and less acidic vacuoles (Codlin, Haines, & Mole, 2008; Codlin, Haines, Burden, et al., 2008; Haines et al., 2009). A similar phenotype has also been observed in JNCL patient lysosomes (Holopainen et al., 2001). Both these effects could be complemented by expression of either btn1 or human CLN3 in btn1Δ yeast strains, indicating that btn1 protein is a functional homologue of CLN3 (Gachet et al., 2005).
Moreover, btn1Δ yeast have a defective response to stressful conditions, such as high temperatures. Under stressful conditions, mutant yeast show impaired growth, characterised by curving of the cells, and elongated and swollen cells at division. Additionally, btn1Δ cells show multiple division septa under normal growth conditions, indicating problems in the final stages of cytokinesis (Codlin, Haines, Burden, et al., 2008; Cotman & Staropoli, 2012; Haines et al., 2009).
This knock-out yeast model displays several CLN3 disease features, and may therefore be very beneficial to determine the protein function of Btn1 and CLN3 on a molecular and cellular level (Bond et al., 2015). However, it cannot provide any insights into the CLN3 function in a multicellular environment, such as the central nervous system, where most JNCL symptoms originate.

3.3 CLN3 mouse models

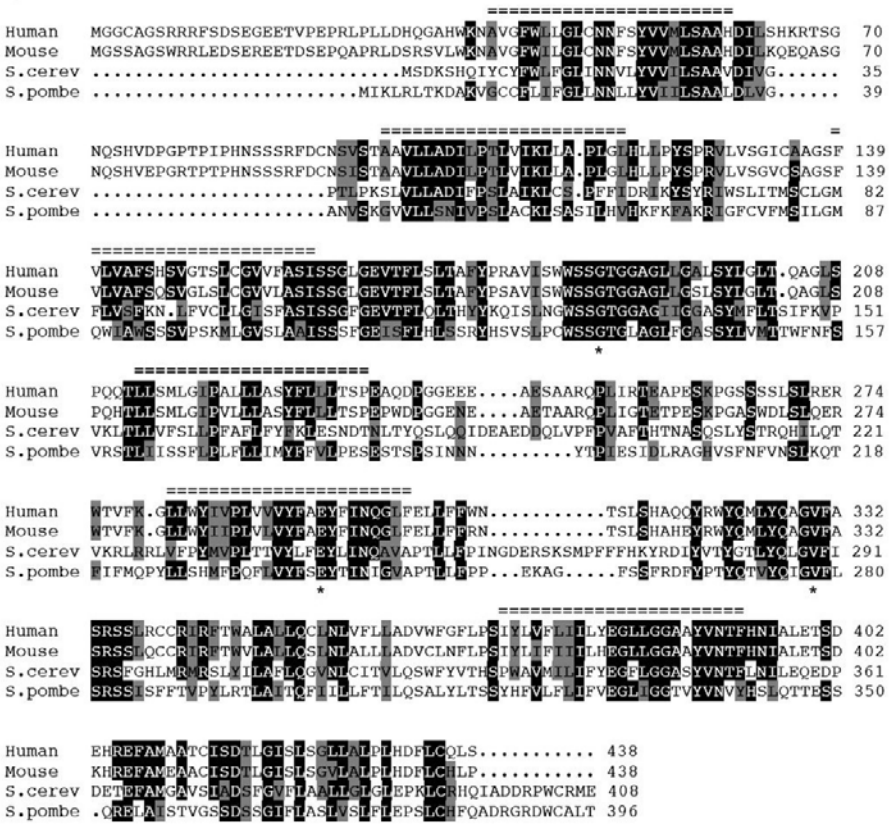

Figure 3. *Protein alignment of human CLN3 with homologues in mouse and two yeast species (Saccharomyces cerevisiae, and Schizosaccharomyces pombe). Dark shading represents identical residues, grey shading indicates similar residues. The proposed transmembrane segments are indicated by ==. Adapted from Gachet et al. (2005).*

Over the years, different kinds of mouse models for CLN3 disease have been developed, all displaying classical JNCL hallmarks, such as accumulation of storage material, neurodegenerative changes and loss of specific neuronal populations. The mouse CLN3 gene is approximately 14 kb long and consists of 15 coding exons. While the exact function of this protein remains unknown, it has been located to mitochondria in some retinal cells in mice (Katz et al., 1997), while other, more recent studies, have located it to late-endosomes and lysosomes in murine neurons (Oetjen et al., 2016).

The original and most widely used is the CLN3Δex1-6 model developed by Mitchison et al. (1999). In this model, exons 1 to 6 are replaced by a neo cassette, resulting in a null mutation and complete loss of the CLN3 protein. Since the mouse CLN3 protein has a sequence identity of 82%, and amino acid similarity of 85% with human CLN3, it was predicted that CLN3Δex1-6 mice would display many of the pathological and biochemical features of JNCL patients. Despite a lack of obvious clinical symptoms, these mice display several pathological JNCL features, such as an accumulation of autofluorescent storage material in neurons and glial cells.

Around the same time, another mouse model was developed by Katz et al. (1999) to mimic the 1kb deletion. In mouse embryonic stem cells, most of exons 7 and 8 was replaced by a neo cassette using a targeting vector, resulting in the CLN3Δex7/8 Katz model. A couple of years later, Cotman et al. (2002) developed a knock-in model displaying a more genetically correct representation of the 1 kb deletion, by removing exons 7 and 8, as well as the neo cassette introduced by Katz et al. completely. This model is referred to as the CLN3Δex7/8 Cotman model, and is used most widely now as it mimics the common 1kb deletion. Finally, there is the CLN3LacZ/LacZ model, developed by Eliason et al. (2007), where the first 8 exons are replaced with the LacZ reporter gene. This reporter mouse can express β-galactosidase under the native CLN3 promoter.

3.4 Simple multicellular organisms

While the yeast model appears to be great for carrying out molecular studies, it cannot relate to tissues or the central nervous system. On the other hand, the nervous system of a mouse model may be too complex for functional studies. Therefore, a simpler multicellular organism is needed. The simple nervous systems of the nematode Caenorhabditis elegans (C. elegans) and the social amoeba Dictyostelium discoideum might fill the gap between the simplicity of yeast models and the neurological complexity of mouse models. Dictyostelium has one functional CLN3 homologue, whereas C. elegans has three homologues, namely cln-3.1, cln-3.2 and cln-3.3; of which single- and triple (XT7) mutants have been generated (de Voer et al.,

2005). However, the appearance of three different CLN3 homologues in this organism complicates the generation of mutant nematodes. Additionally, all three CLN3 homologues are encoded by different genes, and their expression pattern changes with time and location.
Dictyostelium cln3 protein was found to localise both to the contractile vacuole system and the endocytic pathway. Deletion of cln3 in these amoeba results in increased rates of cell proliferation and premature multicellular development (Huber, 2017). These phenotypes are improved upon expression of either Dictyostelium or mammalian CLN3, indicating that CLN3 function is conserved from human to Dictyostelium. Furthermore, results from this study strongly support a role for CLN3 in regulation of the endocytic pathway and calcium-dependent developmental events. These suggested roles are similar to those proposed for mammalian CLN3 (An Haack et al., 2011; Luiro et al., 2004).

4. ADVANCES IN THERAPEUTICS FOR JUVENILE CLN3 DISEASE

Juvenile CLN3 disease is one of many known lysosomal storage disorders. For most LSDs, the pathophysiology is well understood, and over the last years, advances in enzyme replacement therapy have contributed to the development of treatment methods that can reverse or stabilise the progression of many of the somatic symptoms of LSDs. However, despite current knowledge regarding the biochemical processes underlying most LSDs, little is known about the processes that underlie the neurological deficits, and currently available therapeutics do not improve neurological symptoms, leaving LSDs like juvenile CLN3 disease incurable (Ahrens-Nicklas et al., 2019).
Moreover, a great challenge is to target all affected organs, including the brain, simultaneously. The central nervous system often remains untargeted, since many protein-based therapies are unable to pass the blood-brain-barrier or require the usage of highly invasive methods (reviewed by Platt, 2018). This chapter will discuss the advances that have been made regarding the development of therapeutics for JNCL, and will highlight important potential therapeutically intervenable processes that the CLN3 protein is involved in.

4.1 Enzyme-replacement and gene therapy

Most lysosomal storage disorders are caused by mutations in a single gene, resulting in altered activity of the subsequent enzyme. Enzyme-replacement therapy (ERT), whereby the faulty enzyme is injected intravenously, has shown some promising results in several distinct LSDs. Generally, LSDs are practical targets for ERT, because of a unique physiological trait referred to as "cross-correction". This process allows specific extracellular soluble lysosomal enzymes to be taken up and targeted to the lysosomes in otherwise enzyme-deficient cells (reviewed by Rastall & Amalfitano, 2015). However, intravenously injected enzymes cannot cross the blood-brain-barrier and are therefore unable to solve the neurological deficits observed in many LSDs, including the NCLs.
On the other hand, the concept of ERT and its success in some LSDs demonstrates that development of improved LSD treatments might be achieved through gene therapy-based approaches. This technique allows the delivery of an appropriate gene to cells of the patient in order to stabilise or reverse the clinical phenotype (reviewed by Rastall & Amalfitano, 2015). A recently developed recombinant protein therapy, Brineura, has been approved for CLN2 disease, which is caused by mutations in the lysosomal enzyme tripeptidyl peptidase 1.
The development of gene therapy for CLN3 disease, however, is much more challenging. Since CLN3 is a transmembrane protein, gene therapy approaches cannot benefit from the "cross-correction" mechanism. Moreover, CLN3 is not secreted in a precursor form, it cannot be taken up by mannose-6 phosphate receptors, which target newly synthesised enzymes to lysosomes. Considering these implications, it is likely that many more cells need to be transduced with CLN3 in order to show a phenotypic effect (Bosch et al., 2016).
Nevertheless, usage of two types of adeno-associated viruses (AAV) expressing the human CLN3 gene have shown promising results in a CLN3 disease mouse model (Bosch et al., 2016; Sondhi et al., 2014). Prior studies have also used AAV-mediated gene therapy to treat the neuropathology in mouse models for several other LSDs and it has also been used in clinical trials. Virus-based vectors are often used in gene therapy, since they provide for robust gene transfer and expression in several different cell types and tissues (Reviewed by Rastall & Amalfitano, 2015).
Sondhi et al. (2014) hypothesised that distribution of an AAVrh.10 vector coding for human CLN3 would be able to reduce the lysosomal storage in a CLN3Δex7/8 mouse model of JNCL, which recreates the 1kb deletion. Indeed, they demonstrated that the AAVrh.10 treated CLN3Δex7/8 mice showed a rescue of typical JNCL pathology, like reduced numbers of activated astrocytes and less storage material accumulation.
There were, however, some limitations to their approach. First of all, since this vector is unable to pass the blood-brain-barrier and only the cells directly transduced by the AAV vector were likely to be corrected, the vector had to be administered directly to the CNS of new-born mice via intracranial injections in three different areas of the brain, which is quite an invasive method. Secondly, while neonatal distribution of the AAV vector takes advantage of maximum brain plasticity and an immature immune system, it is not the best timepoint when considering onset of CLN3 disease. At this young age, mice do not display any of the JNCL features yet. Lastly, as their selected motor performance tasks did not show any differences between CLN3Δex7/8 knock-in and

wild-type mice (Sondhi et al., 2014), their observations consist merely of morphological and neuropathological examination.

Two years later, the study of Bosch et al. (2016) presented a similar, but more sophisticated approach for AAV-based gene therapy, using an AAV9 vector. Since the AAV9 capsid allows blood-brain-barrier passage, a single systemic intravenous injection of the AAV9 vector carrying human CLN3 cDNA in adult CLN3Δex7/8 knock-in mice was sufficient. Two types of promoters were tested, the MeCP2 promoter driving low levels of gene expression, and the β-actin promoter, which drives high ubiquitous transgene expression. They found that AAV9/MeCP2 but not AAV9/β-actin could rescue motor deficits, glial activation and lysosomal pathology in CLN3Δex7/8 knock-in mice, after a follow-up period of 13 months.

Moreover, a similar gene therapy approach has shown great potential for variant late-infantile or adult onset NCL, caused by mutations in the CLN6 gene, which encodes a transmembrane protein that has been located to the endoplasmic reticulum (Cain et al., 2019). With this approach, CLN6 is expressed under the neuronal β-actin promoter in an AAV9 capsid. Injection of human CLN6 in cerebrospinal fluid shows efficient expression of CLN6 in the central nervous system of mice as well as macaques. While high levels of transgene expression were found, and classical hallmarks of the disease, such as reduced lifespan and motor- and cognitive deficits, were prevented (Holthaus et al., 2019), no adverse effects or pathology were observed in any of the animals. The safety and efficacy of this gene therapy is currently being evaluated in phase I and II clinical trials (NCT02725580).

The results of both gene therapies using the AAV9 vector are very promising in terms of developing a therapeutic strategy, but more research is necessary to determine longer-term effects. Nevertheless, while being administered systemically, CLN3 protein was found to be expressed primarily in the CNS in one study, likely because the MeCP2 promoter preferentially drives transgene expression in neurons. While targeting the brain primarily can be very beneficial regarding the neuropathological phenotype, and for reduction of adverse effects, it will likely not be able to improve the disease phenotype in other organs, such as the cardiac system.

4.2 Targeting the autophagy pathway

It has been suggested that CLN3 is directly involved in autophagy and that the accumulation of SCMAS could be due to deficits in turnover of this protein via the autophagic pathway (Cao et al., 2006). Autophagic pathways, along with biogenesis of lysosomes, are tightly regulated by the coordinated lysosomal expression and regulation (CLEAR) gene network, and its master regulator transcription factor EB (TFEB; reviewed by Settembre et al., 2013). When activated, TFEB translocates to the nucleus, where it regulates the expression of genes involved in lysosomal biogenesis and autophagy. Moreover, a ChIP-sequencing experiment has proven a direct interaction between TFEB and CLEAR-targets in the proximal CLN3 promoter (Palmieri et al., 2011), which positively regulates CLN3 expression (Settembre et al., 2013).

Transcription factor EB activity, and thereby its subcellular localisation, is regulated by the kinase mammalian target of rapamycin complex 1 (mTORC1), which was shown to only exert its activity when located on the lysosomal surface. Phosphorylation of specific serine residues by mTORC1, while on the lysosomal surface, keeps TFEB inactive in the cytoplasm (reviewed by Ballabio, 2016). This pathway was shown to be implicated in many NCLs (Geraets et al., 2019).

During a state of starvation or lysosomal stress, mTORC1 is released from the lysosomal surface, and lysosomal calcium release via the channel Mucolipin-1 is promoted. Freely available calcium in the cytosol activates calcineurin phosphatase, which dephosphorylates TFEB, thereby promoting its nuclear localisation and activation. Upon arrival in the nuclear compartment, TFEB binds CLEAR elements in the promoters of genes involved in lysosomal biogenesis and autophagy, as well as CLN3, to positively regulate their expression (Settembre et al., 2013).

In many LSDs, the function of mTORC1 is implicated. For example, the study of Cao et al. (2006) demonstrated that levels of phosphorylated mTORC1 were reduced in a model of mice homozygous for the CLN3Δex7/8 mutation compared to wild-type mice, resulting in disrupted autophagy pathways in these animals. Studies using overexpression of TFEB have shown improved lysosomal function in many LSDs (reviewed by Ballabio, 2016). Moreover, pharmacological inhibition of mTORC1 has shown potential stimulation of autophagy in CLN3 deficient cells (Settembre et al., 2013). Additionally, it was demonstrated that a loss of TORC1 activity was beneficial in the btn1Δ yeast model, as it corrected most defects arising from a btn1 loss-of-function (Bond et al., 2015). Therefore, pharmacological targeting of this pathway would be a very interesting therapeutic target, which can possibly be achieved by using peroxisome proliferator activating receptor-α (PPARα) agonists (reviewed by Johnson et al., 2019).

PPARα has been shown to be involved in several regulatory and modulatory pathways, and is known to induce TFEB expression (Ghosh et al., 2015). In several different LSDs, including Pompe's disease and Gaucher's disease, PPARα has been reported to be downregulated. Recently, it has been discovered that small-molecule PPARα agonists, such as the fibrates fenofibrate, bezafibrate, and gemfibrozil, have beneficial effects on CLN3 mutated lymphoblast cells. Fibrates are described to exhibit neuroprotective effects to neurodegeneration, for example in Alzheimer's and Parkinson's disease, because of their antioxidant and anti-inflammatory properties. It was found that fibrates could inhibit enhanced cell death, while effectively alleviating defective autophagy and reducing

mitochondrial membrane depolarisation in lymphoblasts of CLN3 disease patients (Hong et al., 2016).
These findings indicate that fibrates could potentially provide effective treatment for juvenile CLN3 disease, likely via the activation of PPARα, and subsequent increasing of TFEB expression. Yet, since such approaches would alter TFEB expression systemically, potential side effects need to be evaluated carefully (Ballabio, 2016).

4.3 Calcium channel antagonists

While the exact pathological mechanisms underlying CLN3 disease, leading to neuronal cell death, remain unknown, some studies have demonstrated that an intracellular accumulation of calcium might trigger apoptosis. Additionally, it has been suggested CLN3 protein might have a modulatory role as anti-apoptosis protein, since defects in distinct signalling pathway proteins interacting with CLN3 have resulted in apoptotic cell death (reviewed by An Haack et al., 2011). Moreover, a strong interplay between CLN3 protein and the neuronal calcium sensor calsenilin was found in both in vitro and in vivo CLN3 knock-down models, which is suggested to result in calcium-induced cytotoxicity. Overexpression of CLN3 in CLN3-/- SH-SY5Y neuroblastoma cells reduced levels of calsenilin and prevented calcium-induced apoptosis, suggesting that calcium homeostasis is dependent on CLN3 activity and is disturbed in absence of the CLN3 protein. Moreover, they showed that six different voltage-dependent L-type calcium-channel modulators, which are able to pass the blood-brain-barrier, can restore calcium homeostasis by a significant reduction in intracellular calcium levels in CLN3 knock-down cells (An Haack et al., 2011).
Using the triple knock-out XT7 C. elegans model developed by de Voer et al. (2005), it was demonstrated that the FDA-approved calcium channel inhibitor Flunarizine rescues the reduced life span and increased mitochondrial mass of these CLN3 deficient nematodes (Kwon et al., 2017). These findings indicate a possible calcium defect due to loss of CLN3, even if CLN3 does not function as a calcium transporter.

4.4 Phosphodiesterase-4 inhibitors

Another potential treatment strategy is based on the second messenger cyclic adenosine monophosphate (cAMP), which regulates a variety of signalling events involved in learning and memory, as well as synaptic plasticity. Expression levels of cAMP are regulated by a balance between synthesis and degradation, which is mediated by adenylyl cyclase and phosphodiesterases (PDEs), respectively. There are eleven known PDE enzyme families, of which PDE4 is most prominently expressed in the central nervous system.
The broad effects resulting from a decrease in cAMP expression, such as the promotion of microglial pro-inflammatory activity, reduced glutamate transporter activity in astrocytes, and overall negative impact on neuronal homeostasis, are all hallmarks that were observed in CLN3Δex7/8 mice previously. These findings suggest there is a possible lack of cAMP activity in these animals (Aldrich et al., 2016).
It has been proposed that PDE4 inhibitors would provide an excellent means in targeting the cellular dysfunction observed in CLN3Δex7/8 mice. Treatment with two different PDE4 inhibitors, Roflumilast and PF-06266047, over a six-month period resulted in a significant downregulation of microglial activation and improved motor function in CLN3Δex7/8 mice, as well as reduced lysosomal pathology (Aldrich et al., 2016). Moreover, all neurological readouts in this study were improved upon treatment with both PDE4 inhibitors, suggesting that these compounds have a disease-modifying rather than only symptomatic effect.
It should be noted, however, that neuronal loss could not be observed in the CLN3Δex7/8 mice. Therefore, the effects of PDE4 inhibitors on neuronal survival could not be tested in this study, and only quantifiable pathological outcomes, such as glial activation and lysosomal pathology, were measured. Future studies should focus on the effects of PDE4 inhibitors on neuronal cell death, as this is one of the main pathological manifestations in Juvenile CLN3 disease.

5. CONCLUSION

Juvenile CLN3 disease has presented a significant therapeutic challenge due to several reasons. First of all, disease onset is in early childhood, and for treatment to be most effective, it should be started as early as possible because lost neuronal cells cannot be replaced. Thus, the optimal treatment window would begin even before neuronal loss has taken place. Second, the main clinical manifestations present in the brain, which is relatively inaccessible due to the blood-brain-barrier. Third, readily established approaches developed for other LSDs do not work for JNCL, as it is caused by mutations in an intracellularly located transmembrane protein. Lastly, the exact function and location of the CLN3 protein remain undiscovered, making it extremely hard to identify specific therapeutic targets.
Nevertheless, the availability of several large and small animal and cell models, as well as patient cell lines and iPSC libraries, has led to many advances in unravelling the function and localisation of the CLN3. Moreover, substantial progress has been made in terms of investigating and developing new therapeutic approaches to halt progression, and perhaps even prevent juvenile CLN3 disease. However, these therapies and their potential side effects need to be further carefully evaluated, before these therapies could be used in the clinic.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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'Interaction of the NPY system with gut microbiota'

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The neuropeptide Y (NPY) system consists of three peptides known as NPY, peptide YY (PYY) and pancreatic polypeptide (PP). This system is present at many levels of the gut-brain axis and forms the main communication between the central nervous system and the enteric nervous system. Outside the brain, the NPY system is involved in gastrointestinal regulation, immunological processes and nociceptive pathways. Within the brain, NPY relieves stress and has anxiolytic properties via the hypothalamic-pituitary-adrenal (HPA) axis. During the last decades, gut microbiota has also been linked to the HPA axis. Neonatal microbiota modulates HPA development and stress regulation in adulthood. Likewise, early life stress can have long lasting effects on microbiota. Given the effects of the NPY system and gut microbiota on the HPA axis and gut regulation, it is relevant to look at microbiota-NPY interaction and its consequences for the HPA axis. On the one hand, the NPY system preserves healthy microbiota by regulating gut-mucosa and promoting immunological response against pathogens. On the other hand, microbiota fermentation results in increased PYY release. PYY counterbalances gut inflammation, enhances nutrient absorption, and increases satiety. Moreover, microbiota imbalance alters Y-receptors and NPY release in the amygdala, hypothalamus and hippocampus. Although the consequences of these alterations are not known yet, they resemble the effects of any stressor: activation of the immunological system and impaired cognitive performance. Irritable bowel syndrome and obesity involve HPA deregulation and gastrointestinal complications. Therefore, this paper will review how these disorders are affected by the interaction between gut microbiota and the NPY system, as well as possible treatments arising from this interaction.

Keywords: *NPY system; microbiota; stress; enteric nervous system; IBS; dysbiosis*

There is a bidirectional communication between the gastrointestinal tract and the central nervous system (CNS), known as the gut-brain axis. The enteric nervous system (ENS) plays a crucial role in this interaction. It belongs to the autonomic nervous system (ANS) and has multiple connections with the parasympathetic and sympathetic systems, although it can operate independently from them (Furness, 2006). ENS is bidirectionally connected with the CNS, mainly via the vagus nerve, pelvic nerve and sympathetic pathway (Furness et al., 2014). This system is involved in controlling different gastrointestinal functions, such as mucosal secretion, immune modulation, gut motility, and blood flow.

During the last decade, different studies have proposed that gut microbiota might have an impact on the brain via the gut-brain axis. Gut microbiota has been related to several psychopathologies, such as depression and anxiety (Foster and Neufeld, 2013). Moreover, there is clear evidence of a reciprocal interaction between microbiota and stress. For example, rodents exposed to early life stress have altered microbiota in adulthood which concurs with an altered stress system (O'Mahony et al., 2009). This is because microbiota play an essential role in the development of the hypothalamic-pituitary-adrenal (HPA) axis and thus, in the stress response during the lifespan (Sudo et al., 2004).

A critical factor for the HPA axis and stress regulation is the neuropeptide Y (NPY). NPY is one of the most abundant peptides in the mammalian nervous system and is known for its stress-relieving properties and interaction with the HPA axis (Reichmann and Holzer, 2016). NPY is implicated in different physiological functions, such as food intake, circadian rhythm or the stress response (Reichmann and Holzer, 2016). This peptide is present at many different levels of the gut-brain axis (Holzer et al., 2012). Within the brain, NPY occurs in multiple areas related to this axis, such as the amygdala, nucleus accumbens (NAc), thalamus or cerebral cortex (Holzer and Farzi, 2014), but one of its main tracks is located in the hypothalamus. Precisely, NPY is released from the arcuate nucleus (ARC) into the paraventricular nucleus (PVH), which is the main source of corticotropin-releasing hormone (CRH) (Reichmann and Holzer, 2016). Via this track, NPY compensates for the distressing outcome of CRH signalling, reducing anxiety and relieving stress. Outside the brain, NPY is mainly released from the enteric neurons of the myenteric and submucosal plexuses, as well as from the postganglionic sympathetic neurons (Cox, 2007).

Although NPY is known for counterbalancing stress, its effects largely differ depending on the location and type of the receptors it targets. Moreover, NPY is a member of the NPY family, which is also composed of peptide YY (PYY) and pancreatic polypeptide (PP). Since all of them target Y-receptors (Rs), the role of NPY also depends on PYY and PP outcomes. There are four Y-Rs identified in humans and rodents, known as Y1-, Y2-, Y4- and Y5-Rs

(Reichmann and Holzer, 2016). NPY mostly targets Y1-, Y2- and Y5-Rs, with the Y1- and Y2-Rs being the most abundant within the brain. The main areas of the brain that express Y-Rs are summarised in Table 1.
Outside the brain, Y-Rs are involved in the immune response, nociceptive perception and ENS (Holzer et al., 2012). Different types of immune cells express Y-Rs, including dendritic cells, mononuclear cells, macrophages, granulocytes and T/B lymphocytes. Additionally, Y1- and Y2-R are found in the spinal cord and mediate nociceptive incoming information. Regarding ENS, Y-Rs are distributed on the gut, primarily targeted by PP and PYY. In particular, Y-Rs are found in enterocytes from myenteric and submucosal plexuses, endothelial and endocrine cells from the intestine, and adipocytes from visceral white adipose tissue (Jackerott and Larsson, 1997; Kuo et al., 2007).
The involvement of the NPY in the gut-brain axis and stress regulation makes this neuropeptide a relevant factor for pathologies, such as irritable bowel syndrome (IBS). IBS is characterised by abdominal pain, cramping, and change in bowel habits (diarrhoea and/or constipation). Importantly, symptoms get worse during stressful periods. IBS patients exhibit altered microbiota and deregulated HPA axis (Elsenbruch and Orr, 2001). Moreover, post-traumatic stress disorder (PTSD) is a significant risk factor for developing IBS (Ng et al., 2019). This piece of evidence suggests that there is an association between the microbiota-stress interaction and IBS symptoms. Since NPY regulates stress and is related to the gut-brain axis, it is possible that NPY is also involved in the aetiology and symptoms of IBS, not only via the HPA axis, but also via the immune system

Table 1. *Main presence of Y-Rs within the brain* ***(Kask et al., 2002)***

Receptor of interest	Main areas of expression
Y1-R	Frontal cortex
	Lateral dorsal septum
	Amygdala
	Dorsal hippocampus
Y2-R	Lateral septum
	NAc
	Bed nucleus of stria terminalis
	Paraventricular nucleus
	Lateral hypothalamus
	ARC
	Amygdala
	Dorsal hippocampus
	Area postrema
Y4-R	Medial preoptic area
	Area postrema
	PVH
	Nucleus tractus solitarius
Y5-R	Limbic brain areas

(Holzer et al., 2012).
In parallel, NPY and stress can alter food intake. Literature regarding the role of NPY in food intake is highly contradictory and inconclusive. Some articles propose that NPY stimulates appetite, while others associate NPY with decreased food intake (Reichmann and Holzer, 2016). Moreover, stress can also alter food intake in absence of NPY changes. These results are concordant with the multiple effects that NPY can display depending on where it is released and which receptor it encounters. However, if NPY variance is related to food intake alterations, its regulation is crucial for preventing other pathologies, such as obesity.
In summary, there is a clear, direct connection between the gut and the NPY system, as highlighted by the presence of manifold sources of this family of peptides, as well as of different Y-Rs throughout the gut-brain axis. In addition, NPY alteration in this axis is related to the stress response and to gut-associated pathologies. Lastly, there is increasing evidence supporting the interaction between gut microbiota, the HPA axis and the stress response. Given these three lines of evidence, it is relevant to understand the direct relationship between the gut microbiota and the NPY system. Their communication may have an influence on the development of pathologies, such as chronic stress or eating disorders. Therefore, the following paragraphs summarise the available literature on the relation between gut microbiota and the NPY system, and its implications for IBS and some psychopathologies.

1. THE EFFECT OF THE NPY SYSTEM ON MICROBIOTA

Most of the interactions between the NPY system and microbiota depend on PP and PYY. Since both of them are released from gastrointestinal tissues, understanding their function is essential when looking at microbiota-NPY communication. Furthermore, as gut microbiota develop in an environment characterised by gut motion and secretion, it is necessary to first look at the effects of the NPY system on microbiota environment.
Gut motility and gut secretion involve all NPY family peptides. Food intake stimulates PP secretion from pancreatic cells and PYY secretion from intestinal L cells (Holzer et al., 2012). Via Y4- and Y5-Rs, PP inhibits intestinal secretion (of electrolytes and water), gastric emptying and intestinal motor activity. Similarly, PYY and NPY inhibit gut secretion via Y1-Rs of endothelial cells and Y2-Rs of primary afferent nerve fibres. PYY and NPY also inhibit gut motility via the Y2-Rs of enterocytes located in myenteric and submucosal plexuses (Tough et al., 2011). In general, the NPY system essentially inhibits gastrointestinal secretion and motility. The three peptides slow gastric emptying, delay intestinal transit and enhance nutrient absorption. Furthermore, these aforementioned functions play a crucial role in preserving gut mucosa, which is essential for optimal

microbiota.

Besides the above-mentioned functions of the NPY on gut motion and secretion, NPY is involved in the regulation of the immune response, inside and outside the gastrointestinal tract. NPY partakes in colonic inflammatory processes by interacting with other molecules and mechanisms. For example, all NPY family members interact with serotonin, which regulates intestinal inflammation (El-Salhy and Hausken, 2016). In particular, NPY administration into the rat's hypothalamus decreases local serotonin levels in this area (measured by microdialysis), while serotonin antagonist administration increases hypothalamic NPY expression. The relationship between NPY, serotonin and somatostatin has been described by El-Salhy and Hausken (2016) and is illustrated in Fig. 1. During inflammation, NPY of ENS and mucosal serotonin secretion are increased, while mucosal somatostatin cells' activity is decreased. Serotonin directly promotes immune cell proliferation, enhances inflammation and stimulates gut secretion and motility. Similarly to serotonin's role, NPY exhibits proinflammatory properties. In turn, somatostatin counterbalances serotonin action by targeting receptors located in immune cells. Somatostatin has anti-inflammatory properties and inhibits gastrointestinal motility and secretion. At the same time, gastrointestinal motility and intestinal secretion (of electrolytes and water) increase the amount of PYY cells and decrease that of PP cells in the gut (El-Salhy and Hausken, 2016). Imbalance between the intestinal microbiota and the intestinal immune system, as well as low levels of intestinal inflammation, enhances permeability of the intestinal mucosal barrier (Holzer et al., 2012). Taken together, NPY can protect from microbiota deregulation (dysbiosis) inside the gastrointestinal system. Moreover, in case of infection, NPY neurons in the hypothalamus help maintain energy homeostasis and other adaptive behavioural responses to immune stress (Holzer et al., 2012).

Furthermore, the NPY system has direct antimicrobial properties (El Karim et al., 2008). NPY has immune properties against potentially adverse gastrointestinal bacteria including Escherichia coli, Enterococcus faecalis and Pseudomonas aeruginosa. It also protects against fungus, such as Candida albicans, usually found in the human gastrointestinal system and oral cavity. NPYs antimicrobial properties also target Cariogenic microorganisms, such as Streptococcus mutans and Lactobacillus acidophilus. Previous research has supported these antimicrobial effects of NPY against Escherichia coli (Hansen et al., 2006); despite the fact that there was no direct activity of this neuropeptide on the Pseudomonas aeruginosa, Streptococcus mutans or Candida albicans. Nevertheless, it is possible that these inconsistencies can be explained by the different stain methods used in each piece of research.

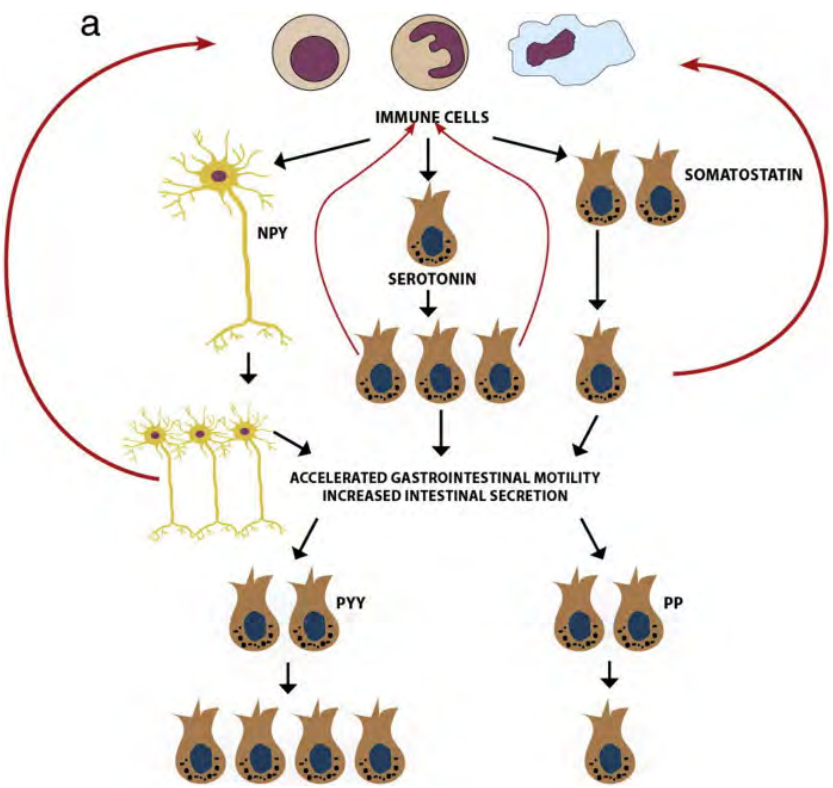

Figure 1. *(El-Salhy and Hausken, 2016): Schematic of the relation between the NPY family and the gut inflammatory process.*

The influence of the NPY system on gastrointestinal microbiota can have implications for related psychopathologies. As previously mentioned, IBS is one of the most relevant conditions linked to these interactions. Some studies show that IBS is characterised by a reduced number of PYY cells in the colon and rectum (Fig. 2 by El-Salhy et al., 2019). In healthy subjects, PYY counterbalances serotonin inflammatory effects by down-regulating serotonin secretion (Kojima et al., 2015). Consequently, the ability to restore balance, in situations where serotonin is increased, might be impaired in IBS patients. Thus, visceral pain and inflammation in IBS patients may be the result of low PYY levels. It has been suggested that lower amount of PYY cells in IBS is a consequence of irregular inflammatory processes during stem-cell secretory lineage (El-Salhy et al., 2017). Therefore, a short-term anti-inflammatory treatment could benefit the patient (El-Salhy et al., 2017). However, as some level of inflammation helps to prevent dysbiosis and infections, promoting PYY release or restoring PYY cells to normal levels might be a more suitable approach. In this regard, a possible long-term solution could be achieved by stem cells transplantation.

2. DYSBIOSIS AND THE NPY SYSTEM

The effects of microbiota on the NPY system have been more broadly studied. Gastrointestinal alterations constantly send signals to the brain via neural roots or via blood pathways. For example, cholecystokinin, ghrelin or PYY can directly activate afferent neurons (Holzer, 2016). Moreover, microbiota produce neurotransmitters typical for the CNS, such as GABA, serotonin, noradrenaline, dopamine, histamine, and acetylcholine (Holzer, 2016). As previously mentioned, there is a bidirectional interaction between microbiota and psychological disorders. Therefore, it can be expected that microbiota alterations can have direct or indirect influences on the NPY system.

One of the most prominent effects of gut microbiota on the NPY system was described by Samuel and colleagues (2008). Considering that the human gastrointestinal system lacks enzymes required for digestion of some complex carbohydrates, gut microbiota fermentation helps in this process. Gut bacteria can synthesise multiple glycoside hydrolases that break down these carbohydrates to short chain fatty acids (SFCAs). SFCAs stimulate L cells through G protein-coupled receptors, inducing PYY release and increasing PYY plasma levels. In support of this process, a study found that after 10 minutes of prebiotic administration, plasma PYY was significantly increased, compared to a control group without administration (Cani et al., 2009). It has been shown that PYY secretion inhibits food intake via stimulation of Y2-Rs on vagal afferent neurons and the hypothalamus (Ueno et al., 2008). As previously outlined, PYY also promotes nutrient absorption via gut motility inhibition. Moreover, PYY signals directly to the brain

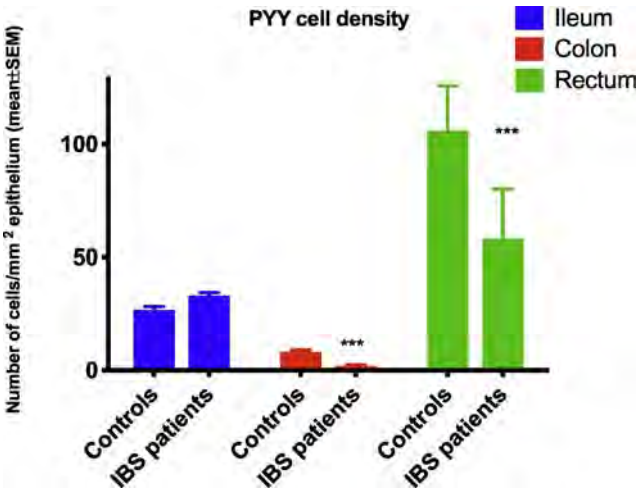

Figure 2. *(El-Salhy, Hatlebakk and Hausken, 2019): density of PYY cells in the ileum, colon and rectum of healthy subjects compared to IBS patients.*

(Dumont et al., 2007) or via the vagus nerve (Ueno et al., 2008), which results in antidepressant effects (Painsipp et al., 2011). Therefore, increased PYY from prebiotic fibre fermentation might regulate food intake, enhance nutrients absorption, and even decrease depressive symptoms.

Given the effects of microbiota fermentation on PYY and thus on food intake, some studies have explored the possibility that probiotics help enhance satiety in obesity patients and promote weight loss. In line with this, a meta-analysis found a reduction in body weight, BMI and fat percentage after short-term probiotic administration (Borgeraas et al., 2018). However, a more recent meta-analysis found that probiotic treatment led to body weight loss in overweight subjects, but not in obesity patients (Koutnikova et al., 2019). Moreover, the weight loss found in overweight individuals was not clinically meaningful, which discards the potential of probiotics as a first-line treatment in these cases. Another approach in obesity treatment could be directly increasing PYY, instead of increasing it via probiotics. In rodents, it has been shown that peripheral administration of PYY decreased body weight gain, glycemic indices and food intake (Pittner et al., 2004). Hence, PYY administration could have positive effects in obesity treatment.

Besides microbiota fermentation, there is an alternative path via which microbiota can influence the NPY system. Some studies have analysed the consequences of gut microbial disruption for the NPY system (Fröhlich et al., 2016). For example, the authors of one investigation reported brain area-specific alterations of Y-Rs and NPY mRNA expression. Specifically, they found that antibiotic treatment reduces Y1- and Y2-Rs expression in the hippocampus. Within the amygdala, dysbiosis led to higher NPY and lower Y5-Rs. Similarly, there was a

significant increase of NPY in the hypothalamus.
On the one hand, higher NPY secretion in the amygdala and hypothalamus depicts anxiolytic effects (Reichmann and Holzer, 2016). NPY is usually released upon stress exposure, and dysbiosis is an internal stress factor for an organism and the NPY. Moreover, it is plausible that the observed NPY increase results from immunological activation, as described above. On the other hand, Y-Rs reduction as a result of antibiotic treatment can have different consequences. First, reduction in Y1-R levels might inhibit the anxiolytic effects of Y1-R stimulation (Olesen et al., 2012; Primeaux et al., 2005). Second, Y2-R reduction has been linked to negative cognitive outcomes (Farzi et al., 2015). Lastly, there is no clear evidence regarding the consequences of decreased Y5-R expression in the amygdala (Reichmann and Holzer, 2016).
Once again, the involvement of the NPY system in the gut microbiota has consequences for IBS treatment. In healthy subjects, dysbiosis activates the NPY system which promotes immunological and inflammatory responses. However, this activation is regulated by PYY that counterbalances serotonin and visceral pain. Given that IBS patients have less PYY cells, it could be possible to treat the symptoms by restoring PYY cell density via microbiota. This can be promoted by the low-FODMAP diet (El-Salhy et al., 2019). This diet restores the microbiota and, as a result, increases SCFA production, which enhances the secretion of PYY.

CONCLUSION

This review summarised empirical research on the interaction between gut microbiota and the NPY system. Implications of this interaction for obesity, IBS and stress were also discussed. As expected, there is bidirectional communication between the NPY system and microbiota, which implicates different pathways and molecules. First, it is confirmed that the NPY system has multiple effects on microbiota regulation. The NPY is essential for preserving gut mucosa by regulating gut motility and secretion. In addition, the NPY system plays an important role in gastrointestinal inflammation by interacting with immune cells and serotonin or somatostatin. Moreover, NPY has direct antimicrobial properties against multiple adverse gastrointestinal bacteria and fungi. Second, literature also supports the influence of gut microbiota on the NPY system through two main paths. On the one hand, microbiota fermentation acts on SFCAs; molecules that stimulate PYY gut release. On the other hand, dysbiosis increases hypothalamic and amygdala NPY secretion and reduces Y1- and Y2-Rs expression in the hippocampus and Y5-Rs in the amygdala. Given the extent to which the NPY system is related to microbiota, this interaction can elucidate treatment possibilities for various health conditions.
First of all, alterations of microbiota on the NPY system can influence HPA regulation and the stress response. The main finding in this regard is the reduction of Y1-R levels in the hippocampus due to dysbiosis. Since Y1-R agonists have anxiolytic properties, reduction of Y1-R levels prevents NPY from mitigating anxiety. Thus, dysbiosis inhibits the NPY anxiolytic ability by Y1-R alteration. However, dysbiosis also increases NPY in the amygdala and the hypothalamus, areas in which dysbiosis does not alter Y1- and Y-Rs. Thus, in the amygdala and the hypothalamus, higher NPY secretion might reduce anxiety. Given the opposite effects of dysbiosis on anxiety and the high number of additional factors affecting the NPY axis, its influences on stress might not be as substantial as other aspects. Nonetheless, long-term dysbiosis could have implications for the whole HPA axis regulation, and hence, facilitate the development of chronic stress disorders.
Second, the influence of microbiota on the NPY system can have consequences for obesity. The relevance of microbiota fermentation for food intake reduction via increased PYY release is worth noting. Although probiotics could be used for enhancing PYY secretion, given the lack of clinical significance, they should not be used as a first-line treatment. Conversely, it is highly recommended to consider PYY administration as a possible treatment for obesity.
Third, the condition that is mostly affected by the studied pathways is IBS. Currently, one of the avenues for symptoms treatment is a low-FODMAP diet. This diet promotes PYY release by restoring microbiota. Alternative treatments could be developed, such as serotonin regulation through interference in NPY-serotonin interactions or potentially PYY administration. However, these alternatives would probably only have short-term effects. Therefore, the best option for achieving long-term benefits could be a stem-cell transplantation of PYY gut cells.
In conclusion, the important role of the NPY system in the gut-brain axis implies direct bidirectional interactions between this system and gut microbiota. Understanding these interactions is essential for developing new treatment possibilities in conditions like obesity and IBS. However, compared to them, stress does not seem to be as highly affected by the connection of the NPY system and gut microbiota.

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CONFLICT OF INTEREST

The author declares no conflicts of interest.

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'Blood-brain barrier leakage in Alzheimer's disease'

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With a world-wide population that is ageing, Alzheimer's disease (AD) is becoming an ever-growing challenge. Many attempts have been made to understand the causes underlying this condition. One aspect that is still controversial is the malfunction of the blood-brain barrier (BBB) during AD pathogenesis. The aim of this review is to outline what studies from mouse models and MRI have revealed so far about the BBB breakdown in AD. Importantly, the emphasis is on the relationship between the neurovascular unit (NVU) dysfunction and its impact on the development of various CNS pathologies, such as AD. Neurovascular unit damage can translate into a reduction of cerebral blood flow (CBF) and an increase in BBB leakage rates. Mild cognitive impairment (MCI) is discussed as a prodromal manifestation phase of the disease, allowing detection of neurodegenerative changes in AD at an earlier stage. In addition, some avenues for further exploration from a neuroimaging perspective are proposed.

Keywords: *Alzheimer's disease (AD); Blood-brain barrier (BBB); Neuroimaging; Neurovascular unit (NVU)*

Alzheimer's disease (AD) is the most common neurodegenerative disorder, contributing to 50% of dementia cases worldwide (Cummings, 2004). It manifests as amnestic-type memory impairment, language deterioration or visuospatial deficits. Typically, the neuropathological hallmarks of AD include abundant amyloid plaques and neurofibrillary tangles, neuropil threads, dystrophic neurites with hyperphosphorylated tau and astrogliosis, as well as microglial cell activation. In addition, loss of neurons and synaptic elements is observed (Serrano-Pozo et al., 2011).

In advanced AD, loss of neurons in the hippocampus and medial temporal lobe is usually observed, and fMRI measurements reveal a hypoactivation of the medial temporal lobe memory system. Interestingly, the same region is hyperactivated in mild cognitive impairment (MCI). This increase of activity in MCI can be seen as an attempt to compensate for an initial functional inefficiency, which then collapses into hypoactivation of the system during advanced AD (Dickerson & Sperling, 2008). Almost half of the individuals with MCI develop dementia and the annual conversion rate to AD amounts to approximately 7% (Mitchell & Shiri-Feshki, 2009). Because MCI precedes the full-blown development of AD, it should be possible to detect the upcoming aggravation changes at an earlier phase. It follows that the characterisation of MCI could be used as a diagnostic clue to determine the future occurrence of AD.

An important limitation when investigating the pathogenesis of AD is that this disorder often occurs in conjunction with other diseases, such as cerebral amyloid angiopathy (CAA) and stroke. Over 80% of AD cases bear hallmarks of CAA with amyloid-beta (Aβ) accumulation and deposition in pial and intracerebral arteries. In one-third of AD patients with the concurrence of CAA in small arteries and arterioles, atrophy of the vascular smooth muscle cells (VSMC) results in rupture of the vessel wall and intracerebral bleeding. This, in turn, contributes to the development and aggravation of dementia and even to hemorrhagic strokes (Zlokovic, 2011). This is particularly true for patients with hereditary cerebral β-amyloidosis and CAA of the Arctic E22G, Dutch E22Q, Flemish A21G, Iowa D23N, Italian E22K or Piedmont L34V type. Undoubtedly, Aβ is involved in AD (Zlokovic, 2011). Currently, different lines of research are being explored to understand the pathogenesis of AD. They focus on the accumulation of Aβ, the involvement of tau protein, local microglial cells and macrophages as a chronic inflammatory factor, as well as on chronic brain inflammation and BBB leakage (Angiari et al., 2015; Heneka et al., 2015).

This review concentrates predominantly on the BBB dysfunction in AD. Firstly, the structure and function of the BBB are described, and then the most important studies of BBB dysfunction in both mouse models and neuroimaging studies of BBB leakage in patients are discussed. Finally, based on the available data, a novel proposition is put forward, namely that characterisation of BBB dysfunction in MCI patients could be used as a potential diagnostic tool to detect early signs of AD in a subset of patients.

1. BLOOD BRAIN BARRIER (BBB)

Although the brain makes up only 2% of the total body mass, it consumes ~20% of the body's glucose and oxygen intake. Moreover, it has the capacity to increase blood flow rapidly when necessary, a process called neurovascular coupling (Mergenthaler et al., 2013). The BBB constitutes the interface between the capillaries of the circulatory system and the central nervous system (CNS) and it is composed of cerebral microvascular endothelium. Together with astrocytes, pericytes, neurons and extracellular matrix, it builds up the so-called neurovascular unit (NVU) (Figure 1). BBB permeability, cerebral blood flow (CBF) and maintenance of the chemical composition of the cerebral brain fluid that supports the functionality of neuronal circuits are

all under the control of the NVU (Zlokovic et al., 2011). Several pathological aspects can influence the NVU, such as (i) circulating substances that can leak from the plasma into the CNS, (ii) uncontrolled nutrient supply, (iii) accumulation of toxins in the CNS, and (iv) altered protein expression and secretion by NVU cells leading to oxidative stress, inflammation and neuronal damage (Figure 2). A large body of evidence underlines the relationship between dysfunctional NVU cells and the development of various CNS pathologies, including AD (Bell & Zlokovic, 2009; Erickson & Banks, 2013; Zlokovic, 2011). Any of the NVU components can undergo functional changes that can result in neuronal injury and cognitive deficits. Therefore, several transgenic animal models of AD, as well as advanced neuroimaging techniques may be used to reveal underlying AD pathology and contribute to finding potential treatments.

2. ANIMAL MODELS

Several AD mouse models have been characterised in the last decades, and collectively they have provided very important information on the pathogenesis of AD. For the purpose of this review, two models that have been most significant in elucidating the role of BBB dysfunction will be discussed in detail.

2.1 Amyloid-β (Aβ) mouse models

The most thoroughly studied players in AD are Aβ proteins. Their function can be altered by many mutations of the amyloid-beta precursor protein (APP) gene, and many mouse models bearing such mutations have been developed. In addition to other crucial pathogenic effects, Aβ dysfunction has been linked to BBB alterations.

In vitro studies on the rat aorta - an established model for studying cardiovascular alterations (Delp, 1985) - have revealed vasoconstrictive properties of Aβ (Thomas et al., 1996). It was found that Aβ attenuates acetylcholine-mediated endothelium-dependent vasodilation in transgenic mice harbouring the App Swedish mutation, which enhances the production and secretion of Aβ. Animals carrying this mutation accumulate high levels of Aβ more easily, leading to the development of amyloid pathology (Zhang et al., 1997). This is relevant because acetylcholine is also a neurotransmitter essential for processing memory and learning, and its concentration is decreased in patients with AD (Francis, 2005). Further studies on AB-deficient mice have demonstrated reduced cerebrovascular reactivity to endothelium after application of vasodilators (e.g., acetylcholine, bradykinin, calcium ionophore A23187). An increased response to vasoconstrictors on vascular smooth muscle cells (VSMCs) and alterations in neurovascular coupling have also been shown (Niwa et al., 2000). Overall, these studies led to the conclusion that Aβ deposition is implicated in the global impairment of vascular responses.

Furthermore, it was found that Aβ works as a suppressor of the advanced glycation end products (RAGE) receptor in brain endothelium, resulting in hypoperfusion and impaired protein synthesis, which is ultimately important for learning and memory. The increase of RAGE in brain microvessels in the APPSw/0 animal model accelerates the return of circulating Aβ into the brain. This results in Aβ accumulation, CBF reductions, neuroinflammatory response (Deane, 2003) and BBB breakdown (Kook et al., 2012). By blocking RAGE, Aβ pathology slows down, which in turn leads to a reduction in CBF (Deane et al.,

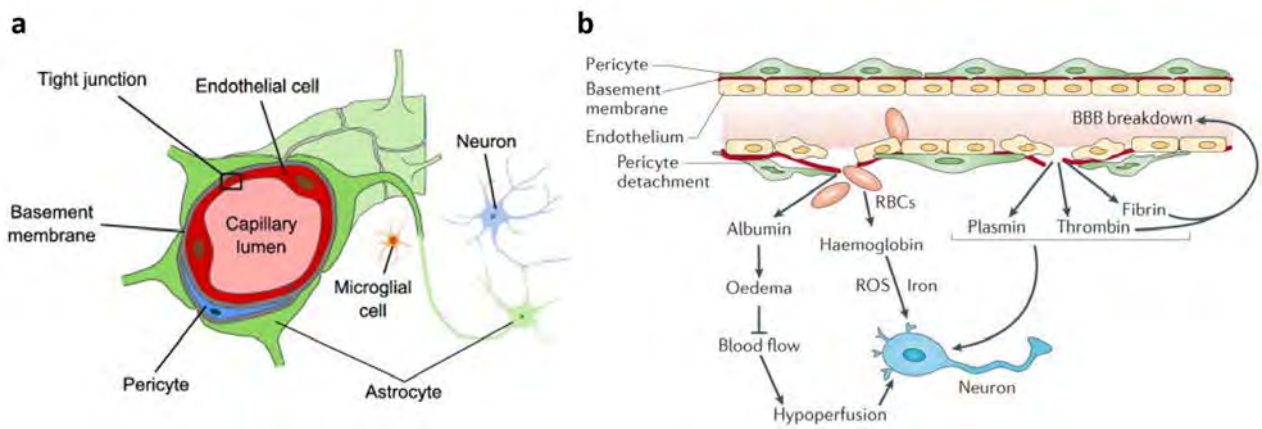

Figure 1. *NeuroVascular Unit. Panel a: schematic of neurovascular unit (NVU) depicting the interaction between astrocytes, pericytes, microglial cells, basement membranes and the endothelium of the BBB (adapted from Heye et al.,2014). Panel b: pericyte detachment-induced BBB leakage. Extravasation of red blood cells which release haemoglobin. The iron present in the heme group catalyses formation of toxic reactive oxygen species, which in turn induce neuronal injury. Leakage of serum proteins such as Albumin triggers vasogenic oedema, causing hypoxia and hypoperfusion, which aggravates neuronal injury. A more permeable BBB enable neurotoxic and vasculotoxic proteins (e.g. fibrin, thrombin and plasmin) to enter (adapted from Zlokovic, 2011).*

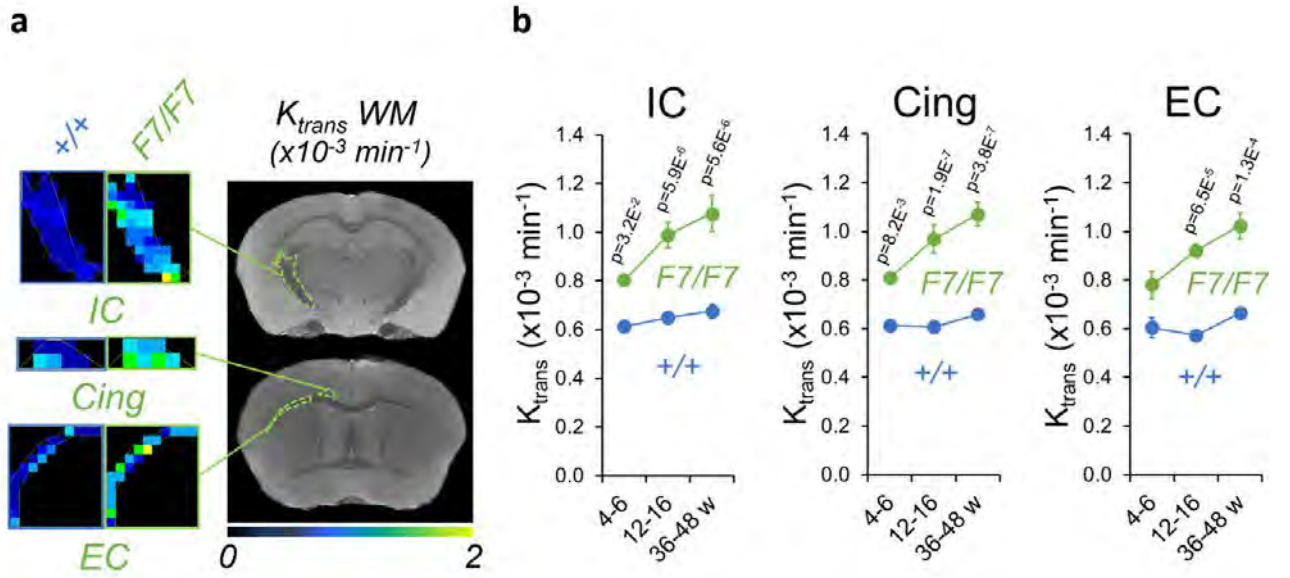

Figure 2. *CDE-MRI data of vascular permeability. Panel a: example of raw data that can be obtained with CDE-MRI and analysis performed in order to calculate the permeability transfer constant (Ktrans). Panel b: difference in [Ktrans] between control mice and PdgfrbF7/F7 mice at different ages (adapted from A. Montagne et al.,2018)*

2003).

Moreover, Aβ contributes to neurovascular dysfunction (in particular, endothelial dysfunction) through the NADPH-oxidase enzyme (Park et al., 2005) by activating the transient receptor potential melastatin-2 channels (TRPM2) in endothelial cells via the poly (ADP)-ribose polymerase pathway. In addition, the CD36 scavenger receptor binds to Aβ and this leads to Aβ-mediated oxidative stress in cerebral blood vessels, decreasing neurovascular coupling (Park et al., 2011). Importantly, neuroimaging studies in AD patients indicate that neurovascular uncoupling precedes neurodegenerative changes (Zlokovic et al., 2011). There is also a degree of crosstalk between these pathways. For example, it has been demonstrated that RAGE regulates CD36 expression (Park et al., 2011). However, the exact relationship between CD36 and RAGE in Aβ-induced CBF dysregulation needs further investigation.

Collectively, the aforementioned studies indicate that Aβ, due to its vasoactive and vasculotoxic properties, can affect different NVU components and thus, in turn, alter the CBF.

2.2 Platelet-derived growth factor B (PDGF-B) deficient transgenic mouse model

From the perspective of the BBB involvement in AD, an interesting model is the Pdgfrβ-deficient transgenic mouse (Lindahl et al., 1997). Mouse embryos with a deficiency in PDGF-β lack microvascular pericytes, which normally form part of the capillary wall. As a consequence, they develop many capillary microaneurysms which rupture at late gestation. It seems that in the mutant mice, endothelial cells of the sprouting capillaries are unable to attract PDGF-β receptor-positive pericyte progenitor cells. This indicates that pericytes may play a significant role in the mechanical stability of the capillary wall. Importantly, these vascular alterations lead to loss of cortical and hippocampal neurons, neurodegeneration, and behavioural deficits. Similarly, pericyte degeneration is present in human AD, thus supporting the notion that vascular-mediated neurodegeneration occurs independently of Aβ pathology (Montagne et al., 2017). Moreover, Armulik and colleagues (2010) observed a direct role of pericytes at the BBB in vivo. For the purpose of the study, a set of adult viable pericyte-deficient mouse mutants were tested. It was shown that higher permeability of the BBB to water and a range of low and high-molecular-mass tracers was strongly linked to pericyte deficiency. Further investigation revealed two important functions of pericytes at the BBB: regulation of the BBB-specific gene expression patterns within endothelial cells, and triggering of polarisation of astrocyte end-feet surrounding CNS blood vessels (Armulik et al., 2010). These findings highlight a critical role of pericytes in the endothelium and of astrocyte function integration at the NVU, and in turn, in the regulation of the BBB.

3. DCE-MRI STUDIES IN LIVING MOUSE MODELS AND PATIENTS

Magnetic resonance imaging (MRI) allows observation of different structural brain changes in AD, such

as hyperintensities in white matter (WMHs), small subcortical infarcts, atrophy, enlarged perivascular spaces and cerebral microbleeds (Thrippleton et al., 2019). For instance, brain atrophy is a characteristic shared by AD and other neurodegenerative disorders. With high-resolution quantitative MRI applied longitudinally, the atrophy in healthy individuals and those cognitively impaired can be distinguished based on regional volume losses and ventricular expansion over time.

There is also growing interest in using this advanced neuroimaging modality to gain quantitative functional information about cerebrovascular reactivity, CBF and pulsatility, as well as BBB integrity and its subtler and chronic disruptions. MRI can be used for disease diagnostics or at the testing phase of clinical drug administration. A particularly interesting type of MRI is the Dynamic Contrast-Enhanced (DCE) MRI. The volume transfer constant (KTrans) is one of the most important physiological parameters reported in DCE-MRI studies. It stands for the rate at which contrast agents arrive in the extravascular extracellular space (EES) per volume of tissue and for the agent concentration within the arterial blood plasma. In this technique, gadolinium contrast agent can be administered to visualise the local leakage from the BBB. Furthermore, the BBB permeability constant can be calculated according to the Patlak analysis method (Heye et al., 2014).

Montagne and colleagues (2015) conducted a series of studies correlating BBB dysfunction with AD. Initially, they investigated the regional BBB permeability in AD patients by applying the DCE-MRI protocol with high spatial and temporal resolution. One examination focused on multiple brain regions. This study suggested that ageing starts in the hippocampal region, which is impaired in early AD patients due to the BBB breakdown. Results indicated worsening of the BBB breakdown in the hippocampus, particularly in CA1 and dentate gyrus subdivisions in people with MCI. The cerebrospinal fluid analysis additionally showed a link with injured BBB-associated pericytes (Montagne et al., 2015).

In a follow up study, the group applied a dynamic contrast-enhanced (DCE)-MRI technique to assess whether pericyte loss underlies white matter (WH) vascular pathology and degeneration. For this investigation, the pericyte-deficient Pdgfrb F7/F7 mouse model was employed. The research was conducted on 11.7T 89 mm vertical bore Bruker BioSpin Avance DRX500 scanner. (DCE)-MRI protocol was followed by post-processing Patlak analysis quantification of regional WM tract vascular permeability in the presence of intravenously administered gadolinium-based contrast agent (Figure 2a). The experiment revealed a progressive increase in the permeability transfer contrast (Ktrans) of capillaries in WM tracts of the corpus callosum, internal capsule, cingulum and external capsule in 4-6, 12-16 and 36-48-week old F7/F7 mice in comparison to the control age-matched Pdgfr13+/+ littermates (Figure 2b). These results imply that pericyte degeneration disrupts WM microcirculation and this, in turn, leads to accumulation of toxic blood-derived fibrin(ogen) and blood flow reduction (Montagne et al., 2018).

According to previous literature, BBB leakage should not be dependent on blood flow, at least within a low-permeability regime. However, new findings from Van de Haar and colleagues (2016) challenge this view. In one of their studies, the neurovascular unit in AD and healthy controls were compared using DCI-MRI and arterial spin labelling magnetic resonance imaging. All DCE-MRI data were collected from 3 Tesla system Philips Achieva. Results suggested that there are two possibilities for BBB breakdown and hypoperfusion. Either the leakage exceeds further than only the permeability surface-area of the barrier, or there is a common mechanism (Van de Haar et al., 2016). In particular, it was found that in patients with early AD with MCI, global hypoperfusion in the brain's Grey Matter (GM) occurs as a consequence of an elevated leakage rate and increase in its respective extent. Most importantly, this study found a correlation between the reduction of CBF and the increase in BBB leakage rates. The latter suggests that there is a common pathophysiological pathway between the two related functional elements of the NVU: the CBF and the BBB.

4. TWO–HIT VASCULAR HYPOTHESIS FOR AD PATHOGENESIS

Histological examination of the brain in AD patients by Alois Alzheimer in 1907 identified Aβ plaques as hallmarks of the disease for the first time (Karran et al., 2011) and consequently, became a principal line of research in understanding the causes of Alzheimer's disease. Postmortem and in vivo studies at an advanced stage of AD, in both humans and mice, have later on confirmed the accumulation of Aβ plaques in the brain. The Aβ animal model showed a relationship between Aβ and receptors over which it exerts control, e.g. RAGE.

Thus, the role of Aβ in AD is not in question, but rather the notion that Aβ deposition is the primary cause of the disease is being debated. The high number of publications on this research line clearly indicates that priority is given to investigating Aβ in AD studies. Aβ certainly can be found in both early and advanced stages of AD. But to gain insight into potential treatments for AD, it is essential to understand the prime source of the disease. So far, there has been no evidence of Aβ presence in people with MCI suggesting that, at least in a subset of patients, Aβ is not necessarily the main cause of AD, but rather that it is a secondary step in a chain of events during AD formation. This is illustrated in the "two–hit vascular hypothesis" which postulates that AD is initially triggered by vascular dysfunction (Figure 3).

This hypothesis is supported by several investigations. The findings of Montagne (2015) established again that BBB disruption is caused by pericytes deficiency, as shown by cerebrospinal fluid analysis. Indeed, it is

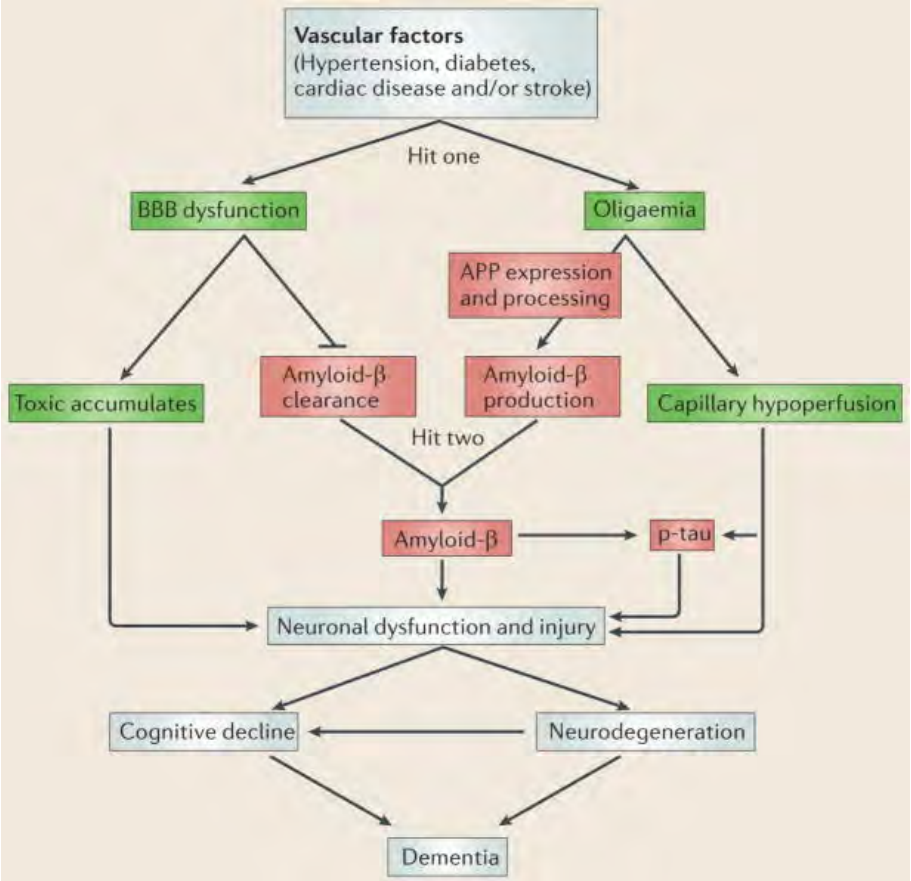

Figure 3. *"two-hits hypothesis". Both Aβ-independent and Aβ-dependent mechanisms converge and lead to neurodegeneration. In the "first hit", vascular risk factors lead to BBB breakdown and reduction in CBF (oligaemia) and start an entire cascade of events which eventually leads to dementia. In this Aβ-independent pathway both capillary hypoperfusion and toxins accumulation induce early neuronal dysfunction. As a consequence of vascular injury, Aβ clearance is reduced leading to its accumulation. This event constitutes the "second hit" which amplifies neuronal dysfunction, speeds up neurodegeneration and dementia, and contributes to disease aggravation. In addition, Aβ and hypoperfusion can trigger hyperphosphorylation of tau resulting eventually in neurofibrillary tangle formation(adapted from Zlokovic, 2011).*

believed that in normal functioning, pericytes together with endothelial cells, limit the entry of neurotoxic substances into the brain, and therefore the connection proposed by Montagne appears legitimate (Iadecola et al., 2015). The studies of Lindahl (1997) and Armulik (2010) on PDGF-β deficient transgenic mouse models also highlight that pericytes fulfil an important role in maintaining the balance of the BBB, and that BBB deficits lead to neurodegeneration.

Indeed, loss of pericytes not only induces an accelerated BBB breakdown, but also accelerates parenchymal Aβ deposition and CAA. This also causes tau pathology and neuronal loss as demonstrated in double-transgenic mice APPSw/0; Pdgfrβ+ with accelerated pericyte loss. Interestingly, early neuronal loss is absent in Aβ-precursor protein transgenic mice (Sagare et al., 2013). These findings imply that pericytes influence the AD neurodegeneration cascade at multiple steps in Aβ APP mice and therefore, they could become a novel therapeutic target for studying AD. In addition to searching for an explanation of the BBB (pericyte) deficiency, Montagne (2015) set out to investigate the occurrence of the first AD changes within the brain using DCE-MR. This neuroimaging technique established that BBB disruption in both ageing and early AD impairs first and foremost the hippocampus, the primary region involved in learning and memory. Importantly, this study showed that BBB breakdown can be detected much earlier than it was previously thought; when people have their first mild cognitive problems. Since MCI commonly develops later in AD, DCE-MRI of BBB leakage could be used for pre-clinical diagnostics and earlier prevention of AD development.

Furthermore, Van de Haar et al. (2016) explored the BBB in AD with 2 different neuroimaging techniques. DCI-MRI and arterial spin labelling magnetic resonance imaging (ASL) enabled the discovery of a correlation between an increase in BBB leakage rates and the reduction of CBF, suggesting that these two NVU functional components have a shared pathophysiological pathway. This evidence highlights the link between BBB dysfunction and CBF reduction. It would be beneficial to gain an understanding of the mismatch between CBF/O2 delivery/neuronal activity, which results in disrupted functional connectivity and neurovascular uncoupling present in the early stages of numerous neurological disorders, such as AD. Therefore, it would be worthwhile to consider aligning BBB permeability results with those of CBF analyses to get a broader overview of the pathogenesis of AD and its development.

CONCLUSIONS AND PROSPECTS

Collectively, the aforementioned evidence sheds light on the course of AD. The reviewed literature suggests that accelerated parenchymal Aβ deposition is preceded by pericytes loss, which contributes to BBB breakdown. The increase in BBB leakage co-occurs with the reduction of CBF, and importantly these changes can be observed already in people with MCI. To better understand the aetiology and development of AD, and to be able to help people before they reach a more advanced stage of AD, the introduction of pre-clinical studies would be highly beneficial.

There is hope that, with the help of new neuroimaging techniques and biofluid markers, AD pathology can be detected already at the pre-clinical stage, before irreversible neuronal loss has occurred. However, there are still some improvements to be made. One of the important factors to consider is image resolution, which relies on the strength of the MRI magnetic field. The difference between the quality of the image obtained with a 3T or 7T MRI can be seen in the example of micro hemorrhages in AD patients and MCI individuals (Sweeney, 2018). Currently, a great number of studies examining MCI and AD apply 1.5T and 3T MRI. For example, high-resolution confocal microscopy of brain tissue allows detection of capillary hemorrhages of 20–30 μm in diameter that can go undetected on 1.5T or 3T MRI (Sweeney et al., 2018). Therefore, for studies of small vessel diseases such as AD, the usage of stronger magnetic field (7T MRI) is paramount.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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'A puzzle to solve: Reconstructing the functional anatomy of C-low threshold mechanoreceptors ascending pathway'

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Unlike other sensory domains, the somatosensory system is composed of several senses, each possessing one or more distinct sensory end organ(s) that decode(s) multiple aspects of its respective sensory domain. In animals, and more recently in humans, the cutaneous C-low threshold mechanoreceptor (C-LTMR; or CT in humans) was introduced as a new candidate for a separate submodality within the somatosensory domain. This notion is supported by the unique physiological, anatomical, and genetic characteristics that identify C-LTMRs as unique and different sensory units. Moreover, the neurophysiological characteristics of C-LTMR punctuate a highly sensitive and selective sensory unit that possesses a unique sensitivity to low velocity, indentation, and force-stroking movements at skin temperature. Taken altogether, this submodality might be dedicated to interoceptive, rather than exteroceptive processes. However, the C-LTM-receptor cannot be understood in isolation but is part of a concentrically organised follicle complex containing several distinct LTMR end organs that innervate different hair follicle types. Costimulation of the follicle complex results in distinct neuronal codes that reflect specific aspects of a sensory event. Although sensory information is initially stimulus-specific and conveyed by distinct ascending channels along the spinothalamic tract, the vast majority of sensory aspects will later be combined in cross-modal sensory networks. Given this early diffusion of sensory information into various networks, individual primary afferents exclusively convey stimulus-specific sensory information centrally ('labelled line') as specified 'labelled-line' postulate. They must be limited to infraspinal afferents. Moreover, the intricate spatial and functional relationship between (C-)LTMR units and the complex somatotopic organisation (e.g. receptor topography, neighbourhood maintenance, information flow, axial organisation, top-down modulation and glomeruli clustering), on both peripheral and central level, highlight a complexity of (C-)LTMR system in hairy skin that is equivalent to other sensory systems. The intricate somatotopic organisation is paralleled by its contribution to various, functionally distinct systems in the spinal dorsal horn. These spinal networks process nociceptive, homeostatic, and other somatosensory information. This is particularly evident in nociceptive modular circuits. These circuits converge and integrate nociceptive processing with C-LTMR information, by regulating the magnitude of incoming nociceptive information through simple, as well as more complex inhibitory connections of the GABA and glycine system. In addition, lamina I projection neurons of the nociceptive pathway might be involved in providing a weighted output between C-LTMR and nociceptive information. This may form the basis for more advanced homeostatic processing in higher cortical areas. The weighted output between C-LTMR and nociceptive information might be transmitted to the parabrachial- and/or the posterior part of ventromedial thalamic nucleus via the spinothalamic pathway, albeit, due to prior integration, not adhering to the widely accepted neo-/paleo-/archeo spinothalamic sub-categorisation. In the light of these recent developments, a new model for the early functional anatomy of the C-LTMR system is proposed in this review.

Keywords: *C-LTMR; CT afferents; LTMR topography; LTMR physiology; Spinal dorsal horn; Nociceptive modulation*

The somatosensory system, often referred to as the "sense of touch", is unique in the domain of perception, as it is composed of multiple senses (or submodalities; e.g. nociception, thermoception and proprioception). Yet, the tactile universe is rich, if not infinite and even a single sensory entity might be defined by a wide range of sensory aspects (e.g. muscle stretch and tactile indentation force for sensing an object's weight. Therefore, the sensory input of the external world must be extracted by multiple highly specialised decoding mechanisms that can be incorporated in a single sense, which endows humans and animals with the remarkable capacity to reconstruct environmental states through the diversity of subjective sensory sensations. These somatosensory senses can be classified according to three major categories: exteroceptive, interoceptive, and proprioceptive functions. The exteroceptive and interoceptive categories are responsible for the perception and reaction to stimuli that emerge from outside or inside the body, respectively. The proprioceptive category allows for perception and control of one's body position, haptic exploration and balance (Abraira & Ginty, 2013; Wolfe et al., 2006; for an overview of somatosensory afferents see table 1).

Sensory Afferent Nerves				
Receptor Type	**Dominant Modality**	**Conduction Velocity[B]**	**Skin Type[C]**	**End organ Type[C]**
Sensory Class Type I: A α-Fiber Class (exclusively proprioceptive)				
Type Ia Aα-Proprioceptor	Rate of change in muscle stretch (important for movement and stability)	80–120m/s	None (muscle)	Dynamic annulospiral ending
Type Ib Aα-Proprioceptor	Level of applied tensile load by muscle force or gravitation (important for proprioceptive reflexes, stability)	80–120m/s	None (tendon)	Golgi tendon organ
Sensory Class Type II: Aβ- (and Aα-) Fiber Class (mostly exteroceptive)				
Type II Aα-Proprioceptor	Static stretch of a still muscle (important for perception of body position)	80–120m/s	None (muscle)	Static annulospiral / flower spray endings
Aβ SAI-LTMR (tactile receptor)	Fine indentation (important for texture discrimination and light touch; discriminative touch) and various other functions	16-96m/s	Glabrous	Merkel cell-complex
			Hairy	Touch dome
Aβ SAII-LTMR (tactile receptor)	Skin stretch (important for hand shape and finger/joint conformation and object motion (proprioceptive and discriminative)	20-110m/s	Glabrous	Unclear (Rufini endings)
			Hairy	Unclear
Aβ-RAI-LTMR (tactile receptor)	Skin movement, fine motion (important for grip control), light touch and hair follicle deflection (discriminative)	26-91m/s	Glabrous	Meissner corpuscle
			Hairy	Longitudinal lancelot ending
Aβ-RAII-LTMR (tactile receptor)	Vibrations (discriminative)	30-90m/s	Glabrous	Pacinian corpuscle
Aβ Field-LTMR (unknown)	Unknown (potentially nociception or sensory modulation)	Unknown	Hairy	Circumferential endings
Aβ-HTMR (nociceptor)	Sharp mechanical pain	16-59m/s	Unclear (possibly both)	Unknown
Sensory Class III: Aδ-Fiber Class				
Aδ-LTMR ('hair units')	Fine hair deflection and cooling (in animals; receptor is not found in humans)	Unclear (probably <59m/s)	Hairy	Longitudinal lancelot endings
Aδ-Thermoceptor	Cooling	Unclear	Unclear	Unclear
Aδ-HTMR (nociceptors)	Sharp mechanical or thermal pain	12m/s	Glabrous Hairy	Free nerve endings
Sensory Class IV: C-Fiber Class (mostly interoceptive)				
C-HTMR (nociceptors)	Mechanical pain	<2m/s	Glabrous Hairy	Free nerve endings
C-Thermoceptor	Warming and Cooling	<2m/s	Glabrous Hairy	Free nerve endings
C-Pruriceptor	Itch	<1m/s	Glabrous Hairy	Free nerve endings
C-LTMR (or CT in humans)	Affective and pleasant touch	<2m/s	Hairy	Longitudinal lancelot / free nerve endings)

Table 1. *Type I-VI Erlanger-Gasser Classification of Primary Sensory Afferents Innervating the Human Skin[A].*

[A]The table content was obtained from various sources, see Abraira and Ginty (2013), Djouhri and Lawson (2004) and Hall (2011).

[B]Conduction velocity is affected by the degree of axon myelination (reflecting axonal diameter); for instance, larger axon diameters (up to 20μm in Aα fibers) corresponds to increase myelination, which in turn correlates with faster conduction velocities.

[C]Many receptor types, especially nociceptors and proprioceptors, also innervate deeper skeletal or muscular tissue and internal organs (e.g. periosteum, joints, arterial and alveolar walls, liver capsules, pleura). However, in general, somatotopic functions demand an interplay between several receptor types. For example, the joint-stretch feedback depends on four types of lamellar LTMRs, such as Pacinian and Ruffini mechanoreceptors, which either detect joint position (proprioception) or respond to reaching joint-limits and injury (nociception).

Abbreviations: SA, Slow adapting; FA, Fast adapting; LTMR, Low threshold mechanoreceptors; HTMR, High threshold mechanoreceptors.

Traditionally, two major sensory neuron classes, the low threshold mechanoreceptors (LTMR) and C-high threshold mechanoreceptor (C-HTMR or C-fibers), were respectively understood as being mostly exteroceptive, serving a discriminative function, or interoceptive in nature, serving affective and visceral functions (McGlone et al., 2014). However, recently a new type of unmyelinated, C-low threshold mechanoreceptor afferent, the C-LTMR (in animals) or C-tactile (CT) afferent (in humans), was discovered. Although these afferents shared the low indentation threshold (LTMR-like) and slow conduction velocity (C-fiber-like; hence being similarly classified), they presumably contribute to substantially different functions. For example, C-LTMR/CT afferents are thought to play a role in socioemotional aspects of touch, pain modulation, and homeostatic processing - as opposed to discriminative touch (Andrew, 2010; Craig, 2002; McGlone et al., 2014).

Although some insight into the functional properties of animal C-LTMR (and less into the human CT) system has been gained in recent years, relatively little is known about its underlying anatomy. Moreover, there are many outstanding questions about its network organisation: How are ascending pathways organised at the neuronal level? How does the neuronal code ascend to, is processed by or integrated in other systems? How does the network organisation give rise to its functional aspects? Thus far, few studies have addressed these questions. Much of what is known about the functional anatomy of the C-LTMR system is limited to the peripheral and spinal cord levels, but studies are yet only loosely integrated. Therefore, this review aims to provide the first complete overview of the peripheral and spinal anatomical network associated with C-LTMR input. For this purpose, central principles, such as C-LTMR's neurophysiology, network structure, spatial organisation and cross-modal system interactions, will be discussed. In addition, this review strives to establish a link between C-LTMR system's anatomy and function and investigates which insights from the C-LTMR system in animals can be generalised to humans, given that research on the human CT network structure is still limited. In terms of a broader scientific perspective, this review also aims to increase the awareness that the first locus of somatosensory processing lies not in the somatosensory cortex but in the spinal cord, questioning the exclusivity of individual, stimulus-specific (C-)LTMR afferents ('labelled lines') at supraspinal level.

First and foremost, a holistic reconstruction of the early C-LTMR/CT afferent system, would not only result in a more exhaustive picture by integrating genetic, anatomical, functional, and behavioural insights, but would also establish a link between multiple levels of explanation. With respect to the societal relevance, identifying the underlying neurobiological mechanisms of the C-LTMR system could contribute to the development of biological interventions for C-LTMR-associated disorders, such as tactile allodynia.

1. THE PERIPHERAL SENSORY SYSTEM OF C-LTMRS

1.1 Neurophysiological characteristics of C-LTMRs: Temporal configurations

Traditionally, C-LTMR fibers have been classified as C-fibers (Zotterman, 1939) due to their similar conduction velocity and axon diameter, related to the degree of myelination. However, recent research concerning the neurophysiological, functional, and morphological aspects of C-LTMR fibers has indicated that this system might differ substantially from C-fibers and other LTMRs (McGlone et al., 2014; Pitcher et al., 2016). The neurophysiological characteristics of C-LTMR's/CT's peripheral sensory system can be objectified according to its (A) stimulus-sensitivity and (B) physiological response to a given stimulus. More specifically, stimulus-sensitivity is quantifiable by investigating the receptors (I) indentation, (II) velocity, and (III) temperature tuning, while physiological response measures contain (I) spiking properties, (II) adaptation rate, (III) after discharge, (IV) neuronal fatigue, (V) action potential shape, and (VI) conduction velocity. These neurophysiological properties will be discussed and compared to C-HTMRs and LTMRs in the following section and are further elaborated on in table 2 (for a more general overview of all sensory neurons see Abraira & Ginty, 2013).

(A) In essence, the sensitivity-optimal stimulus for C-LTMRs that will elicit a maximal physiological response of ?100 impulses/s in animals and in humans is characterised by a low velocity, indentation stroking movement at skin temperature (see table 2A; Iggo, 1960; Nordin, 1990; Vallbo et al., 1999). This specific sensitivity-tuning reflects a highly specialised decoding mechanism for tactile input, that, when paralleled by functional implication, strongly coincides with gentle interpersonal skin-to-skin contact that might occur in the context of affective touch (see the affective touch hypothesis in McGlone and colleagues, 2014). In particular, the thermal aspect of stimulation indicates sampling of socioemotional information as it could be extracted from interpersonal skin-to-skin contact: the correspondence between a thermo-optimal stimulation and the average skin temperature (both 32°C), the preference for cooling and an increased effectiveness of a combined mechanothermal stimulation suggest an implication of C-LTMR in affective touch. CLTMR's higher sensitivity for cooling rather than warming might have evolved around this ecological function, since skin temperature in habitable environments is more likely to be drastically below (rather than above) body temperature and is subject to natural fluctuations across the day (e.g. lower during night) and seasons. With respect to its psychophysical properties, the perceived hedonic quality strongly coincides with dynamic and thermal tuning curves and peak values. This proposes an involvement in affective processes elicited only by

this set of specific stimulus parameters. In addition, place-preference reinforcement mechanisms based on C-LTMR input, further complement this hypothesis (Vrontou et al., 2013). *(B)* With respect to the physiological output characteristics (see table 2B), the intermediate adaptation rate, and more importantly, the slow neuronal conduction velocity could explain the sustained, more state-like qualia that are often reflected in emotional and visceral systems (Craig, 2002) and likewise in the low temporal resolution of this system. Regarding interspecific generalisations, the neurophysiological properties of C-LTMR neurons have been found to be similar in human CT afferents, which suggests some degree of interspecies similarities (Nordin 1990; Vallbo et al., 1999; Wessberg et al., 2003). Nevertheless, neurophysiological parameters differ between species and the C-LTMR population in animals is not homogenous, but may instead consist of several subgroups (Pitcher et al., 2016; Vallbo et al., 2016). Furthermore, inference solely based on receptor physiology is limited and many questions remain open, such as: how does the integration of distinct physiological aspects contribute to function? Addressing such questions is imperative for future investigations.

Taxonomic considerations are another important aspect that can be derived from neurophysiology. As reflected in the comparisons depicted in table 2, C-LTMRs/CTs possess some characteristics that are similar to C-fibers (most prominently conduction velocity) and LTMRs (most prominently similar indentation force). Interestingly, the C-LTMR and the Aδ-LTMR ('hair unit'), that is generally associated with visceral processes, share similar physiological (and, as discussed later, topographical) configurations. Both units have similar indentation thresholds and are more tolerant to cooling, implying a degree of similarity in function, that might be visceral in nature. However, multiple neurophysiological and later described topographical, parameters, such as thermal/velocity tuning, as well as aspects of neuronal fatigue, adaptation rates, spiking properties and after discharge suggest that C-LTMRs are temporally and spatially distinct from other afferent sensory systems. In addition, an immense neurophysiological heterogeneity within the C-LTMR type is evident, and when complemented by genetic labelling studies, research suggests that this afferent sensory group could be further differentiated into, at least, two subclasses, mrgb4- and th/vglut3/tafa4- expressing afferents (Abraira et al., 2013, 2017; Seal et al., 2009; Vrontou et al., 2013). Interestingly, Tzschentke (2007) postulates that the different subclasses might even serve distinct functions, although this is not validated by other studies (see Lou et al., 2013).

1.2 Receptor geography of C-LTMRs: Spatial configurations

Despite that not much is known about molecular processes and cutaneous terminal anatomy, some recently accomplished major breakthroughs have revealed a remarkable relationship between end organ anatomy and receptor organisation (Kuehn et al., 2019; Li et al., 2011). Li and colleagues (2011) utilised a CreER knock-in into the locus to visualise the LTMRs respective terminal endings connected to a subset of three hair follicle types (guard, awl/auchene, and zigzag) in the hairy skin of mice (see also figure 1A and table 1 for a detailed description). *(I)* Guard hairs, the longest but least abundant (1%), receive Aβ RA-LTMR lanceolate nerve endings and are associated with Aβ SAI-LTMRs that innervate touch domes. *(II)* Awl/auchene hair follicles (25%) are triply innervated by interdigitating nerve endings of C-, Aδ- and Aβ-RA-LTMRs. *(III)* The most abundant hair follicle type is the zigzag hair follicle (74%), that is innervated by both C- and Aδ-LTMR lanceolate nerve endings in a remarkably morphological interdigitated manner. Moreover, all three hair follicle types also receive circumferential endings from Aβ Field-LTMRs, surrounding the palisades of the longitudinal LTMR endings (Kuehn et al., 2019; Millard & Woolf, 1988). Therefore, each mouse hair follicle type is innervated by a "unique and invariant combination" of morphologically and physiologically distinct sensory neuron subtypes, making each hair follicle type a distinctive mechanosensory end organ (Abraira & Ginty, 2013). Together, these units constitute a cohort of a concentric, organised sensory complex that is structured in a reiterative, but partially overlapping pattern, containing one centrally located guard hair and an assorted surrounding of awl/auchene and zigzag hairs types (Kuehn et al., 2019). Given the natural complexity of the tactile universe, a decoding machinery of equal complexity is necessary to analyse its states by defragmenting the sensory composite. Thus, "touch perception is the product of how these [sensory aspects] meld together to translate complex touch into ensembles of activities of individual LTMRs subtypes" (Abraira & Ginty, 2013, p. 626). Consequently, these hair follicle units are not functionally decoupled from each other. The hair follicle complex rather represents an extensive decoding machinery in which each end organ, "be it ending rigid set of LTMR palisades circumferencing a hair follicles or a free nerve, represent a distinct sensory unit that [...] extract and interpret several salient and distinctive features of the tactile landscape" (Abraira & Ginty, 2013, p. 626).

Nonetheless, the longitudinal lancelot ending at the hair follicle is not the sole determinant to transducing tactile events into C-LTMR-associated responses. Evidence from genetic tracing studies shows that free nerve endings of C-LTMR neurons that exclusively express mrgb4+ (stained with genetic markers), are also located in the neighbouring epidermis. Notably, mrgb4+ afferents are similarly organised in discontinuously arranged responsive patches covering about 50–60% of hairy skin, with an increasing terminal arborisation density from distal to proximal body sites and an absence in pad skin (analogous to glabrous skin in humans) or genitalia (Liu et al., 2007). Additional cutaneous

Table 2. *Neurophysiological Characteristics of C-Low Threshold Mechanoreceptors.*

		C-LTMR	Other Mechanoreceptors	
			C-HTMR	LTMR
(A) Stimulus sensitivity profile				
(I)	**Indentation force threshold**	Very low indentation force responsiveness (range = 0.3-2.5 mN; median =1.3mN; Iggo 1960; Kumazawa and Perl 1977; similar in humans see Vallbo et al., 1999; Nordin 1990). However, C-LTMRs are also sensitive to hair deflection.	High indentation force responsiveness (range = 10-80mN; median = 40mN; Iggo 1960; Kumazawa aet al., 1977; Vallbo et al., 1999)	Similar indentation force threshold (lower threshold at 0.5-1.3mN for subtypes; Ackerley, Wasling & McGlone 2016). Similar, Aβ-RA-LTMRs and Aδ-LTMRs are both sensitive to hair deflection.
(II)	**Stimulus velocity**	Low stimulus velocity responsiveness (1-10 cm/s^{-1}; mode = 3 cm/s^{-1}; Vallbo et al., 1990) that is characterized by an inverted U-shaped stimulus-response relationship with high dynamic sensitivity (Pitcher et al., 2016; Kumazawa et al., 1977; Nordin, 1990 in humans), stimulus response relationship is proportional to stimulus-response curve of the perceived hedonic quality (with 1-10 cm/s^{-1} being rated as most pleasant; Essick et al., 2010; Löken et al. 2009).[A]	Not responsive to low stimulus velocity (Vallbo et al., 1990); stimulus response relationship is linear that coincides with anociceptive affective qualities.	Broad stimulus velocity responsiveness; mean firing increases monotonically with stimulus velocity in all myelinated afferent types (Löken et al. 2009).
(III)	**Thermal**	Strongly confined intermediate thermal sensitivity (maxima = ~32°C) that follows, similar to the velocity tuning, an inverted U-shaped stimulus-response relationship that is proportional to the stimulus response curve for hedonic ratings, reaching its maximum for stimuli at skin temperature (McGlone et al., 2014). The inverted U-shaped stimulus-response relationship is reflected by an lower sensitivity of the receptor for innocuous cooling and absent sensitivity in innocuous warming, noxious cooling and noxious warming (Ackerley et al., 2014a; Kumazawa et al., 1977), suggesting that cooling is more tolerated than warming (Nordin, 1990).	Polymodal C-HTMRs signal both mechanical and thermal (heating, sometimes cooling) events, but pure mechanical C-HTMRs have no thermal sensitivity (Bessou & Perl, 1969, Ackerley, et al., 2016; Nordin 1990 in humans).	Thermal sensitivity is substantially different for Aβ-LTMRs that show no temperature modulation (McGlone et al., 2014), but similar to the functionally and spatially related Aδ-LTMRs, which tolerate cooling more than heating (Abraira et al., 2013).
(B) Physiological response profile				
(I)	**Spiking properties**	High frequency responses after sensitivity-optimal stimulation (maxima = 110 impulses/s^{-1}; Pitcher et al., 2018; Vallbo et al., 1999; Nordin 1990); in contrast baseline activity show little or no spontaneous impulses (Bessou et al., 1969).	Peak spiking is higher for C-nociceptors (maxima = 300 impulses/s^{-1}; Pitcher et al., 2018).	Peak spiking is substantially higher for Aβ-LTMRs (maximum can be up to 800 impulses/s^{-1} or more; Pitcher et al., 2018).
(II)	**Adaptation rate**	Intermediate adaptation rate (McGlone et al., 2014) that is characterized by an initial brief burst of approximately 100 impulses/s followed by a period of relatively stable spiking in the 20–65 impulse/s range that lasts approximately 5 to 10s and a cessation of impulse frequency that returns to baseline firing at 20s (Pitcher et al., 2018). After activity has decreased back to baseline firing can reoccur in non-fatigued neurons (see after discharge and neuronal fatigue).	C-fibers can be slowly adapting, while (C-)HTMRs in particular exhibit little or no adaptation (Lewin & Moshourab, 2004), but can produce a persistent generation of action potentials signaling the stimulus magnitude for several minutes or hours (Vallbo, Löken & Wessberg, 2016).	Aβ-LTMRs are either slowly adapting (SA), exhibiting maintained discharge during sustained skin deformation for minutes, albeit at a decreasing impulse rate over time. Fast adapting (FA) exhibit an initial discharge after indentation, but lacking static sensitivity (Abraira et al., 2013).
(III)	**After discharge**	A subset of C-LTMRs shows a high incident of an after-discharge in non-fatigued or perithreshold stimulated receptors: responses can increase after the initial period of adaptation, with firing continuing for 1 to 2 minutes, even in the absence of the stimulus (Kumazawa et al., 1977; Vallbo et al., 1999).[B]		Aβ-LTMRs do usually not exhibit after discharge activity (Vallbo, 2016).

(IV)	**Neuronal fatigue**	CTs (Vallbo, 2016) and C-LTMRs (Pitcher et al., 2018) possess a highly fatigable end organ; after repeated application of identical stimuli unit impulse generation is strongly decreased around a submaximal level ('end-organ failure hypothesis' formulated by Iggo and Kornhuber, 1977). The margin of neuronal fatigue can vary, depending on interstimulus interval and number of repeated exposures. Postactivation fatigue may be very long lasting and full recovery may take several (4-30) minutes; in addition, the response after the longer resting period is substantially larger in terms of impulse rate, number of impulses, as well as duration of discharge (Iggo, 1960, Vallbo et al., 2016). The minimal contact time to elicit a spike was also reduced by previous stimulation. For example, after strong stimulation was followed by an 5-10-minute interval a contact time of 100-150ms was required (Bessou et al., 1971). [C]		Aβ-LTMRs generally exhibit little changes in spike output after repeated stimulation (Vallbo et al., 2016), hence are (almost) nonfatigable. For instance, minimal contact times remain as low as 3ms (or less) to evoke robust responses (Bessou et al., 1971).
(V)	**Action potential shape[C]**	C-LTMR have a small action potential amplitude (61mV; 1ms ascending phase; 1.5ms descending phase; 2.5ms total duration; Pitcher et al., 2018) and their action potential shape is comparable to other C-fibers, however unlike C-HTMRs, they lack the characteristic 'hump' on the descending phase, which might be due to underlying differences in ion channels types, more specifically, the type 2 hyperpolarization activated cyclic nucleotide-gated channel (HCN2; Andrew & Craig, 2016). The duration of after-hyperpolarization to 80 % of baseline is 5ms.	C-HTMRs have a moderate action potential amplitude (75mV; 2.5ms ascending phase; 3.5-4.75 descending phase; 7ms total duration; Pitcher et al., 2018). The predominant phase of C-fiber impulses is always negative, and impulses are usually triphasic (Vallbo et al.,2016). The duration of after-hyperpolarization to 80 % of baseline is 15–20ms.	Aδ-LTMRs have large action potential amplitude (90mV; 5ms total duration; Pitcher et al., 2018) The initial predominant phase in A-fibers is mostly positive and impulses are usually diphasic (Vallbo et al., 2016).
(VI)	**Conduction velocity**	C-LTMRs have a relatively slow conduction velocity (0.5-2 m/s; 1m/s on average; Nordin, 1990; Vallbo et al., 1999). In addition, C-LTMRs also exhibit conduction velocity slowing (14% slowing after repeated stimulation; Gee, Lynn, & Cotsell, 1996) and unlike other C-fibers they show more abruptly slowing over the first 6s followed by a plateau (approximating the time required for adaptation).	The conduction velocity of C-HTMRs are comparable to C-LTMRs (0.5-2 m/s; 1m/s on average). The conduction velocity slowing in C-HTMRs is more pronounced than in C-LTMRs (27-29%; Ge et al.,1996) and assumed to underlie some forms of persistent pain (Pitcher et al., 2018)	The conduction velocity is substantially higher for heavy myelinated Aβ-LTMRs (20-80ms; 60ms on average) and less for the thinly myelinated Aδ-LTMRs (12–60 m/s).[D]

[A]*Some receptor types might show overlapping tuning curves for slow brushing movements (e.g. C-HTMRs; McGlone et al., 2014).*
[B]*Interestingly, after-discharge phenomena is only elicited in neurons that are sufficiently stimulated (by optimal stimuli that are tuning-congruent) but not exposed to prolonged stimulation. Does this reflect an additional filter layer of stimulus-tuning that might enable the sensory system to discern continuous but irrelevant low velocity/indentation simulation (e.g. soft tissue used in clothes) from a highly specific tactile aspect of stimulation - eventually gentle interpersonal/affective touch?*
[C]*Even more surprisingly, units have also been found that show baseline enhancement rather than fatigue (McGlone et al., 2014) highlighting an enormous physiological heterogeneity of C-LTM-receptor properties that are unique within the somatosensory domain.*
[D]*However, some differences across species exist (Pitcher et al., 2016, see also table 1 in Abraira & Ginty, 2013).*

terminals also explain observations from early studies (e.g. Iggo, 1960) that one type of C-LTM- receptors is sensitive to hair movements in cats, while other C-LTMR populations are not. Besides the evidence for two anatomically distinct end organ terminals, this notion supports further differentiation of C-LTMR subtypes: mrgb4- and th/vglut3/tafa4-exclusive types that are found to innervate the epidermis and the hair follicle cell, respectively (Abraira et al., 2017; Li et al., 2011; Liu et al., 2007). Despite the fact that the location and end organ morphology of CT afferents in human skin remain unknown, preliminary evidence is provided by the close topographical correspondence between both C-LTMR first-order afferent types in animals (Li et al., 2011; Liu et al., 2007) and a CT afferent population that was quantified in humans by Wessberg and colleagues (2003) using microneurography. Similarly, the receptive field (RF) of CTs was described as a series of discontinuously arranged small responsive hotspots that are oval or roughly round in shape, with no preferred orientation. This congruence demonstrates that C-LTM- and CT-receptors might have a similar cutaneous topographical arrangement.

Considering the peripheral organisation of different LTMRs (C-, Aδ-, Aβ SAI-, Aβ Field- and Aβ RAI-LTMRs), Kuehn and colleagues (2019) recently observed several important characteristics that demonstrate how LTM neurons innervate hair follicle across the skin: (I) Mono/

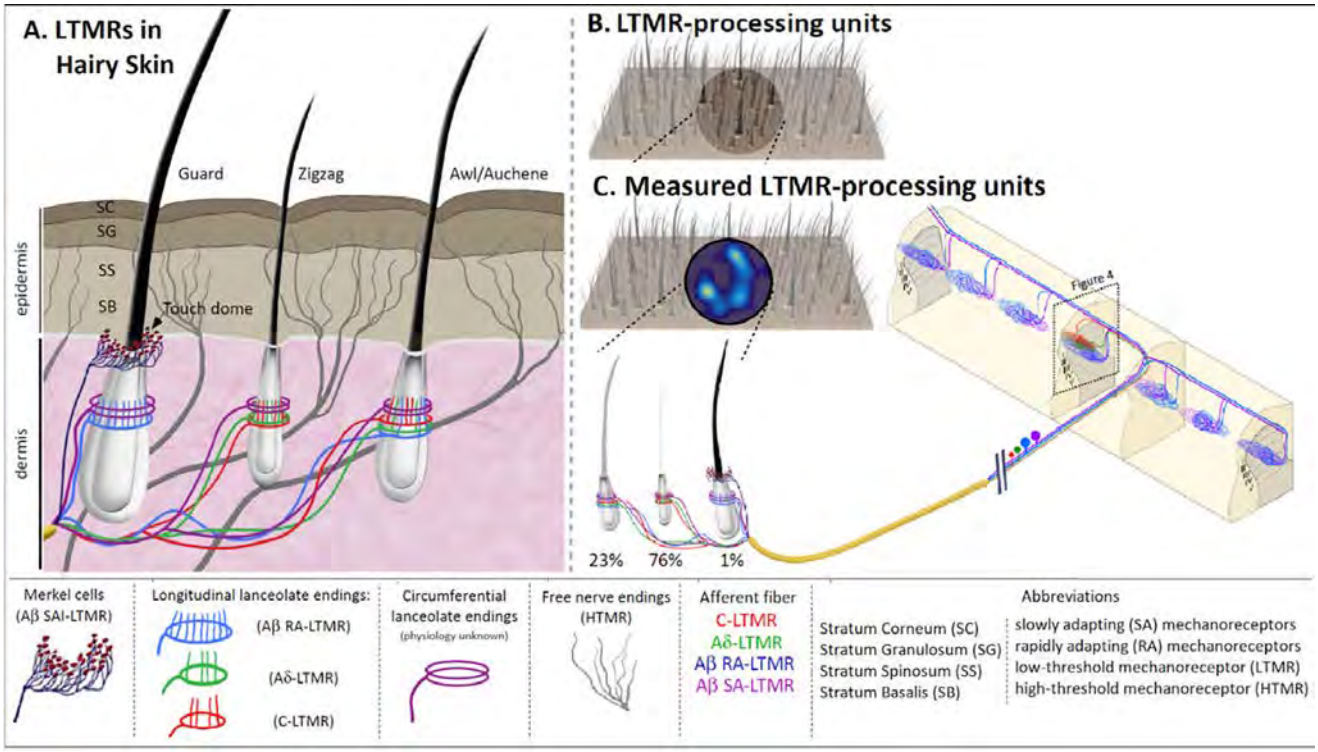

Figure 1. *A simplified schematic illustration of the mechanoreceptor topography in hairy skin and the spinal dorsal horn*
(A) In the hairy skin of the mouse, tactile stimuli are transduced through different C-LTMR (red), Aδ-LTMR (green), Aβ RA-LTMR (blue), Aβ SA-LTMR (violet), Aδ/C-HTMR (gray) and Aβ Field-LTMR (not depicted here) receptor types. Each end organ is associated with one (or more) of the three hair follicles types, defined as guard, awl/auchene, and zigzag. The longest hair, the guard hair follicle type, is dually innervated by Aβ-RA-LTMR longitudinal lanceolate endings at the base and Aβ SAI-LTMR touch domes at the apex. Awl/auchene hairs are of intermediate length and are triply innervated by C-, Aδ-, and Aβ-LTMR longitudinal lanceolate nerve endings. Zigzag hairs are the shortest and are dually innervated by C- and Aδ-LTMR longitudinal lanceolate nerve endings. In addition, circumferential lanceolate nerve endings are connected to all three hair follicle types. Besides the hair follicle associated-LTMRs, Aβ Field-LTMRs and both Aδ- and C-HTMRs free nerve endings detecting noxious touch innervate the epidermis. (B) Hair follicles across the hairy skin are organised in reiterative partially overlapping clusters, containing 1 centrally located guard hair follicle, surrounded by about ~74% interspersed zigzag and ~25% surrounding awl/auchene hair follicle types. Each mouse hair unit within a given follicle cluster receives a ''unique and invariant combination of morphologically and physiologically distinct somatosensory subtypes". Each of those units translate a specific aspect of the tactile landscape into a sensory neuron to propagate to the spinal dorsal horn and converges onto columnar LTMR-processing systems in a somatotopically organised manner. (C) The measured receptive field topography of CTs afferents was superimposed on the schematic representation of the follicle organisation. Note the considerable match between the receptive field topography of CTs-units and the aforementioned concentric follicle organisation. The electrophysiological activity map and the conceptual illustration were respectively adopted from Wessberg and colleagues (2003) and Abraira and Ginty (2013); originally from Brown, 1981a) and modified to fit the specificity of this review. Abbreviations: SA, slowly adapting; RA, rapidly adapting; LTMR, low-threshold mechanoreceptor; HTMR, high-threshold mechanoreceptor; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum; SB, stratum basalis.

poly-fiber innervation of hair follicle cells, (II) isoneuronal axon branching, and (III) innervation across body regions. (I) Unlike other relatively tilted LTMRs (excluding Aβ RA LTMRs) that exhibit exclusively mono- and dual-fiber end organ expression with respect to the hair follicles they innervate, C-LTMRs display the highest level of dual-neuron innervations across all body parts (~25%; see figure 2A and 2C). (II) Regarding isoneuronal axon branching, C-LTMRs differ substantially in terms of their multi-axon branching pattern from the other three LTMRs subtypes (Aβ SA-LTMRs exhibit no multi-branching), since 70% of C-LTMRs, compared to <15% of the other LTMRs (see figure 2B), display a multiple-axon branch innervation pattern. This might indicate early divergence (or diffusion) of sensory input, explaining the low spatial resolution of C-LTMRs found in neuropathic pain patients (McGlone et al., 2014). (III) The extent of innervation overlap for neurons of the same LTMR subtypes differs, albeit subtly, according to hair follicle type and body region (see figure 2C).

Thus, different LTMR subtypes in hairy skin (except for Aβ RA-LTMRs) are relatively tiled with respect to both skin regions and the hair follicles with which they associate. Therefore, they are more or less assigned to their own unique anatomical territory implying a tiled cutaneous RF organisation. However, when specifically considering C-LTMR's peripheral fiber configurations, both innervation and axonal branching patterns exhibit a lesser tiled ('non-overlapping') topographical organisation, also in terms of RF arrangement. Because tiling of receptors ensures maximal and non-redundant coverage of sensory space that would be otherwise metabolically costly, a highly 'redundant' isoneuronal branch innervation pattern therefore must possess a functional implication (Grueber & Sagasti, 2010). For C-LTMR, this might allow a more sensitive detection of an extremely slow brushing stimulus (Kuehn et al., 2019). Unlike divergence, prespinal convergence between somatosensory units has not been found and since LTMRs impulse propagation shows different spiking properties, velocity rates and signal propagation patterns, (C-)LTMR signals are peripherally conveyed along distinct channels ("labelled lines").

2. SPINAL CORD

2.1 Infraspinal pathways and spinal cord terminalisation sites

The spinal dorsal horn (SDH) receives axonal projections from all LTMRs, including C-LTMRs, that innervate non-facial skin (Abraira et al., 2017). The specific SDH region is therefore defined as the LTMR recipient zone (RZ; see also figure 3 for a detailed anatomical illustration of the SDH).

The axon of C-LTMR's lancelot endings, also called first-order (1°) afferents, pass through its soma and the pseudo-unipolar cells of the posterior dorsal root ganglia (DRG) to enter the spinal cord in the lateral division of the posterior DRG. After passing through the posterior DRG, the afferents collaterally branch in the gray matter at the segment of entry and into the tract of Lissauer. From the Lissauer's tract, the 1° C-LTMR afferents arborise directly into the substantia gelatinosa of Rolando (SGR) to synapse on the interneurons in lamina IIiv. This area on the dorsoventral axis determines the C-LTMR-RZ (Kuehn et al., 2019; Li et al., 2011; Sugiura

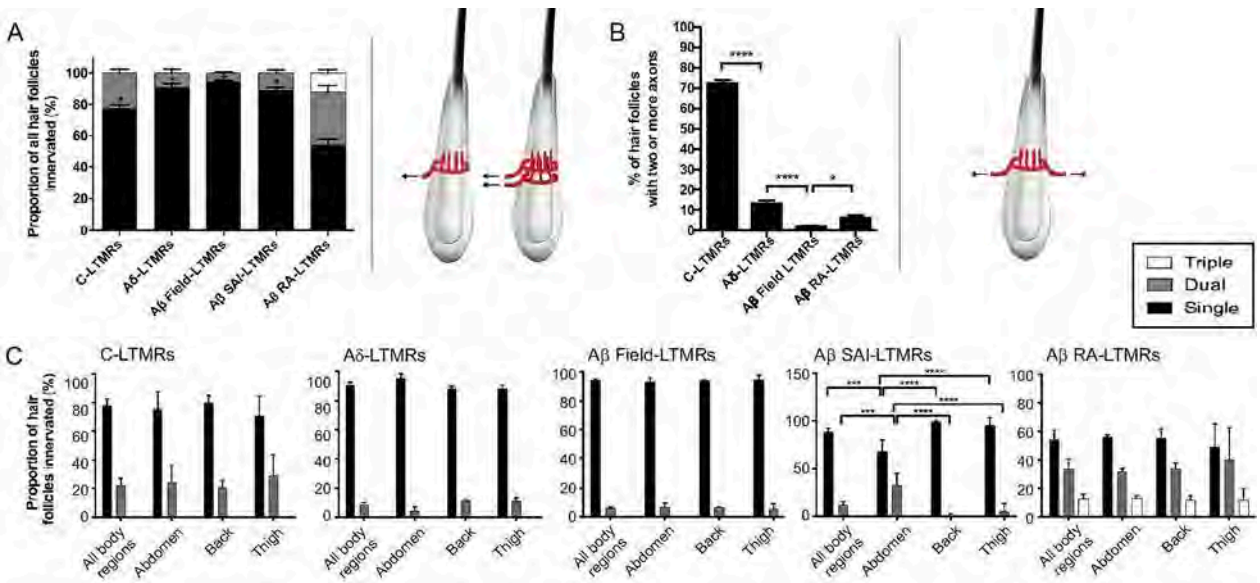

Figure 2. *Peripheral innervation pattern of hair follicle for each LTMR subtype in hairy skin and across body regions*
*The bar plot quantifies the data that was obtained, while the schematic illustration depicts the specific concept of hair follicle innervation. (A) The first figure quantifies the relative peripheral overlap of individual neurons for each specific LTMR subpopulation. Regarding the bar plot, of the total hair follicles innervated by each LTMR subtype, bars represent the relative fraction that receive single (black), dual (gray), or triple (white) innervation by that subtype. Note that, among the non-triple innervating LTMRs, C-LTMRs exhibit the highest dual-fiber innervation. (B) The second figure quantifies the magnitude of isoneuronal axonal overlap within individual LTMR receptive fields. Here, bars indicate the relative fraction of innervated hair follicles that receive innervation by poly-axonal branches of individual neurons for each specific LTMR subpopulation. (C) The third figure shows the relative peripheral overlap within specific LTMR subpopulations according to body region. Original data and figures were derived from Kuehn and colleagues (2019) and modified to fit the specificity of the article. Significance level: *P < 0.05, **P < 0.01, ***P < 0.0005, ****P < 0.0001.*

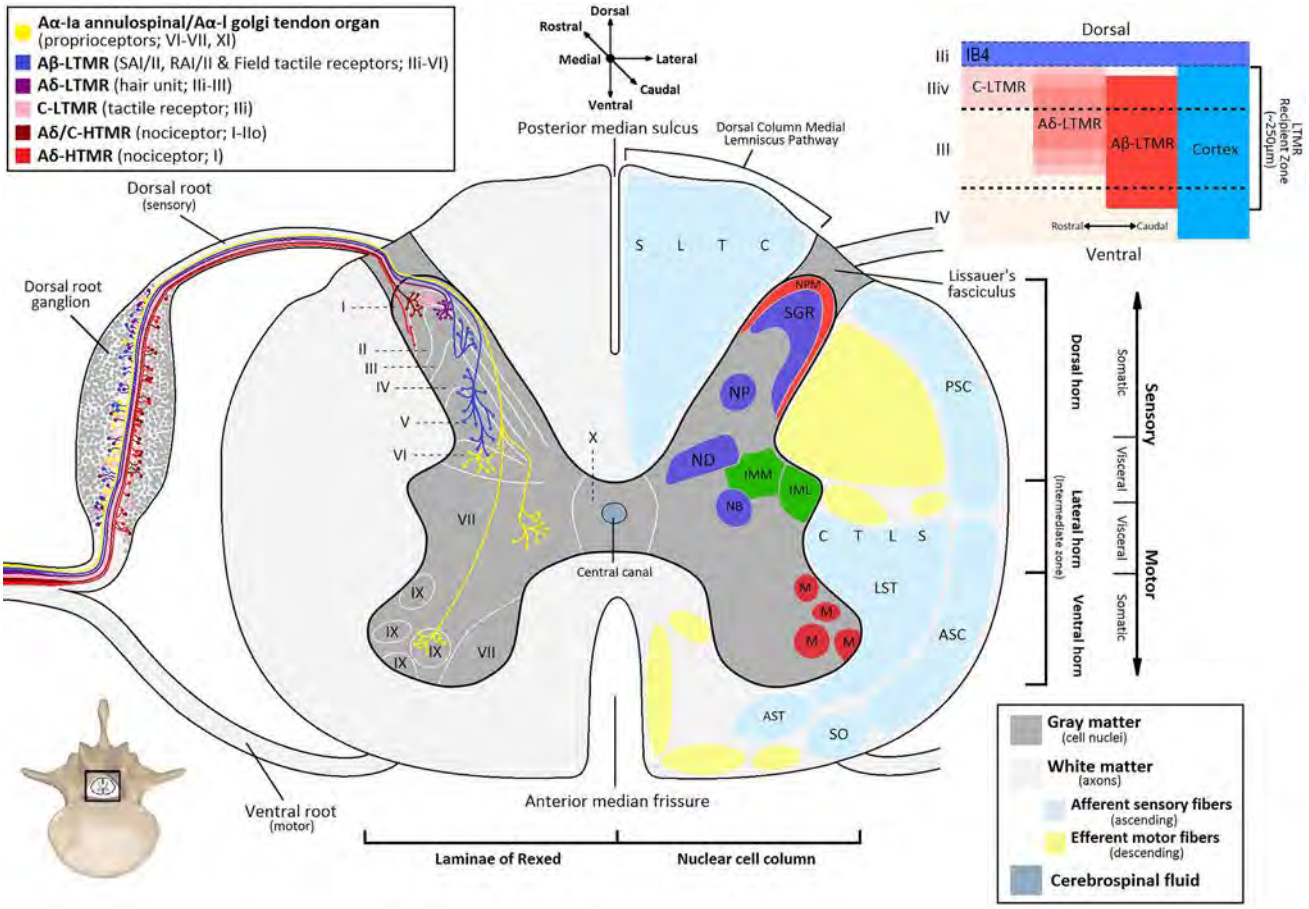

Figure 3. *Schematic illustration depicting 1° LTMR terminalisation site and recipient zones of a transversally dissected spinal cord Various 1°somatosensory afferent subtypes terminate in different Rexed laminae (I-X) that define the dorsoventral axis of the spinal gray matter. LTMR subtype terminalisation sites are spatially adjacent and limited to their specific layers along the dorsoventral axis, forming the specific LTMR recipient zone. Besides unimodal topographical organisation, 2° LTMR neurons are multimodally interconnected at their terminalisation site, (forming spinal glomeruli) and across layers (forming more complex circuits). With regards to the C-LTMR signaling pathway, information might converge with Aδ-/C-HTMR signals along a lamina II-to-I nociceptive amplification/integration pathway by exerting regulatory control on bottom-up nociceptive signaling. The spinal connectome, however, involves a more complex circuit that can involve top-down corticospinal projects to regulate and integrate bottom-up inputs. LTMR neurons within the gray matter of the spinal cord are organised according to somatotopic principles that define the concentration of C-LTMR associated neurons along the three axes (depicted at the top) and information flow. The vast majority of ascending spinal projections leave the gray matter, enter their specific ascending tract in the white matter and propagate towards supraspinal systems. For instance, C-LTMR signals propagate from laminae IIiv postsynaptic partners to 3° lamina I Wide Dynamic Range projection neurons. The majority of signals of the C-LTMR signaling pathway are likely to be transmitted through the spinothalamic tract (neo-/paleo spinothalamic pathway) or diffuse into various ascending pathways (potentially archeo spinothalmic pathway). The original illustration of gray matter was derived from Halldin (2006); the illustration of LTMR recipient zones was derived from Abraira and colleagues (2017). Abbreviations: NPM, nucleus posterior marginalis; SGR, substantia gelatinosa Rolandi; NP, nucleus proprius; ND, nucleus dorsalis; NB, nucleus basalis; IMM, intermediomedial cell column (parasympathetic); IML, intermediolateral cell column (sympathetic); M, motor neurons; S, sacral; L, lumbar; T, thoracic; C, cervical; PSC, posterior spinocerebellar tract; ASC, anterior spinocerebellar tract; LST, lateral spinothalamic tract; AST, anterior spinothalamic tract (Neo/Paleo); SO, spino-olivary tract.*

et al., 1986). However, some existing evidence suggests that C-LTMR-RZ might exceed the junction between the ventral SGR and the dorsal part of the nucleus proprius (Gatto et al., 2019). Interestingly, C-LTMRs and Aδ-LTMRs terminate in similar RZs, paralleling, or maintaining, the remarkable relationships between their end organs (see section Receptor geography of C-LTMRs: Spatial configurations). Due to the C-shaped collaterals of Aδ-LTMRs that arborise into flame-shaped endings, both sensory units overlap in lamina IIiv that can, however, extent into lamina III (Abraira et al., 2013, 2017; Gatto, et al., 2019; Light & Perl, 1979). According to the principle of 'neighbourhood maintenance', that can be observed in many sensory systems (e.g. retinal, striate, and extrastriate cells), an evolved adjacent organisation allows efficient interaction and thus suggests a close functional relationship (Wolfe et al., 2006). Besides their closely related spatial characteristics (see sections Receptor geography of C-LTMRs: Spatial configurations and Infraspinal pathways and spinal cord terminalisation sites) and similar temporal characteristics (see figure 2.1 and table 2), C-LTMRs and Aδ-LTMRs seem to contribute to the same nociceptive modulation circuits in the SDH by projecting to pkcγ-expressing interneurons (Abraira et al., 2017). This further complements the proposition that Aδ-LTMRs and C-LTMRs have a close, but yet unexplored, functional relationship.

2.2 Principles of somatotopic arrangement in the spinal cord

The sensory information conveyed by spinal cord postsynaptic partners of 1° afferents has been found to be guided by (A) information flow and (B) somatotopic organisation principles. (A) Sensory signals are generally determined to be steered towards either the opposite pole of the dorsoventral axis to terminate in two major, but functionally distinct output pathways. (I) The lamina III-to-II-to-I axis (ventral-to-dorsal) steers information, that is mostly related to nociceptive processes (specifically nociceptive; Craig, 2002), via dorsal-oriented axons of interneurons towards projection sites associated with the (anterolateral) spinothalamic pathway (STP; see figure 4). The STP consists of three different subtypes, which differ in the location of their projection neurons along the lamina I-III axis. The 'fast' conducting neospinothalamic (NST) pathway conveys information of sharp pain and crude touch, that ultimately contributes to the immediate awareness of a painful sensation and its exact location, via few contralateral synapses along the lateral spinothalamic tract. The phylogenetically older, 'slower' conducting paleospinothalamic (PST) pathway conveys dull pain, burning sensation, temperature and simple touch, via multiple bilateral synaptic connections along the anterior spinothalamic tract. The 1° afferents follow the general somatosensory pattern of ascendance (e.g. similar to the C-LTMR) until the entry into the Lissauer's tract. The 1° nociceptive spinothalamic neurons (pseudo-unipolar posterior DRG) may end in the segment of entry or one or two segments up on 2° afferents in the lamina I (nucleus posteromarginalis), for the neospinothalamic-, or on 2° afferents in the lamina II (SGR), for the paleospinothalamic afferents. The 2° NST axons immediately leave the SDH at lamina I, decussate in the anterior white commissure, at the same level of entry, and ascend in the contralateral anterior and (predominantly) lateral quadrant of the white matter tracts. The majority of 2° PST afferents, on the other hand, progress towards lamina III (nucleus proprius) to terminate on their 3° afferents, while other 2° PST neurons synapse in lamina IV-VIII. The 3° nucleus-proprius-afferents decussate in the anterior white commissure to collect bilaterally in the spinothalamic tracts of the anterior (predominantly) and lateral quadrant. The third and photogenically oldest pain tract, is the yet poorly defined archeo spinothalamic (AST) that mediates visceral, emotional and autonomic reactions to pain along a multisynaptic and diffuse tract. Initially, 2° nociceptive neurons convey sensory signals from lamina II (SRG) to laminae IV to VII. From lamina IV to VII, fibers ascend and descend in the spinal cord via the multisynaptic propriospinal pathway (Almeida et al., 2004; Dafny, 2000).

(II) The other major pathway, the dorsal column medial lemniscus pathway, steers information from lamina III to VI via vertically oriented interneurons towards the second major output pathway and is more exteroceptive dominant (e.g. discriminative touch; Abraira & Ginty, 2013; Craig, 2002; McGlone, 2014). Nevertheless, additional layers of complexity and crosstalk between the major output pathways of the spinal cord exist, as exemplified by the nociceptive modulation circuits (see section Spinal Modular Circuits: C-LTMR and HTMR System). The spinal cord interconnectivity and modulation networks imply that the C-LTMR system adheres to the first information flow pathway. In addition, there is some preliminary evidence showing that C-LTMR sensory information ascends mainly through the STP (discussed later). For instance, surgical sectioning of the STP for treatment of chronic intractable pain in humans is demonstrated to not only affect nociceptive-, pruriceptive- and thermoceptive sensations, but also to diminish feelings of pleasure, displeasure and erotic sensations, usually elicited through low-velocity/ indentation forces-stroking movements (Foerster & Breslau, 1932; Lahuerta et al., 1994). Since clinical and non-clinical observations in humans have indicated that C-LTMR system activation elicits modest-to-moderate pleasantness (McGlone et al., 2014), the fading of pleasurable tactile sensations after spinal cordectomy, suggests that the STP conveys sensory information that contribute to CT's hedonic aspects.

(B) The somatotopic organisation of 1° afferents terminating on 2° spinal cord interneurons is structured along 3 axes - (I) the dorsoventral, (II) rostrocaudal, and (III) mediolateral axis (Abraira et al., 2017). (I) The

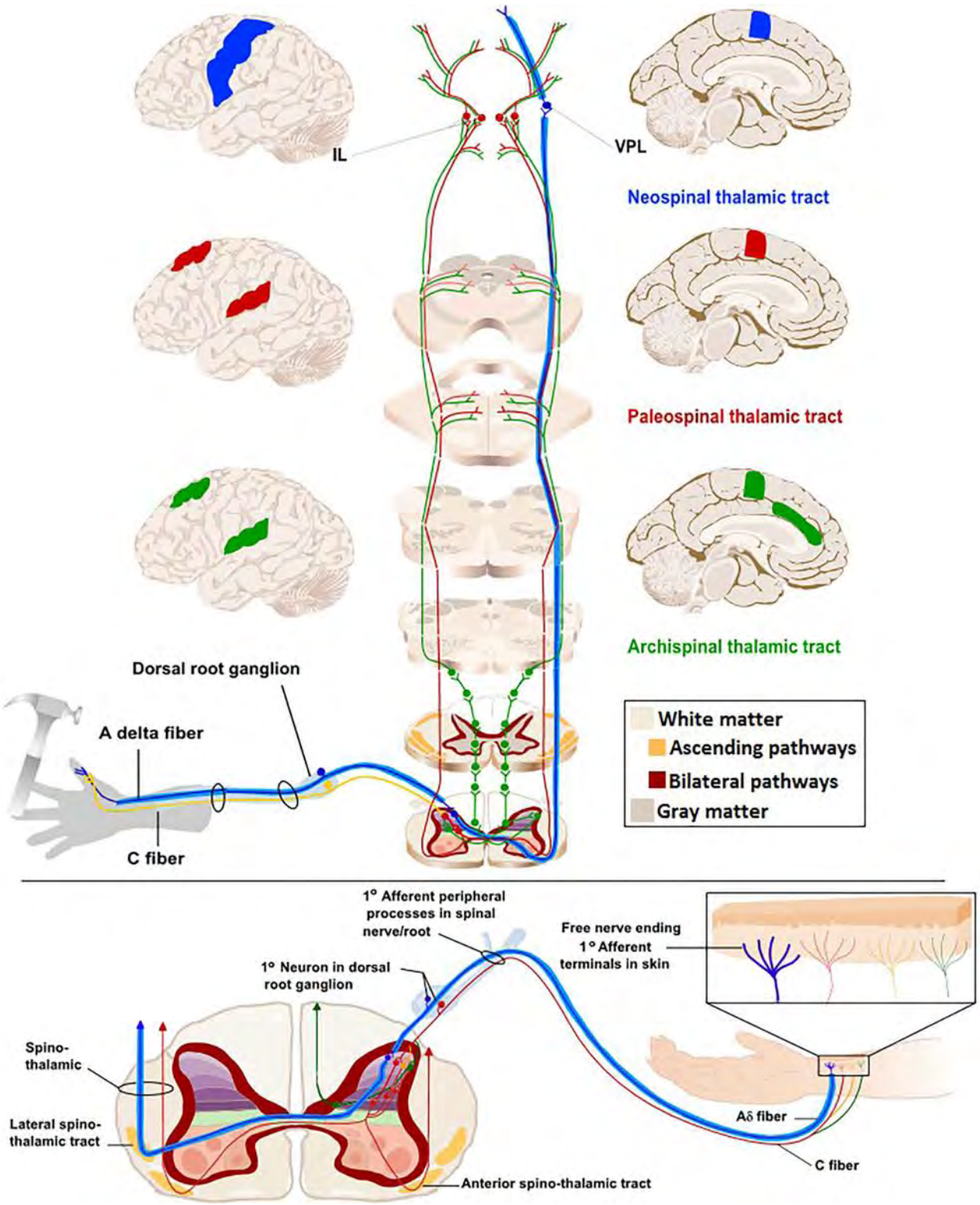

Figure 4. *Three ascending sub-spinothalamic white matter tracts conveying peripheral somatic nociceptive sensations to cortical systems First order myelinated neospinothalamic (blue), paleospinothalamic (red) archi spinothalamic (green), travel through different laminae of the dorsal horn (light violet-to-purple) and eventually decussate through the anterior white commissure to enter either the lateral or anterior spinothalamic tract (yellow) or the multisynaptic propriospinal pathway. Original image was retrieved from Dafny (2000). Abbreviations: IL intralaminar (IL) thalamic areas VPL, ventroposterolateral thalamic nucleus.*

segments along the dorsoventral plane of the spinal cord receive input from different sensory subtypes, thus relay different somatosensory information, defining different mechanoreceptor RZs (Abraira et al., 2017). Furthermore, these segments can be categorized according to their cytoarchitecture (Rexed laminae I-VI); prominent throughout this review, C-LTMR terminalisation, processing and projection is mostly, if not exclusively, limited to lamina II-I (see the dorsoventral axis in figure 3). (II) Caudal and rostral inputs are integrated by caudal or rostral spinal cord regions, that are also known as the cervical-to-sacral spinal cord compartments. Along the rostrocaudal axis, C-LTMR's and Aδ-LTMR's branching morphology differ from Aβ RA- and SA-LTMRs, since they do not bifurcate upon entering the spinal cord, but instead travel one or two segments rostrally before entering and arborising within the SDH (Abraira & Ginty, 2013; Li et al., 2011; e.g. rostrocaudal axis in figure 3). (III) Inputs from distal to proximal skin regions are integrated along the medial-lateral axis of the spinal cord (Abraira & Ginty, 2013; see the mediolateral axis in figure 3). The somatotopic organisation of C-LTMRs along the medial-lateral axis might differ substantially from other LTMRs, since C-LTMRs are not expressed in glabrous skin and, in addition, might be further distorted through somatotopic magnification (see somatosensory homunculus; Dominy, 2009; Wolfe et al., 2006).

The topographical organisation within the central nervous system strongly corresponds to the peripheral receptor organisation in hair follicle and epidermis of the hairy skin - a key feature of this mechanosensory system. Although all types of LTMR cell bodies are found to be widely and randomly distributed throughout individual DRG, and occasionally in adjacent DRG, central axonal projections of all LTMR subtypes exhibit striking spatial adjacency in the SDH, despite that some variation in adjacency and overlap exists across and within subtypes (e.g. are more pronounced in Aδ-LTMRs). C-LTMRs, whose cutaneous RFs are predominantly nonoverlapping (i.e., tiled), are the most remarkable in that they display immediately adjacent SDH termination patterns, with remarkably little or no overlap (Kuehn et al., 2019). Interestingly, an enlarged volumetric expansion along the rostrocaudal, compared to the mediolateral axis, was observed for all LTMR subtypes. This demonstrates a topographic difference between central axial arrangements and their respective peripheral cutaneous RF (Kuehn et al., 2019; see figure 5A). This topographical bias differs, albeit subtly, according to the LTMR subtype and innervated body region (see figure 5B and C). Similarly, the LTMR-associated central circuits and the dendrites of many, if not most, LTMR-RZ projection neurons and interneurons are disproportionately biased towards the rostrocaudal axis (Kuehn et al., 2019; see figure 5C). According to Kuehn and colleagues (2019), these organisational features suggest that postsynaptic partners of LTMRs integrate across multiple LTMR subtypes that are commonly found in the same skin area.

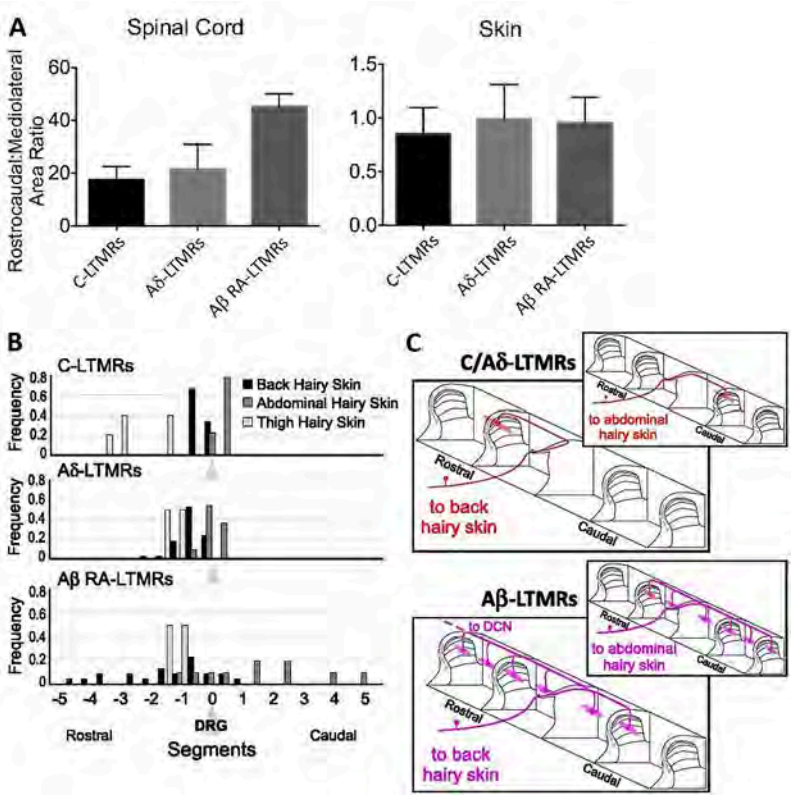

Figure 5. *Somatotopic organisation along the rostrocaudal, mediolateral, and dorsoventral axes*
(A) The first figure depicts the rostrocaudal: mediolateral ratio of area occupied by individual C-, Aδ-, and Aβ RA-LTMRs in the dorsal horn of the spinal cord (Left) versus hairy skin (Right). LTMR central projections show rostrocaudal elongation and mediolateral compression compared to their peripheral projections. (B) The central projections of LTMR subtypes display morphological differences according to the innervated body region. The magnitude of individual C-, Aδ-, and Aβ RA-LTMRs are quantified with respect to the location relative to the dorsal root ganglion, according to the innervated body region (back hairy skin, black; abdomen hairy skin, gray; thigh hairy skin, white). (C) Schematic illustration depicting C-, Aδ- and Aβ-LTMR's central projection organisation originating from back versus abdominal hairy skin.

Integration between subtypes, rather than convergence within subtypes, can also be observed for the role of C-LTMRs in nociceptive modulation circuits.

2.3 General Spinal Interconnectivity of the C-LTM System

The interneurons in the DSH do not simply reflect a linear pathway steering somatosensory information towards higher cortical areas, but can be better understood as being part of one or multiple integrational networks. Different somatosensory modalities, and even visceral aspects of the autonomic nervous system, are connected to the DSH (Andrew & Craig, 2016). Although laminar II (SGR) is not a broadly interconnected area, the SGR contains many interneurons with specific excitatory (e.g. glutaminergic) or inhibitory (e.g. GABAergic and glycinergic) connections (Abraira et al., 2017; Lu & Perl, 2003). This highlights the SGR, or more generally the SDH, as a potential integration area of the C-LTMR system.

Several spinal cord interconnectivity principles were observed by Abraira and colleagues (2017). (I) The LTMR-RZ is highly complex and contains at least 11 cell types that can be distinguished from each other by their unique combination of genetic, physiological, morphological, and neurotransmitter properties. The input to these cell types comes from various sources that involve other LTMR-RZ interneurons, such as spinocortical neurons and corticospinal neurons. (II) Each LTMR subtype forms many synapses with other LTMR-RZ interneuron classes. With regards to the connectivity of C-LTMRs, they have selective postsynaptic partners, forming the majority of their synapses to rorβ+, cdh3+, pkcγ+, pve+ cell types (for an overview, see Abraira et al., 2017). (III) Each LTMR-RZ interneuron class receives convergent inputs that originate from 1° afferents of various LTMR subtypes, descending corticospinal projections, and other LTMR-RZ interneurons. Thus, LTMR-RZ interneuron subtypes sample unique combinations of ascending LTMR inputs, that might result in an output of weighted averages between distinct input modalities. Given that LTMR subtypes differ in their tuning properties, action potential, conduction velocities, RF sizes, and adaptation properties, the outputs of LTMR-RZ interneuron subtypes have the potential to reflect many tactile ensembles (Abraira et al., 2017). With respect to the corticospinal projections, the excitatory synaptic input from corticospinal neurons is diverse and directly connected to LTMR-RZ interneurons (lamina II-III), but not to the superficial dorsal horn (see the illustration of LTMR-RZ in figure 3; Abraira et al., 2017; Gatto et al., 2018). Corticospinal projections that might engage in pain modulatory circuits seem to be relayed through axoaxonic inhibitory synapses upon LTMR terminals of cdh3+ interneurons - a selective postsynaptic partner of C-LTMR afferents. LTMR-RZ interneurons receive inputs from both 1° LTMR afferents and cortical efferents to sensitise or desensitise tactile pathways, possibly in a modality-specific and somatotopically organised manner, to differentially process tactile inputs during tactile exploration and passive touch. Regarding the submodality of affective touch, C-LTMR signals might already modify nociceptive and socioemotional processing as early as at spinal cord level. As previously described, C-LTMR/CT stimulation evokes a moderate feeling of pleasure and provides a pleasant opponent sensation that can affect nociceptive processing (Leknes & Tracey, 2008). Similarly, both top-down corticospinal efferents, as well as bottom-up infraspinal afferents that convey modulating signals to nociceptive processing circuits in the SDH are potential mediators for pleasure-related analgesia. In humans, top-down nociceptive modulatory effects are likely to be, at least partially, mediated through descending μ-opioid pathways from the periaqueductal gray or rostral ventral medulla terminating in the cortical recipient zone of the SDH (IIiv-IV; see figure 3; Mayer & Saper, 2000). In addition, C-LTMR/CT afferents are thought to be optimised to signal caress-like touch, providing a sense of support, reassurance, attachment, and social support that can alleviate pain (Dunbar, 2012; McGlone et al., 2014). Similarly, interaction with socioemotional systems might depend on μ-opioid top-down signaling pathways and oxytocin exostosis (Dunbar, 2012; McGlone et al., 2014). Furthermore, endogenous opioid signaling is disrupted in patients suffering from both depressed mood and chronic pain conditions (Willoch et al., 2004; Zubieta et al., 2003). Moreover, comorbidity between depression and chronic pain, often involving anhedonia, has been observed in several patients (Marbach & Lund, 1981). Thus, compelling neurological evidence points towards the direction of early C-LTMR related top-down and bottom-up (described later) modulation of nociceptive and socioemotional processing. Furthermore, the descending corticospinal pathway might convey other modulatory factors, for instance by recruiting memory and perceptual systems, semantic or visual contextual cues might descend to the SDH to affect bottom-up processing (McGlone et al., 2014; Olausson et al., 2010; for sensory modulation, see Abraira et al., 2017). Interestingly, anterolateral SPT neurons do not only project contralaterally to the periaqueductal gray, but also to the reticular formation, establishing a spino-cortical-spinal loop that is implicated in pain modulation (Coste et al., 2008). (IV) A sparse LTMR input allocation distributed broadly across the LTMR-RZ describes a synaptic architecture best exemplified by parallel LTMR input modules. It has been found that individual LTMR subtypes diverge to directly contact four or more postsynaptic LTMR-RZ interneuron classes. However, considering the entirety of the excitatory connectome for each LTMR-RZ interneuron type, individual LTMR subclasses represent only a minor fraction of the inputs, ranging from 0% to 30%. An implication of parallel channels is increased network interconnectivity (Abraira et al., 2017). Performing LTMR input computations in

a parallel fashion, rather than hierarchically, enables an enormous cellular and circuit-level substrate for integration, plasticity and context-specific output, potentially enabling selective gating of certain modalities under particular physiological states.

Synaptic arrangements between LTMR subtypes and their postsynaptic targets can be quite complex and can often form synaptic glomeruli. Usually, these synaptic clusters consist of glutamatergic, postsynaptic connections to the 1° primary afferents, but also synapse with neighboring (2°-) interneurons. Taken altogether, the interconnectivity of these anatomical units endows them with the capacity to modulate presynaptic primary afferent input at the very first synapse within the SDH. At SDH level, two glomeruli types have been described (Abraira & Ginty, 2013). (I) Type I glomeruli are located in lamina II, receive input from unmyelinated fibers and have axonal contacts to purely GABAergic interneurons. (II) Type II glomeruli are located in the lamina II/III boundary, receive mostly inputs from myelinated fibers and have axonal contacts to both GABAergic and glycinergic interneurons. Interestingly, 1° C-LTMR afferents often terminate in particularly complex structures with many axoaxonic synapses that resemble both type I and II glomeruli. Moreover, both C-LTMR associated glomeruli types seem to perform nociceptive modulatory computations, directly at a terminalisation site (Abraira et al. 2013, 2017; see also the next section). Indeed, C-LTMR-mediated signaling might already be locally regulated at the first synapse; both the central terminal and postsynaptic dendrites are subject to inhibition, resulting in primary afferent depolarisation (Larsson & Broman, 2019). Given the implication of C-LTMRs in homeostatic processing and the elicited state-like percept in clinical and healthy subjects, primary afferent depolarisation in this pathway might prevent hyperactivity of LTMR systems (Larsson & Broman, 2019; McGlone et al., 2014). This mechanism might be relevant for regulating and balancing C-LTMR feedforwarded signaling contributing to homeostatic states.

2.4 Spinal Modular Circuits: C-LTMR and HTMR System

Throughout the literature, several models for specific bottom-up nociceptive modulation circuits at SDH level have been described (Lu & Perl, 2003, 2005; Zheng et al., 2015). Interestingly, two essential computational units can be identified across several SDH-interneuron models, each expressing different anatomical and functional properties: (A) a nociceptive amplification pathway and (B) various regulating modules, which will be explained in the following section.

(A) The nociceptive amplification pathway represents a lamina II-to-I nociceptive integration module that is capable of amplifying and manipulating primary afferent input from DRG Aδ- and C-HTMRs, through successively integrating nociceptive input in the process of forwarding signals to lamina I projection neurons (an 'output-' pathway of the SDH; Arcourt & Lechner, 2015; see Lu & Perl, 2005, 2013). Generally, this amplification mechanism relies on excitatory, monosynaptic, glutamatergic AMPA-mediated interconnections between lamina II-IIo-I interneurons and presynaptic partners in the DRG. For instance, lamina IIo vertical cells receive input from DRG Aδ-HTMR fibers, whereas most lamina I and lamina II transient central (TC) neurons maintain connections to DRG C-HTMR fibers (see figure 6A and the blue circuit in figure 7; Lu & Perl, 2005, 2013; Zheng et al., 2015). Interestingly, low velocity/indentation stimulation evoke a C-LTMR associated physiological profile in lamina IIo vertical cells, which is presumably transmitted by lamina IIi C-LTMR interneurons expressing vglut1, as well as in lamina I projection neurons (Andrew, 2010; Larsson& Broman, 2019; Light & Perl, 1979). This physiological profile indicates that nociceptive computations contain C-LTMR features and should therefore rather be understood as an integrated signaling in the foregoing modulation process.

(B) This combined signal of HTMR and C-LTMR input seems to originate from modulation mechanisms that regulate the output magnitude of nociceptive amplification pathways through GABA and glycine-mediated inhibitory connection. (I) The GABA mediated pathway, first described by Lu and colleagues (2003), is characterised by a simple inhibitory connection between a lamina IIi transient central neuron - an early unit of the nociceptive amplification pathway - and a presynaptic lamina IIi islet cell. The transient central cell and islet cell receive monosynaptic, glutamatergic AMPA-mediated input from 2° primary DRG C-HTMR and C-LTMR afferents, respectively (see figure 6A and the yellow circuit in figure 7). (II) The more complex glycine-mediated pathway, described in a later study by Lu and colleagues (2013), involves glutamatergic (excitatory) connections of Aβ-LTMRs projecting DRG neurons to lamina II pkcγ+ and glycine-releasing neurons. The glycine neurons have an active glycinergic (inhibitory) connection to pkcγ-expressing cells, exerting a strong inhibitory control via a shared feed-forward mechanism, located at the spinal laminae II/III junction. This glycine-mediated feedforward mechanism silences the otherwise active glutamatergic synapse between the pkcγ+ and TC neuron and thus serves as a gate control mechanism (Lu et al., 2013; see Melzack & Wall, 1965; see figure 6B). Any factor that attenuates, or even abrogates, the glycine-mediated feed-forward inhibition on the pkcγ-expressing neuron, recruits various polysynaptic LTMR signals that evoke action potential outputs in the nociceptive pathway, which in turn induces an inflated pain sensation. Interestingly, the terminals of C-LTMRs expressing vglut3 show dense overlap with pkcγ+ neurons and a direct connection has recently been validated (Abraira et al., 2017; Hughes et al., 2003 in rats; Seal et al., 2009).

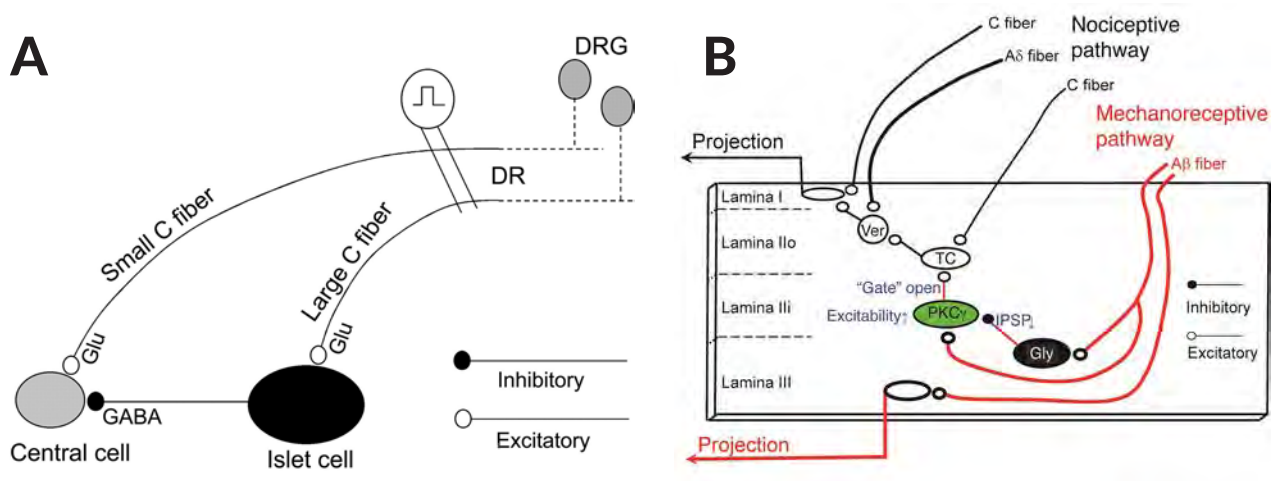

Figure 6. *(A) GABA and (B) glycine-mediated inhibitory circuits implicated in pain modulation in the spinal dorsal horn*
(A) GABA (inhibitory) mediated pain modulation circuit. The presynaptic islet cell maintains a GABA projection to the transient central neuron; both neurons receive monosynaptic glutaminergic AMPA-mediated input from different primary afferent C-fibers. The islet cell type receives projections from C-LTMR afferents (conveying innocuous touch), while the transient central cell receives input from nociceptive C-HTMRs (conveying noxious touch). Original is retrieved from Lu and colleagues (2003). (B) Glycine-mediated pain modulation circuit. The pkcγ+ cell maintains a preexisting, but normally silent excitatory connection to the TC neuron. Mechanoreceptive afferents project to both the pkcγ+ and glycine cell resulting in a strong inhibition of this connection. The glycine-mediated inhibitory connection forms a feed-forward inhibitory circuit. Original is retrieved from Lu and colleagues (2013). Abbreviations: SDH, spinal dorsal horn; DRG, dorsal root ganglion; TC, transient central cell; Ver, vertical cel; Gly, glycine; GABA, gamma-aminobutyric acid; pkcγ+, protein kinase C gamma.

Furthermore, 2° C-LTMR afferents synapse in pve+ spinal interneurons. Pve+ interneurons are a subtype of pv+ interneurons and are implicated in GABA and glycine exostosis (Abraira et al., 2017). Thus, beside GABA, glycine neurons are an essential component in bottom-up modulation networks that integrate C-LTMRs input into the HTMR signaling pathway to regulate nociceptive output for cortical projection.
Therefore, it can be proposed that C-LTMR stimulation could have some beneficial effects, such as alleviating the abnormal or chronic pain associated with pathological conditions. For instance, distortions in glia-mediated neurotrophin exocytosis (e.g. brain-derived neurotrophic factor, glia-produced proinflammatory cytokines, peripheral nerve injury, and pharmacological substances altogether characterise pathological conditions that compromise glycine-mediated inhibitory control (Coull et al., 2005; Kawasaki et al., 2008; Lu et al., 2013; Takazawa & MacDermott, 2010).
However, these simplified SDH somatosensory networks are more complex than depicted, far surpassing the scope of this review. On the one side, the nociceptive pathway is certainly represented in various anatomical and functionally different circuits receiving inputs from various somatosensory sources. In addition, different somatosensory-nociceptive modulation subunits seem to exist that converge nociceptive modulation information at different levels, or these subunits are at least interconnected to some extent (Arcourt & Lechner, 2015; Sewards & Sewards, 2002; see Zheng et al., 2015) - it is most likely that nociceptive processing is extended beyond simply amplifying nociceptive information. On the other side, C-LTMR sensory neurons seem to project to networks that serve functions other than pain modulation (Abraira et al., 2017). Only recent developments in the field target the complex interconnectivity between cell types processing tactile information and the impact of their physiological, morphological, and genetic features on network function; yet, not all cell features (morphology, physiology, neurotransmitter, genetic profile) are cohesively integrated to identify separate and distinguishable cell types. Even in lamina II, the most extensively studied region of SDH, a substantial proportion of interneurons remains unclassified (Abraira et al., 2017; Grudt & Perl, 2002; Maxwell et al., 2007).

2.5 Spinal (WDR-) projection neurons

In the previous section, several nociceptive modulatory networks were presented that converge (C-) LTMR and HTMR sensory information in the nociceptive amplification pathway steering the information towards lamina I projection neurons. Lamina I neurons contain several subpopulations, classified according to their sensory input. Roughly 70% are categorised as nociceptive specific (NS), receiving mechanical and/or

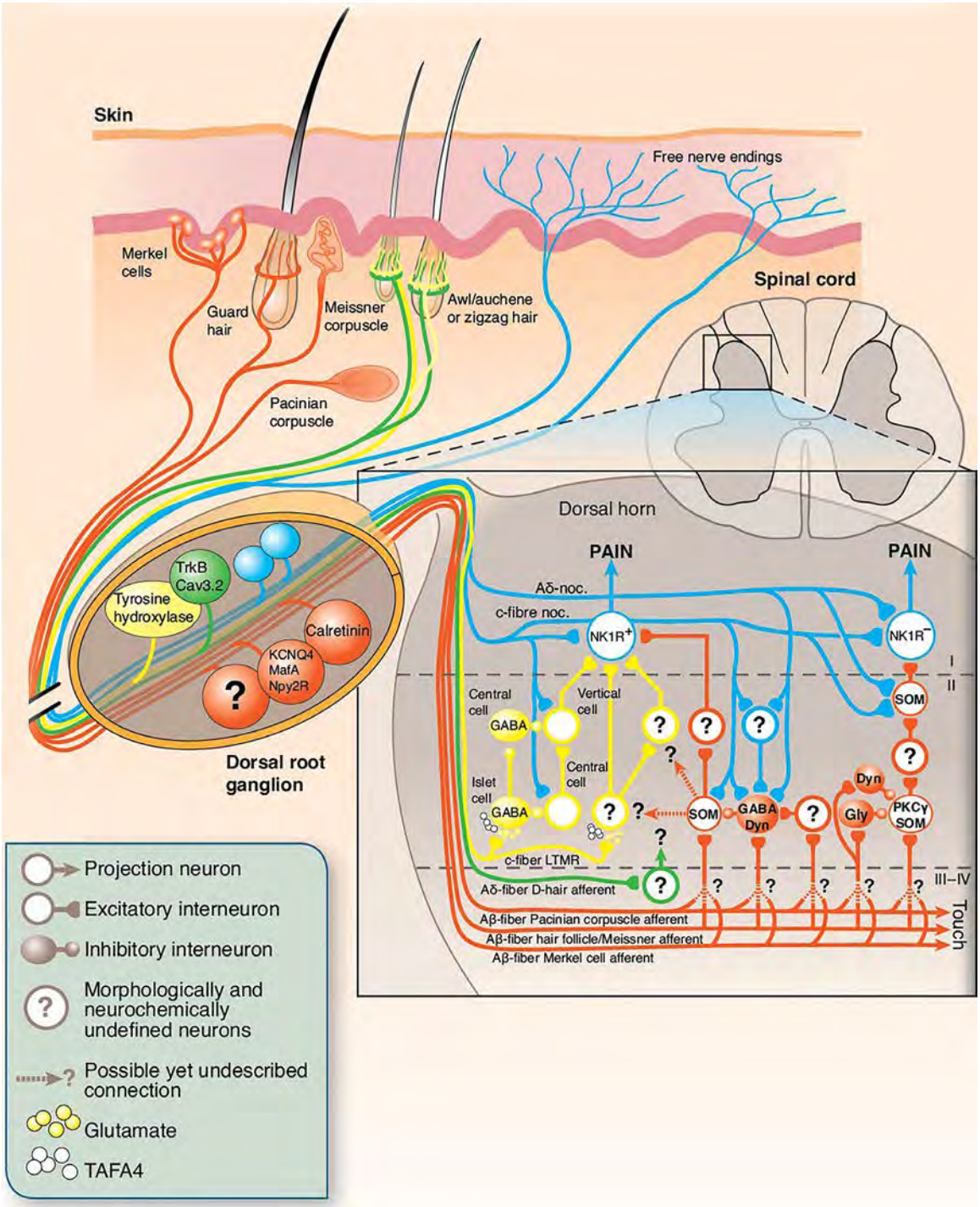

Figure 7. *Peripheral and spinal circuits involved in mechanical allodynia*
This figure depicts separate C-LTMR and Aβ-LTMR related nociceptive modulation networks in the dorsal horn of the spinal cord. Under normal conditions, touch and nociception are processed separately. However, under pathophysiological conditions, such as trauma or nerve inflammation, touch can amplify nociceptive processes, for instance through a long-term depressed glycine-mediated control mechanism in the case of C-LTMR sensory input, which eventually results in tactile allodynia. Originally published by Arcourt and Lechner (2015), which also provides a more detailed description of the circuits described in their model.

heat coding input, 15% as polymodal nociceptive (HPC), receiving noxious heat, pinch and noxious cold coding input, 10% as 'wide dynamic range' neurons (WDR), receiving LTMR and nociceptive coding input and 4% as cooling thermoreceptive (COOL), receiving innocuous cooling coding input (Andrew, 2010). Interestingly, lamina I and lamina II WDR neurons both co-express a physiological property of the peripheral C-LTMR system - a peak discharge at slow brush velocities (1–10 cm/s), with firing declining exponentially after linear increasing brushing velocity (U-shaped) and neuronal fatigue (Andrew, 2010; Sewards & Sewards, 2002). These WDR neurons therefore must represent the neuronal units that receive the integrated signal of HTMRs and C-LTMRs transmitted along the nociceptive amplification pathway of the modulatory circuits, first described by Lu and colleagues (2013).

Interestingly, besides lamina I/II WDR neurons, other WDR populations are identified in lamina III spinoparabrachial neurons of the nucleus proprius (following the PST) and lamina V neurons at the neck of the dorsal horn (following the AST; Andrew, 2010; Sewards & Sewards, 2002). However, both lamina III and V WDR neurons demonstrate a linear monotonic increasing velocity-response relationship (Andrew & Craig, 2016; Sewards & Sewards, 2002). This indicates an interconnection of 2° primary Aβ-LTMR, rather than C-LTMR afferents, that might originate from their terminalisation sites in lamina III-V of the SDH. Lamina III and V interneurons might be part of different nociceptive modulation networks (figure 7; Arcout & Lechner, 2015). This difference in physiological profile of different WDR neurons suggests that C-LTMR sensory input is exclusively relayed through lamina I projection neurons. Generally speaking, all lamina I WDR neurons receive convergent inputs from heat/mechanical nociceptors, as well as innocuous mechanoreceptors, but no lamina I cells are identified that received inputs exclusively from C-LTMRs (Andrew, 2010). Similar results are obtained from lamina I trigeminothalamic and spinothalamic neurons in primates and carnivores (Andrew & Craig, 2016).

Concluding, C-LTMR sensory information is exclusively relayed along the lamina II-to-I axis, while converging with HTMR sensory input, to ultimately terminalise in WDR lamina I projection neurons that react to both HTMR and C-LTMR input. This suggests a weighted integration of both ascending channels. But what might a weighted integration of nociceptive and presumably affective information represent? Andrew and Craig (2016) extend the evidence of converging signals to a functional idea: the affective value processed by the C-LTMR system lies within a homeostatic spectrum depending on the weighting of NS and WDR channels. The homeostatic response can shift from positive when only lamina I WDR neurons are activated, to negative when both WDR and NS/HPC neurons are activated (see Sewards & Sewards, 2002). The signal would therefore depend on the ratio between WDR and NS/HPS activation. In order to functionally decouple C-LTMR sensory information from HTMR input, Andrew and Craig (2016) argue that there must be a neuronal comparator that receives the output of both lamina I WDR neurons, as well as NS neurons. Although evidence for a neuronal comparator in humans is minimal, it has been suggested that this comparator in rodents could be located within the brainstem or posterior triangular nucleus of the thalamus (Gauriau & Bernard, 2004). However, interspecies differences in C-LTMR subtypes and magnitude of lamina I spinoparabrachial and spinothalamic projection neurons exist, potentially implying differences in processing these weighted signals; spinoparabrachial pathway and sensory type variety is more pronounced in sub-primates (Andrew & Craig, 2016).

3. SUPRASPINAL PATHWAYS

So far, the neuronal code of C-LTMRs processed along the lamina I/II axis might be integrated by multiple and functionally distinct networks connecting the 2° afferents to lamina I projection neurons of nucleus posteromarginalis. Further ascendance towards higher areas is likely to be conveyed by 2 major 3/4° pathways - (A) the spinoparabrachial and (B) the spinothalamic pathway.

(A) The ascendance from lamina I SDH neurons via the spinoparabrachial pathway, was revealed by Andrew (2010; see sections Spinal Modular Circuits: C-LTMR and HTMR System and Spinal (WDR-) projection neurons) by utilising antidromically stimulation of the contralateral parabrachial nucleus in rats (in vivo). This demonstrates that WDR lamina I neurons project to the parabrachial nucleus. (B) The spinothalamic pathway involves C-LTMR signal conveyed via thalamic nuclei (see figure 4): (I) the posterior triangular and/or (II) the posterior part of the ventromedial thalamic nucleus (VMPO; Andrew & Craig, 2016 in (sub-) primates; Gauriau & Bernard, 2004, in subprimates). (I) Interestingly, about half of the somatosensory neurons in the posterior triangular thalamic nucleus can be excited by innocuous brushing stimuli, indicating that the input might be derived directly from WDR lamina I neurons. However, collaterals from lamina I spinoparabrachial neurons also project to the posterior triangular nucleus (Al-Khater & Todd, 2009). (II) Concerning the VMPO, fMRI experiments in two patients suffering from a rare neuropathic disease (lacking Aβ-fibers) have shown that selective CTs stimulation activates the posterior contralateral insular cortex (Olausson et al. 2002). The insula, specifically the fundus of the superior limiting sulcus, receives mostly input from afferents of the VMPO, which in turn receives exclusively input from lamina I NS and WDR projection neurons (Andrew, 2010; Craig, 2002).

Both pathways reflect a transmission of C-LTMR-related sensory information through the anterolateral spinothalamic pathway. However, as it has become evident throughout this review, contradictory evidence prevents a definite allocation of the C-LTMR system to one of the three subtypes. On one side, C-LTMR projection neurons are yet exclusively located in lamina I, a characteristic of the NST. On the other side, C-LTMRs terminalise in lamina II and multiple research lines indicate that C-LTMR is physiologically, anatomically, and functionally more closely related to emotional, visceral and homeostatic qualities, involving processing in the neuromodulatory brainstem nuclei. These considerations suggest projection via the PST. Presumably, due to its integrative nature, C-LTMR signals diffuse in multiple systems and therefore might diverge into both, or eventually three, subtypes of the ST-pathway.

3.1 Labelled-line Theory?

Historically, sensory modalities have been modelled as physiologically and anatomically discrete channels ('labelled lines') that convey particular modalities of cutaneous sensory information from the peripheral receptors to the somatosensory cortex. Therefore, sensory information propagates through a 'direct pathway' in a separated manner - even along the highest ascending afferent fibers. According to this view, most if not all LTMR integration and processing begins in the somatosensory cortex (Mountcastle, 1957).

As previously mentioned, there is no doubt that tactile events are initially fragmented in distinct sensory aspects to ascend along anatomically and physiologically discrete 1° channels ('labelled lines'; Pitcher et al., 2016; Vallbo et al., 1999). However, initially separated sensory aspects must be reassembled to reinstate the various aspects of the complex sensory event that form the holistic character of the particular perceptual quality. And both anatomical and physiological measurements indicate that sensory integration begins at subcortical levels. Aforementioned evidence of early sensory integration urges a more integrative view, as opposed to a strictly 'direct pathway' to the somatosensory cortex to process and integrate sensory information (Mountcastle, 1957). Indeed, often a combination of many distinct features is needed to give rise to tactile percepts, as exemplified by the tactile perception of liquid (Bentley, 1900). Wetness perception depends upon the sensory input that is derived from activation of both temperature and pressure receptors, but does not require the skin to be wet, punctuating the relevance of somatosensory integration in order to account for the richness in tactile percepts. Similarly, evidence exists depicting the hair follicle complex as a complex decoding machinery that operates as an integrated functional unit (Abraira et al., 2017) and, as shown throughout this review, early diffusion of C-LTMR signals into various functional networks is the rule rather than the exception, reflecting the integrational nature of this system. This anatomical interconnectivity has been demonstrated to be extended to functional implications, since stimulation of CT afferents in neuropathic patients does not evoke clear qualia but rather elicits a more diffuse state-like sensation that is consistent with the low spatial and temporal resolution often observed in this submodality (Abraira, 2017; Kuehn et al., 2019; McGlone et al., 2014; Pitcher et al., 2016; Vallbo et al., 1999). More specifically, the distinct physiological parameters, peripheral and central somatotopic arrangements, SDH interconnectivity, and WDR projection neurons suggest a more elaborated, early somatosensory processing and integration, and therefore contradict the cortical integration dogma and exclusive supraspinal 'labelled lines', as postulated by the 'labelled-line' theory (Mountcastle, 1957).

Analogous to the early visual system, different sensory aspects are extracted by differently configured sensory neurons (e.g. possessing large RF sizes, dynamic adaptation and high sensitivity), are then later recombined to contribute to a single perceptual quality (e.g. motion perception). Similar to the retina, the key locus of early visual preprocessing, the SDH might be identified as the first area that is dedicated to representation, integration, and processing of somatosensory ensembles, at least for hairy (C-)LTMRs. Altogether this sensory network, originating from the hair follicle complex in hairy skin, highlights a level of complexity, organisation and sensitivity in hairy skin, previously thought to only exist in glabrous skin and other sensory domains (Abraira & Ginty, 2013).

CONCLUSION

With respect to the recent developments in the field, a new model for the early functional anatomy of the C-LTMR system is proposed. Both fiber innervation patterns and the physiological profile of C-LTMRs point towards a highly sensitive and outermost selective sensory unit, tuned to low-velocity/indentation forces, such as stroking movements at skin temperature. This unit is part of a cohort of concentrically organised sensory units that are arranged in a reiterative but partially overlapping pattern, exclusively across hairy skin. Specifically, longitudinal lanceolate endings are identified as sensory end organs of C-LTMRs that innervate zigzag and awl/auchene hair follicle types of the sensory complex in a morphological interdigitated manner, demonstrating a close relationship with Aδ-LTMs nerve endings. This is even reflected in the somatotopic organisation of the SDH. The latter suggests a potentially functional, but yet unexplored relationship between both sensory units. Similar to many other LTMRs, C-LTMRs project their unique sensory code to the RZ in the SDH in a remarkably consistent peripheral-

to-central somatotopic manner, adhering to rostrocaudal, mediolateral and dorsoventral organisational principles, highlighting a level of complexity similar to the anatomical organisation found in glabrous skin and in other sensory domains (e.g. the retina).

Although each neuronal code is initially conveyed by distinct ascending channels and limited to distinct somatosensory subsystems, it is very likely that a vast majority of sensory aspects meld together in functionally distinct sensory networks, contrasting the idea that the somatosensory cortex is the first locus of somatosensory integration, as it was postulated by the 'labelled-line' hypothesis (Mountcastle, 1957). The spinal cord interconnectivity does not simply reflect a linear path but resembles an intricate network structure that consists of several simple and more complex glomeruli networks, top-down corticospinal networks, and bottom-up spinocortical networks. While sensory input of C-LTMRs flows along the limina II/I axis towards the output locations of the spinothalamic projection neurons, sensory information of the C-LTMR system is integrated in nociceptive modulation pathways. More specifically, the integration with nociceptive information, might, at least partially, depend on the C-LTMR dependent GABA- and glycine-mediated regulation of the nociceptive amplification pathways. The research on lamina I projection neurons suggests two possible ascending pathways terminating either in the VMPO or the parabrachial nucleus. The responsiveness of WDR spinoparabrachial projection neurons to both nociceptive and C-LTMR-related inputs implies a weighted output between both channels. Together with NS neurons, shifts in activation of those neurons might have an implication for further processing of homeostatic information in higher cortical areas. The last highlight of this review punctuates another aspect of the integrative nature of the C-LTMR system. Since C-LTMR afferents diverge into different functional systems that process nociceptive, homeostatic and other somatosensory information, C-LTMR information flow does not adhere to spinothalamic sub-categorisation.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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'Can't touch this: studying human touch interaction in times of social distancing regulations'

Dr. Anouk Keizer

Anouk Keizer is an Assistant Professor at the department of Experimental Psychology at Utrecht University. She is a member of the Helmholtz Institute, the Utrecht Research Group for Eating Disorders, and supervisor of the Journal of Neuroscience and Cognition. Her field of interest focuses on affective touch perception and the role that touch may play in mental health. Her work focuses also on novel and innovative paradigms and technology to study body image disturbances in both healthy individuals and psychiatric patients - with a strong emphasis on translating science to clinical practice.

Human touch interactions are fascinating to watch. We probably all felt a surge of awkwardness when president Trump just would not let go of president Macron's hand. I find such interactions not only fascinating to watch on YouTube, but interesting enough to study scientifically. For the past few years, one of my research lines focused on the role of interpersonal touch in the development and maintenance of psychiatric disorders, such as personality disorders, dissociative disorders, and trauma-related disorders. In my research I typically stroke the hand or the arm of a participant with a soft brush and ask them to rate how they experienced the touch in terms of pleasantness. When the COVID-19 pandemic hit, it was soon clear that my research would come to an abrupt halt. After all, being in close contact with participants was no longer allowed, let alone actually touching them. So, I had to come up with a COVID-proof alternative. Before I will explain what that alternative was, I first want to provide you with a little bit of background by discussing some fascinating previous work that provides a glimpse into the basic functions of human social touch interactions.

The human skin houses the so-called C-tactile afferents (CT afferents). These are unmyelinated afferents that respond specifically to slow-paced, caressing-like, soft touch at a speed of 3 – 10 cm/sec. These CT afferents are directly connected to the insula through interoceptive pathways that signal feeling rather than sensing states (see e.g. McGlone et al., 2014). The insula is mainly important for processing information related to self-consciousness, interoception, empathy, and emotions. As such, human-to-human touch, at a CT-optimal speed, bypasses the somatosensory cortex (in fact, deactivation of the somatosensory cortex has been reported (Olausson et al., 2002)), and is directly labelled with an emotional valence by the brain. Many studies have shown that healthy participants generally perceive

CT-optimal touch as more pleasant than CT-non-optimal touch (Morrison et al., 2010). Having a route from the skin to the brain, that primarily conveys social touch information, may help us understand why we might sometimes be at loss for words, while still able to comfort someone simply by holding them. Interpersonal touch is a strong tool for communicating emotions.

In the field of neuroscience, the CT afferent system has been studied for a few decades now. More and more studies show that CT-optimal and social touch actually have beneficial effects on our mental and physical health (e.g. Morrison et al., 2010).

For instance, research has shown that CT-optimal touch can decrease feelings of social exclusion (Von Mohr et al., 2017). In a study on this topic, participants played

We probably all felt a surge of awkwardness when president Trump just would not let go of president Macron's hand

the "Cyberball game" which has been extensively used to study the effects of social exclusion on pain and well-being. In this task, participants are asked to toss a ball with two other (computer-generated) players. They initially receive the ball two times, after which they are excluded from the game for the remaining tosses. Playing this game has been found to result in negative affective, cognitive, and physiological responses in healthy individuals. Remarkably, when the researchers touched

their participants at a CT-optimal speed after the game ended, they experienced a reduction in feelings of social

exclusion compared to participants who were touched at a CT-non-optimal speed. This indicates that the CT system might be important for the sense of belonging and for promoting social closeness.

Another investigation showed that parental touch reduced social vigilance in children (Brummelman et al., 2019). In the experiment conducted in this study, children were asked to complete a computer task that assessed attention to social threat. Before the children started the task, their parents told them that they themselves would be working on a different computer. Half of the children were briefly touched on their shoulder (similar to a pat on the back) by their parents before they turned around and walked away. The results showed that children who were touched, compared to those who were not touched, showed a diminished attentional bias towards socially threatening stimuli. The latter indicates that even a very brief touch interaction can signal that the environment is safe for exploration, which reduces social vigilance. Interestingly, this effect was only found for children aged between 8 and 10 years, not for children aged 11 to 14. It can be argued that the relation of the latter group with their parents was shifting, due to the transition into adolescence. This nicely illustrates that the context in which touch occurs is crucial, as well as the relationship with the toucher.

There has also been some research on the effects of social touch on pain perception. In one of these studies, tonic heat stimuli were applied to female participants (Goldstein et al., 2016). They underwent the painful stimulus either by themselves, while the experimenter was touching their hand, or while their partner was touching their hand. The results revealed that subjects reported the lowest levels of pain when their partner was touching them, indicating an analgesic effect of social touch by a loved one. This could explain why children go running after their parents after hurting themselves; it is likely that a hug from your parents actually reduces the pain you experienced.

The summarised message of this brief overview on social touch literature is that social touch works as a buffer against mental and physical discomfort, as well as stress. So… What does that mean for our socially (physically!) distant post-COVID society? Do we all miss out on the beneficial effects of social touch, or could we even experience negative effects due to touch deprivation?

At this point we do not know a lot about the impact of touch deprivation on humans, simply because it is unethical to set up an experiment in which half of the participants are instructed to refrain from touch interactions for a prolonged period of time. In that respect, the current pandemic provides a very unique situation for science. It is now possible to study on a community level the potential effects of touch deprivation. This is why I have set up an online study with a few colleagues right after the lockdown started in the Netherlands. We aim to investigate if people report touch deprivation and which people suffer the most from this. The usual suspects could be the elderly in nursing homes or young singles living by themselves. But perhaps it is even possible to be touch deprived when you live together with your partner and possibly a few children. And how do feelings of touch deprivation relate to quality of life? Besides assessing levels of touch deprivation, we are also interested in how individuals perceive CT-optimal and CT-non-optimal touch interactions. Obviously, we cannot touch our participants, so we ask them to watch short video clips of two individuals touching each other. Do we find it more pleasant to watch movie clips in which we see CT-optimal touch stimulation, or could it be that touch is becoming a taboo for us, something we associate with the possibility of getting infected with a virus? Notably, a limitation of this research is that we do not have data on subjects' levels of touch deprivation before the pandemic started. This is why we have been recruiting participants for a few months, to take into account the effect of time.

As the study is still running at the time of writing, I cannot draw any conclusions from the data yet. At this point the number of infected individuals outnumbers 4000 already, which for me is an indicator that this topic is something that is on people's minds right now. A quick glimpse into the dataset does show that on a scale from 0 ('currently I would prefer to be touched less') to 10 ('currently I would prefer to be touched more') participants score on average a 7.82 (SD = 2.33). When asked to indicate if they would prefer to touch others less or more, they score 7.55 (SD = 2.50). So, it seems that in my community sample people do miss being touched and touching others. What the potential negative influence of a strong longing for touch is remains yet to be seen.

My advice for anyone who feels touch deprived would be to stay safe, abide by the rules, and read the paper by Jakubiaki and Feeney (2016). Real social touch interactions have beneficial effects, but interestingly, these authors suggest that even imagining previous social touch experiences with a loved one can actually be the key to reducing our stress levels.

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Mindblowing Interviews

For this edition, we interviewed six local researchers about their mindblowing field and novel research. We asked them three questions:

1) *'What topic/field do you work in?'*

2) *'What do you find most fascinating in your field?'*

3) *'How would you envision the future of this field and its development in the next five years?'*

We tried to include as many different fields as possible to make sure we will cover a good variety of mindblowing topics. Below you find the six short interviews about circular RNA's, CRISPR/Cas9, 22q11.2 deletion syndrome, non-invasive brain stimulation, prediction models, 7T MRI and genetic imaging. Enjoy!

'The hidden role of circular RNA'

Mateja Rybiczka-Tesulov, PhD

My name is Mateja Rybiczka-Tesulov, I am a PhD candidate of the Pasterkamp lab and I am interested in unravelling the function of circular (circ) RNAs during prenatal brain development. CircRNAs can be found throughout our bodies but also in other animals, plants and microbes. However, only a few circRNAs have a known function, leaving a lot to yet be characterised. The brain is the organ with the highest and most diverse circRNA expression, which together with the observation that circRNA levels differ between developmental time-points, builds the foundation of my research. We investigate the function of these novel molecules in developing dopaminergic neurons, the cells that are implicated in Parkinson's disease.

The most fascinating aspect of circRNAs is probably their discovery. CircRNAs derive from many host genes and partly share the sequence of their corresponding messenger RNA. This similarity has led to their long-lasting hidden existence and belated discovery. With the increasing use of RNA sequencing over time, researchers became curious to know whether some ever-returning scrambled mismatches, later named "back-splice junctions", were not artefacts after all. It was not until less than a decade ago that computational tools were adapted, and allowed for the detection of circRNAs. Then a new research field was born. This back-splice junction is the result of the circularisation process itself and is now used as the unique identifier of circRNAs. It shows how trouble-shooting can sometimes lead to great discoveries with a big impact.

For the future, I think that circRNAs will be integrated in many different fields of research, including single-cell RNA sequencing and host-pathogen interactions. Another important aspect is the potential use of circRNAs as biomarkers or drug targets, which could aid early diagnosis or support treatment of various diseases, respectively. From a functional perspective, we are only scratching the tip of the ice-berg as we are beginning to understand the degree of involvement of these special molecules in our biochemical processes.

'Studying the remarkable molecular architecture of synapses'

Dr. Arthur de Jong

My name is Arthur de Jong and I work at the section of Cell Biology, Neurobiology and Biophysics in the Biology department at Utrecht University. I study the molecular architecture of synapses, and how this organisation coordinates rapid communication between neurons. The precise localisation of synaptic proteins is essential for efficient synaptic transmission, and mislocalisation of only tens of nanometers can severely impact communication between neurons. Using our recently developed CRISPR/Cas9 techniques, we can now label endogenous proteins with fluorophores, such as GFP or mEos, and visualise them at nanometer precision with superresolution microscopy. In particular, I use these tools to investigate how ion channels are targeted to specific locations within the synapse, and to test how manipulation of ion channel localisation affects synaptic function.

The assembly of the synaptic protein machinery is remarkably precise, but by no means static. Neurons adapt synapse structure and composition based on neuronal activity; and this synaptic plasticity may underlie memory formation. This link between molecular composition and brain function motivates me to push the boundaries of microscopy techniques, and of the molecular tools required to visualise synapse architecture in living cells. The molecular and spatial accuracy we now achieve with CRISPR-based tools were unimaginable just 5 years ago. I continue to be fascinated by the mechanisms used by neurons for synapse assembly and maintenance, as a building block for learning and memory.

The challenge in this field is to study synaptic architecture in vivo. How does synapse composition differ between cell types and brain areas? Do changes in composition contribute to animal behavior? In principle, we have the genetic, behavioral and microscopy tools to answer these questions, but combining them in a meaningful experiment remains hugely complex. In that respect, future advances in biology will be largely driven by the ability to refine these tools. In parallel, defects in synaptic architecture contribute to psychiatric disorders, and CRISPR has the potential to treat this. Fundamental and clinical research go hand-in-hand here, to the benefit of both.

'Interdisciplinary research into language development'

Emma Everaert, MSc

My name is Emma Everaert and I started my PhD project in January 2019. The project is a collaboration between the Utrecht Institute of Linguistics and the UMC Utrecht, and it combines the fields of psycholinguistics and cognitive/developmental (neuro)psychology. Our team consists of a developmental psychologist (PhD), cognitive neuroscientist (PhD), linguist (post-doc), psycholinguist (PI), speech and language therapist, speech and language pathologist, paediatrician, and a psychiatrist. The project (3T Onderzoek) focuses on both the linguistic and cognitive development of children with the 22q11.2 Deletion Syndrome and children with a Developmental Language Disorder. We compare their performance on various behavioural tasks to that of a control group of typically developing children while considering other factors (socio-economic status, medical problems, etc.). By doing so, and by relating these measures of language and cognition to one another we aim to better understand the factors implicated in both typical and atypical language development.

As a researcher studying cognitive development it frequently amazes me how the human brain has the capacity to rapidly learn and acquire very complex

skills. How do you study that? Development, especially of language, is still predominantly studied through behavioural tasks with children and research with children can be challenging. They might not adhere to your neatly-thought out study protocol, so sometimes you have to get creative. What I like about this practical work is that it helps me better understand my data and may even provide me with observations that can lead to new hypotheses. Moreover, the interdisciplinary nature of the project enables me to constantly evaluate and consider all the different factors that might be at play. A single observation can be explained by many factors or theories depending on the research field.

In the coming years, I hope to see more and more interdisciplinary projects. I think people tend to stay in their own (niche) field, which is a shame because I firmly believe that combining insights and techniques from different disciplines will strengthen scientific findings. By combining behavioural data, bio-data, and imaging techniques I hope we will slowly but surely learn more about the complex pathway from genes to human development and behaviour.

'Current directions in non-invasive brain stimulation'

Dr. Dennis Schutter

Research performed in the BrainStim lab at the department of Experimental Psychology is concerned with the neurobiological and functional basis of cognitive and affective information processing. Transcranial magnetic (TMS) and direct/alternating current stimulation takes a central role in my studies and allows for studying the relation between brain and function in a direct manner by perturbing neural activity in vivo. The effects of non-invasive brain stimulation methods are read out by electroencephalography, hormone levels, psychophysiology, and performance on cognitive and affective-behavioral test batteries.

TMS studies have, for example, not only established the frontal lateralisation theory of motivational direction but have also provided a mechanistic account for the therapeutic efficacy of TMS in the treatment of major depressive disorder. The fact that meta-analyses have demonstrated comparable therapeutic efficacy of TMS and pharmacotherapy has contributed to the current implementation of TMS in the Dutch care system. Further to modulating neural tissue, TMS can also be used to examine callosal signal transfer between the left and right frontal cortex. In addition to finding predictive patterns of callosal signal transfer related to anger and aggression in healthy volunteers, our group was the first to show deficient interhemispheric signal transfer from the right-to-left frontal cortex in violent offenders with psychopathic tendencies.

Alongside cortical dysfunctions associated with motivational control and emotion regulation, evidence has been accumulating which suggests that the cerebellum plays a significant role in these processes as well. Breaking with the conventional idea of the cerebellum being primarily devoted to motor-related functions, a series of TMS studies was performed that confirmed direct involvement of the cerebellum in emotion processing, behavioral inhibition and regulation. These findings corroborate evidence about the existing closed cerebello-cortical loops and cerebello-limbic connections in the mammalian brain. They have further shaped the cerebellum-oriented model of psychopathology.

With regards to the cerebellar connection with the limbic brain circuit, an ongoing post-mortem study identified a monosynaptic white matter tract between the cerebellum and the hypothalamus in humans. While this finding needs to be replicated and further examined, this connection will likely provide an essential link between the cerebellum and the subcortical emotion circuit. Taken together, these results will not only broaden our understanding of the workings of the brain, but simultaneously will result in the development of novel testable protocols for adjuvant treatment of mental disorders using non-invasive brain stimulation.

'Improving patient diagnosis and treatment with novel prediction models'

Dr. Mathijs Raemaekers

My name is Mathijs Raemaekers and I'm working as a Postdoc in the department of Neurology and Neurosurgery. One of my research lines focuses on applying neuroimaging to the fields of Neurology and Neurosurgery, including techniques, such as functional Magnetic Resonance Imaging (fMRI) and Diffusion Tensor Imaging (DTI). With the use of fMRI, we can measure brain activity through vascular responses, and with DTI we can model white matter fibers by measuring the diffusion of water in the brain. These measurement techniques have the potential to radically increase our understanding of the human brain's functioning, and support the treatment of neurological patients. One of the big challenges is to translate research results obtained with these techniques into approaches that can be directly beneficial to patients, thereby transforming the way in which medical care is given to patients, thereby transforming the way in which medical care is given.

Our research group is currently developing a toolbox that predicts which white matter fibers will be damaged by a stroke. The approach is based on the notion that degeneration along any location of a single fibre-bundle will result in an identical functional deficit. As the stroke location for a particular deficit is interchangeable, there is a less straightforward link between a stroke location and its symptoms, making it harder to predict functional outcome and the optimal therapeutic intervention. The output of the toolbox does not have this caveat, as it maps out the entire length of the damaged fiber-bundles. The toolbox can thereby help facilitate diagnosis and treatment of stroke, but it can also be used as a tool in neurological research.

'The exciting potential of using 7T MRI'

Dr. ir. Jeroen Siero

My name is Jeroen Siero and I am an assistant professor in Neuroimaging at the Vascular Brain Imaging Group at the UMCU. As the PI of the Vascular Brain Imaging Group, my research interest is to study the brain's vasculature and function with Magnetic Resonance Imaging. MRI is a very versatile imaging technique that uses magnetic fields to create detailed images of the human body, in particular its organs and soft tissues. Using the powerful 7-tesla MRI system we have at the UMCU, we can generate images in even more detail and observe processes that were previously 'invisible'. One of my goals is to detect any subtle damage to the brain's vasculature and tissue in an early stage.

What is fascinating in the field of MRI research is the rapid development of new non-invasive methods to measure more rapidly, more efficiently, and more specifically. This involves a wide range of themes - from dedicated hardware for the MRI system, image acquisition and reconstruction approaches to analysis strategies, such as machine learning. I am fortunate to be in a group that is involved in all of these themes. These novel tools allow new opportunities to study brain vessels and function in healthy subjects and under diseased conditions.

In terms of the future of this field, I think we will increasingly see different types of information coming from MRI machines (and other modalities) being directly combined in a single form of analysis. Such a multimodal approach will significantly aid the interpretation of the data as a whole, and will enable us to better link results to underlying physiology. For this purpose, machine learning techniques which have already become quite popular in scientific research, will be instrumental in combining the tons of data involved. Such an analysis platform will allow us to design smart prediction models for conditions, such as neurodegenerative diseases. I hope this will yield crucial new

'Insights from longitudinal imaging-genetics in Psychiatry'

Dr. Rachel Brouwer

Why does one individual develop a psychiatric disorder and another one does not? Why does one child develop into a healthy adult and another child experiences difficulties? Since many psychiatric disorders display the first symptoms in a period in which the brain is still under development, it might not be a surprise that these disorders are accompanied by alterations in the structure of the brain. When studying psychiatric disorders and structural brain deficits observed in patients, we may pose the question of whether these brain deficits are the result of a genetic predisposition for the disease, or whether they are the result of the illness itself.

People are different, there is huge variation in brain structure, even in healthy populations. It has been well-established that brain structure, as measured through magnetic resonance imaging, is a heritable phenotype. We recently showed by means of twin studies that structural brain changes are heritable throughout the lifespan, using longitudinal imaging data several years apart. This implies that the speed of brain development or aging is influenced by genetic factors. In a next step, we identified the genetic variants involved in longitudinal brain changes through a genome-wide association study. Here, we investigated whether single-nucleotide polymorphisms were associated with the extent of brain structural changes, and whether the effect such polymorphisms exert was dependent on the age at which the brain changes took place. We found that genetic variants that influence the change in brain structure overlap with those that represent a vulnerability for psychiatric diseases, such as depression and schizophrenia.

'Cerebral organoids provide new ways to unravel neurodevelopmental disease mechanisms'

Astrid van der Geest, PhD

Astrid van der Geest is a PhD-candidate in the laboratory of professor dr. Pasterkamp. Her work focuses mainly on the genetics of Amyotrophic Lateral Sclerosis (ALS), a neurological disorder which selectively affects motor neurons. By using cerebral organoids induced from patient-derived cells, Astrid van der Geest is able to study patient-specific genotypes of ALS.

Neuroscientists have used neural three-dimensional (3D) cultures called spheroids for many years. Especially since the Yamanaka lab published the protocol for human induced pluripotent stem cells (iPSCs) in 2007, human neural models have been increasingly developed. These 3D protocols have however always been directed towards certain neural cell types, e.g. motor neurons. The research questions that one can answer using them are generally concerned with whether iPSCs with a certain genetic background respond to stimulation with small molecules and are able to differentiate into the intended cell type.
The cerebral organoid protocol developed by Lancaster and colleagues (2013) sheds further light on this previous work by distinguishing the self-organising nature of the organoids. The stem cells are first aggregated to a so-called embryoid body (EB). After taking away the growth factors – proteins that influence the stemness of a cell – the EBs undergo neural induction in a medium that contains hormones and components which help steer the differentiation into the neural direction. To support the growth of the organoid, the EBs are coated with matrigel, an ECM protein gel derived from murine Engelbreth-Holm-Swarm (EHS) sarcoma. Eventually, the organoids are introduced to their long-term culture conditions after two weeks. In this medium, their neural maturation is supported. The organoids are kept spinning in it to enable nutrient access, and within a short space of time, they grow a few millimeters in size, containing millions of cells.

This self-organising nature of cerebral organoids allows for the manifestation of functional consequences of genetic defects. It is possible to analyse aberrant cortical development in organoids derived from patient iPSCs. Moreover, the effects of single genes on human neurodevelopment can be evaluated by combining iPSCs with Crispr-Cas9 genome editing, as an example. Altered development can manifest itself via increased or decreased generation of certain cell types in the neurodevelopmental cascade from radial glia cells to upper-layer cortical neurons. It is also possible that the organoids exhibit a tendency towards certain cell types only present in specific brain areas.

Unfortunately, there is a trade-off between the complexity of a model and its degree of heterogeneity. Neural spheroids are a simple cellular model, but also quite homogeneous in composition. The opposite holds for cerebral organoids: there is a large degree of heterogeneity. There are efforts tailored to decrease this heterogeneity - both in the protocol, e.g. by selecting for uniformly sized EB's, as well as in the analysis, by for instance using data analysis pipelines of single-cell sequencing experiments especially developed for cerebral organoids.

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For this edition, we decided to enrich the Methodology section. In addition to the expert piece by Astrid van der Geest on organoids, we included two perspectives of students who worked with the technique for their Major research projects. They shared their most important insights into the use of organoids in research.

Ari Hauser:

Through their ability to adapt to the genetic profile of their donors, cerebral organoids can be used to mimic cell development of healthy neural networks, but also of neurological diseases. For example, amplification methods, such as quantitative polymerase chain reaction (qPCR) can be applied to analyse the transcriptomic properties of in vitro organoids. Ultimately, this technique allows us to draw conclusions about the degree to which genes and cell functions differ depending on their pathological background. Furthermore, organoids with their 3-dimensional structure, offer the possibility to study cerebral network formation. For this reason, organoids are sliced up to nanometer-thin layers using cryostat technology and marked with antibodies to determine cell morphology, functionality, or interaction.

Jeske Hoogeboom:

To elaborate on Ari's piece, cerebral organoids offer the potential to assess early human development and understand diseases, such as neurodevelopmental or psychiatric disorders. In addition, by using cerebral organoids, we can diminish the use of animals in experiments, and more specifically targeted approaches become available that use human induced pluripotent stem cells (hiPSC)-derived cerebral organoids. This enables us to examine the effects of drugs or other (environmental) factors on early human brain development. One way to investigate this is by making use of Western blot analysis to study protein expression at each stage of development, e.g. to compare stimulating/enriched and unstimulating/impoverished conditions.

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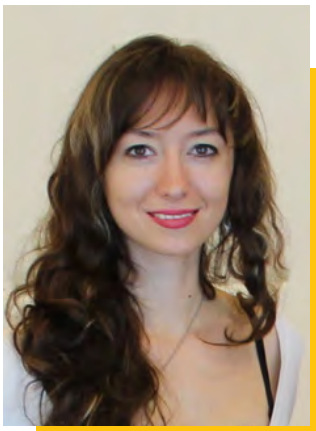
Online PhD Experience

Dr. Julia Berezutskaya



Dr. Julia Berezutskaya did her PhD at the UMCU Brain Center lab of professor dr. Nick Ramsey. The lab focuses on Brain-computer interfaces and her PhD topic was: 'Speech perception in the human brain.' Due to the COVID-19 pandemic, the plans regarding her PhD defence suddenly changed and she decided to do her defence online. She shares her experience of what defending her PhD online felt like.

Like for any other PhD candidate, my PhD experience was not an easy ride and I faced many challenges over the course of the past five years. In December 2019, I finally handed in my completed thesis and thought that the deed was done. However, early 2020 the world faced a big challenge: the COVID-19 pandemic put most things on hold – going to work, stores, hospitals, going almost anywhere at all. And there I was with my PhD defence scheduled for April 14th 2020, only three weeks after nearly everything in the NL was shut down. Luckily, Utrecht University reacted very quickly and offered to arrange an online ceremony even before the official quarantine began. This gave me plenty of time to prepare for the changes, and my online defence was confirmed on April 3rd. All the preparations and the defence itself went very well for me. I had a mock virtual defence in my lab and sent around hard copies of my thesis to the opponents, who preferred a real book over a pdf. On the day itself, I had the luxury of waking up half an hour before the defence (the fellow night owls among you will surely understand), and I found presenting my work from home very stimulating and calming at the same time. The Internet connection was good for everyone involved. I wore formal clothes and arranged a sunny corner that looked good in the frame. Overall, the preparations were minimacompared to what it would have been in real life. The next day, I got a confirmation that I had successfully defended my thesis, signed by the rector.

And after all, it may be easier than you think to make this day feel special and big.

Personally, I found the whole experience pretty amazing Here are some of the definite upside: 1) The atmosphere at home was definitely more relaxing with no pressure of presenting in front of a large audience. 2) Many people, especially from abroad, including my parents in Russia, were able to join the livestream. 3) I got a video of the defence later that day and shared it with people who could not make it. 4) I got a glimpse at my opponents' surroundings. If you are lucky, they might have a cute pet in the back. 5) No costs spent on reception.There were of course also certain downsides: 1) No costs spent on reception. 2) No defending at the amazing Academiegebouw. 3) No fancy pictures. 4) No ceremonial handing in of the diploma. 5) Most of my PhD books are still in the box I received from the publisher. In general, defending my PhD online was flexible, relaxed and all-including, yet seemingly at the expense of feeling special, celebratory and a big deal.Personally, the latter part did not bother me much. After the ceremony, I was on the phone for two hours receiving warm congratulations from family, friends and colleagues. That felt great and special nonetheless. It was later followed by a great dinner, cake and wine with my partner, which I prefer to a crowded bar anyway. The online defence did not affect my plans after graduation either. I had already started my postdoc contract in February and having graduated on time only made things simpler. All in all, I am glad the university offered me a chance to graduate as planned and I would recommend it to everyone. Getting your degree on time makes things easier for you and helps the university manage their graduation requests. And after all, it may be easier than you think to make this day feel special and big without having to go too far outside your house.

Career Perspectives

'Remember: a completed N&C Master's opens many doors, both within, as well as outside the academic world.'

Senior Medical Writer
at Oxford PharmaGenesis

Dr. Tim van Hartevelt

Studying for a BSc degree in neuroscience confirmed my desire to be a scientist and to pursue a career in discovery and innovation. So, I followed the usual educational trajectory, starting with the Neuroscience & Cognition Master's programme at Utrecht University, followed by a PhD (Biomedical Sciences at Aarhus University, Denmark and the University of Oxford, UK), before a period of working as a postdoctoral researcher at the University of Oxford. Although I enjoyed life as a researcher, I felt that a long-term career in this type of job was no longer what I wanted, partly because of the challenges in finding funding and job security. I had to re-evaluate my goals and ambitions and find out what types of careers were suitable, and interesting, for someone with my background and expertise.

My search led me to medical writing which, it seemed to me, would utilise my scientific training, satisfy my intellectual curiosity, and enable me to fulfil my career aspirations. I have been a medical writer at Oxford PharmaGenesis for approximately 2.5 years now and there has not been a single day where I have regretted my decision to leave academia and join the company.

I found the switch from academia to industry a very positive experience, and not nearly as shocking as might be expected. I immediately felt valued and respected for the work I did and for the knowledge I brought to the table. I had an extensive background in neuroscience when I switched to work as a medical writer, a substantial part of which was developed during the 2-year Neuroscience & Cognition research Master's programme at Utrecht University. This enabled me to immediately bring valuable knowledge into the company and further develop my writing and communication skills on the job.

I feel that my years of experience and learning in neuroscience, including the Neuroscience & Cognition Master's I completed, prepared me very well for a job working across myriad topics within neuroscience. They have provided me with the ability to understand, value, and translate scientific and clinical research into scientific publications, as well as medical communication and training materials. The latter often involves adapting the tone and complexity level of the materials to reach different audiences, such as healthcare professionals, researchers and patients. It is very exciting to also be able to work in different therapeutic areas thanks to a broad neuroscience and scientific background. There is always a new skill to learn or new knowledge to acquire, irrespective of whether you work in academia or in the industry.

Looking ahead, it is exciting to contemplate new opportunities for learning and career development in different job roles. Although it is often considered important to find or create a niche for yourself, broadening your skills early in your career through courses or additional training could lead to many different and exciting opportunities.

Coordinator Research Data Management
at UMC Utrecht

Irina van Dijk, MSc

When the N&C journal's board approached me for a contribution on my career path, I didn't hesitate to say Yes. When I started the N&C Master's programme, I was convinced that I would become a researcher, as I'm sure many of you are today. However, after my graduation, I turned down PhD positions and started an alternative career journey. But why?

First of all, I realised during my internships that becoming a successful and satisfied scientist requires one to be truly passionate about a certain topic. Therefore, as I have many different interests, it was difficult to put my heart and soul into one specific project. Additionally, I talked to many people who did not pursue an academic career after obtaining their Master's degree. Thanks to their candour, I realised that the main advantage of pursuing a PhD -continued learning- was available in many workplaces. Lastly, during my internship in New York City, I became more familiar with the Open Science movement. I was truly inspired by this new culture which emphasized the huge benefits of sharing data and other research output. Besides, it made me slightly more sceptical about pursuing a PhD. Imagine what we, the scientific community, could achieve together if we reward fruitful collaborations more than completing a single PhD project…

Back in the Netherlands, I had a side job at Utrecht University's YOUth study: a large-scale, longitudinal cohort following children in their development. After finishing the N&C Master's, I started in a full-time position at YOUth. Here, I could apply my knowledge from internships about Open Science, the medical-ethical review process and research policies.

Currently, I work at the UMC Utrecht in the team Data solutions & Research IT as a Coordinator Research Data Management (RDM). In short, I create awareness on FAIR data (Findable Accessible Interoperable Reusable) and I explore new needs, opportunities and trends in RDM within and outside the UMCU. My N&C time has enabled me to easily empathise with different researchers, from both fundamental and clinical research, and to understand their needs. In addition, I acquired skills such as critical thinking, efficiency with processing large amounts of information, and taking different perspectives.

One piece of advice: talk! Get to know your fellow N&C students from previous years, colleagues from your internship and people working elsewhere with an interesting career path. Most of them are very willing to talk about their careers – you only need to ask! These conversations may broaden your horizon and allow you to consider different career options. Remember: a completed N&C Master's opens many doors, both within, as well as outside the academic world.

Good luck on your career journey and feel free to contact me on LinkedIn.

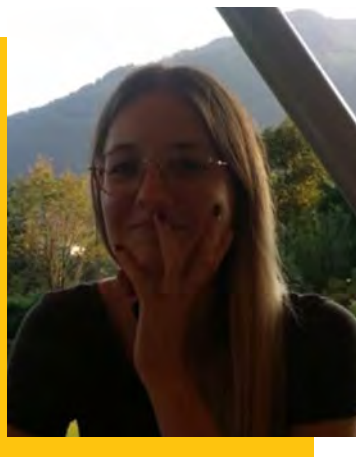
Research with Psychedelics

'Currently we can speak of a psychedelic Renaissance'

'Microdosing with psychedelics, the future of psychiatric treatment?'

by Nadia Hutten, MSc

Department of Neuropsychology & Psychopharmacology, Faculty of Psychology & Neuroscience, Maastricht University, the Netherlands



During the last decade there has been increased interest in the therapeutic potential of taking low doses of psychedelic substances, such as lysergic acid diethylamide (LSD) and psilocybin, a practice called microdosing. Patients report that microdosing reduces the symptoms related to their diagnosed condition, including attention deficit hyperactivity disorder (ADHD), depression, and anxiety. Microdosing has been reported to be more effective than conventional treatment options (Fadiman & Korb, 2019). For instance, ADHD patients describe that the effects of a low dose of LSD last longer without the sudden drop to fatigue, as experienced with conventional medications (Family et al., 2020). Of note, this is not reported for all diagnosis-related indications. Microdosing is a practice intended not to cause extreme alterations of the senses (Hutten, Mason, Dolder & Kuypers, 2019a) and due to the uncontrolled nature of self-medicating, it can be difficult to distinguish from a placebo effect (Hutten, Mason, Dolder & Kuypers, 2019b). However, patients report that low doses of psychedelics do relieve their symptoms and improve their quality of life (Fadiman & Korb, 2019). The question then would be whether continuing this practice does them any harm.

While scientific evidence regarding the safety of microdosing is lacking to date, one study in an elderly population demonstrated that low doses of LSD were well tolerated. Negative effects, such as dizziness and headaches were documented, but were mild and transient (Hutten et al., n.d.). Furthermore, anecdotal reports show that some users experience negative physiological and psychological effects and are sometimes a reason to discontinue microdosing (Kuypers, 2020). Next to that, the long-term impact of microdosing on mental and physical health is still unknown. Research has yet to show whether positive effects outweigh potential negative effects.

If indeed patients do benefit from microdosing, the question is how this will be implemented in the current medical system. It should be kept in mind that even though microdosing entails using low doses, it still involves using a psychedelic substance which might induce latent emotions coming to the surface. Therefore, it is favourable to guide patients in this process, to provide psychological support, and not solely prescribe microdosing as a medication for symptom reduction.

While research shows that microdosing can have a positive influence on mood and cognition (Fadiman & Korb, 2019; Family et al., 2020; Szigeti, 2020), evidence also suggests that not everyone will benefit from this practice (Fadiman, 2019; Szigeti, 2020). Therefore, future research needs to examine the biological underpinnings of people's individual responses to microdoses to fully understand their underlying mechanisms. Consequently, patients could be screened in the future on these biological markers and in turn, receive effective personalised therapy.

'This is your brain on drugs'

by Floor van der Does, PhD

PhD student, study coordinator psilocybin-assisted psychotherapy for treatment-resistant depression
Leiden University Medical Center

Ketamine, MDMA, LSD, magic mushrooms, ayahuasca, DMT and ibogaine are compounds that are mostly known as party drugs or recreational psychedelics. In recent years, however, the field of psychedelic medicine has emerged. These powerful psychotropic compounds are now being researched as tools in psychotherapy for a host of conditions, including depression, anxiety, chronic pain, addiction, and post-traumatic stress disorder. This is not a new idea. For centuries, psychedelic mushrooms, mescaline and ayahuasca have been used by indigenous people in medical and spiritual ceremonies. In the fifties and sixties, a large body of research showed very promising results in treating various psychiatric illnesses, like end-of-life anxiety and substance abuse disorders, with psychedelics. However, as LSD was adopted by counterculture, and leaked from the research labs into Western society, the 'War on Drugs' was started and funding for psychedelic research dried up.

Currently we can speak of a psychedelic Renaissance. As I am writing this piece, the Interdisciplinary Conference on Psychedelic Research is taking place. This is an online conference with more than 1000 attendees and three days of talks, workshops and discussions on topics related to psychedelic medicine. This illustrates the progress that has been made on the many potential applications of psychedelics. The public view of these compounds is shifting away from their negative associations with other drugs and towards a greater understanding of their healing potential. The FDA in the United States has even awarded a 'breakthrough' status to MDMA-assisted psychotherapy for PTSD and psilocybin-assisted therapy for depression. Within the next couple of years, MDMA and psilocybin are likely to be available on prescription and to be administered by carefully-trained clinicians in a treatment setting as part of a larger treatment plan.

The mechanisms behind these compounds' medicinal value are still a topic of research and debate. Part of the effects may stem from the subjective psychedelic experience, which often involves deep feelings of connection, and an opening up of the mind's usual patterns of thought and emotions to allow for new ideas. The compounds have also been shown to have effects on the brain. They interact with 5-HT2A serotonin receptors and are associated with decreases in the activity of the default mode network, and changes in brain-wide connectivity. Neuroimaging studies have mostly been conducted with small numbers of participants so far, however, and - as we often like to conclude in science - more research is needed.

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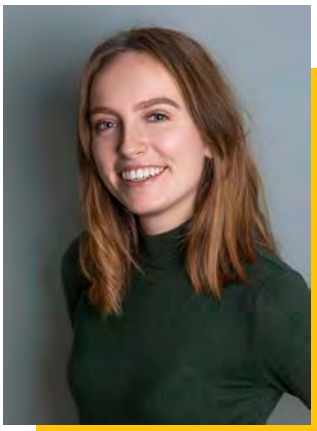
Alzheimer's Association International Conference 2020

Esmée Verburgt



As a student who is discovering the neuroscientific field, your first conference or congress will always be a precious memory. In the week of July 27th - 31st the Alzheimer's Association International Conference (AAIC) 2020 took place. In normal circumstances, this conference would have taken place in Amsterdam and I would have been unable to attend due to my student position and high costs. When the pandemic hit, the members of AAIC decided to move the conference to an online platform and make registration free! Meaning that being a Master's student, I was still able to attend the conference from my own home. This is of course not how I imagined my first conference experience, since there was no overwhelming feeling of walking in a room full of experts in this field or a chance to network. Nonetheless, I was able to navigate online through every talk I thought might be interesting. Normally, you would have to choose one talk and miss out on the other if they were scheduled at the same time. However, I did not have to miss a single thing, since I could just watch it back at any moment!

This conference truly is promised to inspire you and broaden your view on your pre-existing knowledge.

This platform provided 2-to-3 live sessions per day, 15 minutes on-demand talks, which were recorded in advance, and published posters. They assigned a theme to the different days throughout the week. The conference kicked off with the theme 'Basic science and pathogenesis' on Monday. The theme of Tuesday was 'Biomarkers', followed by 'Clinical manifestations and drug development' on Wednesday. On Thursday, people could watch talks on the topics of 'Public health, dementia care, and psychosocial factors and dementia care practice'. Finally, the closing theme for Friday was: 'Professional development'. With over 100 talks a day, every aspect of every type of research was covered. The biggest names in Alzheimer research gave an update about their groundbreaking research, but what I found similarly interesting were those young investigators who got a chance to talk about their research. This conference truly is promised to inspire you and broaden your view on your pre-existing knowledge. It may even direct you into finding a topic or research group you would like to perform your minor internship at, like in my case! By combining all these amazing researchers and their work, this conference aims to direct new Alzheimer research into uncovered grounds and work together to get one step closer to understanding and treating Alzheimer's disease!

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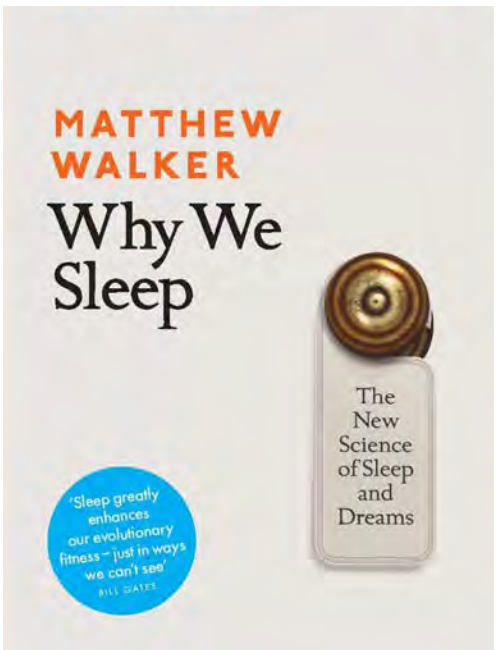
'Why we sleep: The new science of Sleep and Dreams' by Matthew Walker

Charlotte van Dijk

Have you ever wondered why being in a coma-like state for six to eight hours every night would ever be evolutionarily beneficial? Not being able to move, gather food, socialise, mate, or stay away from enemies - it doesn't seem to make much sense from an evolutionary perspective. Then why does every single animal on Earth, without exceptions, require sleep? In this book, Matthew Walker tries to explain what sleep is and why we so desperately need it. Dr. Walker is a Professor of Neuroscience and Psychology at the University of California, Berkeley where he founded the center of Human Sleep Science - a place where a lot of sleep research is performed. His research focuses on the impact of sleep on human brain function in healthy people, but he also tries to identify the role of sleep in diseases, such as Alzheimer's disease, Parkinson's disease, and depression.

In this book, Walker provides scientifically supported information about what sleep is, why we need it, and what happens when you don't get enough of it. The book is divided into four sections which you can read in any order you like. The first one tries to demystify sleep by explaining what it is, but also how much we should sleep and how sleep changes as we age. The second part describes why we thrive when we get enough sleep and the destructive effects of sleep deficiency. In the third part, Walker dives into the world of dreams and everything that happens in your brain when you reach a hallucinatory state. The focus of the final passage is on society. He describes why doctors should not be prescribing sleeping pills and he tries to raise awareness surrounding the high percentage of people suffering from sleep deprivation, as well as its effect on public health.

In addition, the author beautifully combines this content with practical tips, derived from scientific research, on how to improve one's ability to sleep.



Walker really emphasises the importance of sleep, he even suggests putting his book down if one starts to feel sleepy!

Throughout the book, Walker really emphasises the importance of adequate sleep, so much that he even suggests putting his book down if one starts to feel sleepy, and going to sleep instead!

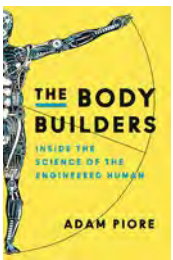
The book was recommended to me by no other than Bill Gates. It is no surprise that sleep is a subject of great interest to someone successful, as you could argue Bill is, because it is important for almost every biological process taking place in your body right now. You don't have to be Bill Gates to profit from knowing what a good eight hours of sleep can do for your physical and mental health. So, besides sleep being a fascinating subject on its own, I, together with Bill, highly recommend that you read this book to improve your health and performance!

Ten books revealing hidden treasures in neuroscience

The N&C Journal Board

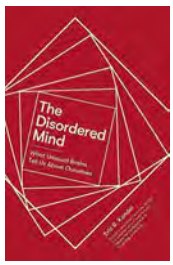
Alongside our traditional book impression by a fellow Neuroscience and Cognition student, we compiled a list with ten books worth reading. They teach us about the beauty and complexity of the human brain, and about shifts in thinking that have left a legacy in the field or are yet to shape it. Each Journal board member recommended a book and described why they found it impressive and enjoyable to read. The list does not follow any specific order of priority as all reads are captivating and interesting in their own way. We hope you will get to read and enjoy at least some of them!

'The Body Builders' *by Adam Priore*



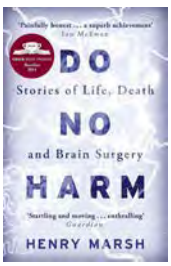
In this book, Adam Priore takes the reader on a journey in the field of neuro-bioengineering. From hacking our genetic codes to using telepathy to assist those who can no longer speak, these new techniques are helping individuals everywhere around the globe by building better bodies and better lives. The author guides the reader through these fascinating cutting-edge discoveries and

'The disordered mind' *by Eric Kandel*



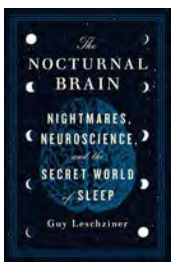
In this versatile book, Nobel prize winner Eric Kandel explains how normal brain processes can become dysregulated, resulting in devastating illnesses like autism, schizophrenia and Alzheimer's disease. In the past, neurological and psychiatric disorders were considered as separate conditions because it was accepted that the former were caused by dysregulation of the brain, and the latter by dysregulation of the mind. Here, Kandel argues that in fact, both types of disorders have a shared cause because the brain and the mind are inseparable. He describes how brain diseases can provide a window into the healthy brain - the more scientists and clinicians learn about them, the more they understand about healthy brain function. This inevitably leads to the development of more effective

'Do No Harm' *by Henry Marsh*



This is the story of a modern neurosurgeon, recounting what it is like to probe the line between life and death of a patient. How it feels to hold someone's life in one's hands and cut into the organ that creates thought, feeling and reason, or live with the consequences of a life-saving operation if something goes wrong. If you are not afraid of graphical descriptions and want to have a look into the true (British) medical system, this is your book!

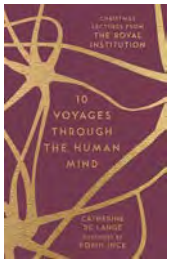
'The Nocturnal Brain: Nightmares, Neuroscience and the secret world of sleep' *by Guy Leschziner*



This book describes various fascinating and extraordinary sleep disorders, such as insomnia, night terrors and sleepwalking, from the view of a neurologist in a captivating way. As a glimpse into some of the extreme cases, there is a description of a woman in a state of deep sleep who gets dressed, unlocks her car, and drives for several miles before returning to bed, or a man who cleans out kitchens while 'sleep-eating'. The book also outlines the biological and psychological factors necessary to get the rest that we need in order to maintain our physical, mental and cognitive abilities, as

'10 voyages through the human mind'
by Catherine de Lange

The science journalist and editor Catherine de Lange has summarised the ten most interesting Christmas lectures about neuroscience and cognitive science held at the Royal Institution in London, ranging from Archibald Vivian Hill's early experiments on electrical signal transmission in the nervous system to Sophie Scott's fascinating exploration of human language. De Lange's lively descriptions and the wealth of background information she provides almost make you feel like you have attended the lectures yourself

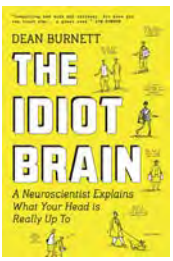


'Are we smart enough to know how smart animals are?'
by Frans de Waal

Frans de Waal is a biologist and he researches the depth and scope of animal intelligence. This book provides stories about animal cognition and shows that we have been underestimating the non-human brains for quite some time. It outlines interesting perspectives about what separates the human mind from an animal's mind and provides the reader with fresh new insights into what is known about human and animal intelligence.

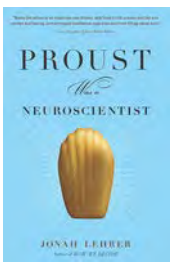
'The idiot brain'
by Dean Burnett

In this very recent and popular neuroscience book, Dean Burnett guides his readers through the human brain and explains its normal functioning based on the peculiarities we experience every day. For instance, why do some people sleepwalk? And why do we sometimes think of something, get distracted by a younger brother and then forget why we went to the kitchen? Questions like these will be answered, and Burnett makes it clear that most of these situations are just some imperfections that enable the brain to function properly. So we should get used to them...



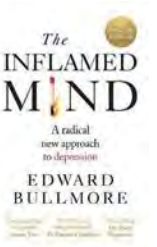
'Proust was a neuroscientist'
by Jonah Lehrer

In his sparkling debut, Lehrer shows the necessity of art and imagination in neuroscience, linking the ideas of famous writers, philosophers and painters to modern ideas in neuroscience. This book argues that often, artists have formed ideas about the mind and the brain before modern neuroscience had even arrived at the same theories and conclusions centuries later! It urges science and art to listen more closely to each other because combining the



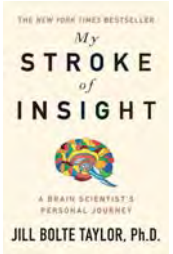
'The inflamed mind'
by Edward Bullmore

This thought-provoking read by University of Cambridge Professor Edward Bullmore reveals the breakthrough-new science on the link between mental depression and physical inflammation. He explains how and why mental disorders can have their roots in the immune system and explores a whole new way of looking at how mind, brain and body all work together in a sometimes misguided effort to help us survive in a hostile world.



'My stroke of insight'
by Jill Taylor, PhD

In this book, neuroanatomist Jill Taylor provides a special account of stroke - not just as a professional working in this very field, but also as a human being and patient who managed to get awareness into the experience of her own stroke! Whilst the neuroanatomical details seem easy to understand for a neuroscience student, the real virtue of the book lies with the shared first-hand insights as the author provides a new perspective on stroke as something beautiful, spiritual, and ultimately awakening to experience.



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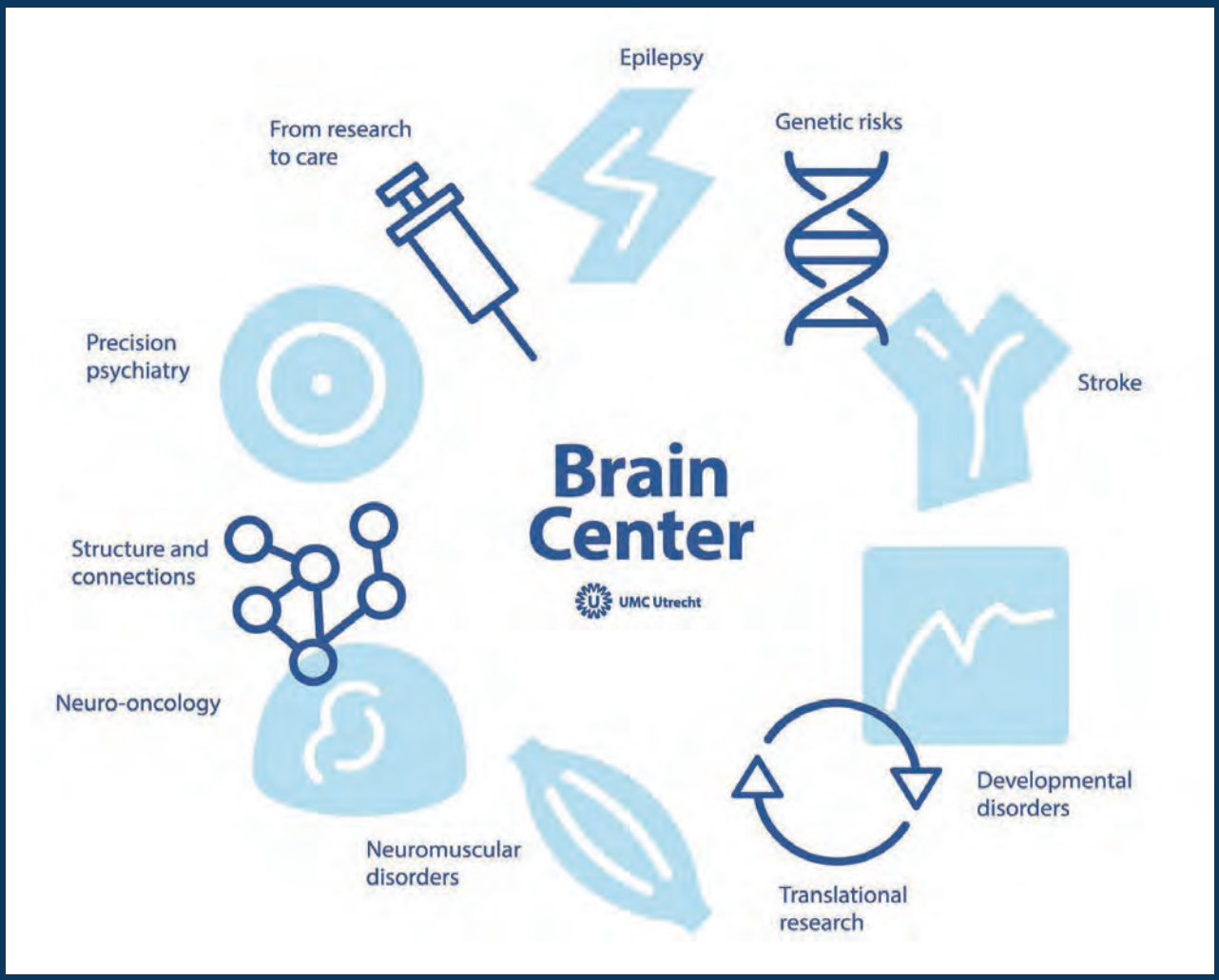

Rudolf Magnus Young Talent Fellowship

The UMC Utrecht Brain Center invests in junior scientific talent. The Rudolf Magnus Young Talent Fellowship ((€200,000 to be shared between the two applicants) allows junior researchers to develop a strong and recognizable research profile and set up interdisciplinary collaborations.

Contact

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Education

Internships: students can work under supervision of researchers in the field of Translational Neuroscience, Neurology and Neurosurgery, Psychiatry, Rehabilitation.

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www.umcutrecht.nl/braincenter

Mind the Brain Symposium 2020

Eat your brain out!

The first online Mind the Brain symposium ever which took place in May was a great success! Due to COVID-19, it was nothing like we planned, but we still considered it an amazing experience. After the symposium we felt proud of what we have accomplished. Even though we were disappointed that we did not get to experience organising a real-life event, we still learned a lot from behind-the-scenes of an online symposium as well. The most valuable lesson being that communication is key. It is difficult to communicate well from behind your computer, as non-verbal communication is reduced to a minimum. I think I can speak for everybody when I say that working from home was a challenge, but it was also a positive experience, and we will probably see more of this in the future, even when the pandemic is over.

After the symposium our job was not done yet! We still had to deal with the assessment of the symposium and the evaluations people gave for it. We also had to start preparing for the poster session. Student involvement in the form of presentations and poster sessions has always been a great part of the Mind the Brain symposium. Of course, we did not want to remove this part due to the change in format, so we decided to have the symposium, including the student presentations, and the poster session, separated into two online events. At first, we had some hope left that we could host the poster session in real life, but unfortunately it was not possible. This resulted in an online poster session, which was attended by 115 people! During the event, posters were presented in smaller groups to facilitate discussion, where it was easy to talk about the different posters together. In addition, we also had three poster presentations in a plenary session. They had been selected by our poster judges and the public had the chance to vote for the best one afterwards, which resulted in the 'Winner of the best poster' awarded to Robin Haak! Our abstract judges also awarded the prize 'Winner of the best abstract' to Teuntje Pelgrim. Congratulations to both!

Now that we are finished with our Mind the Brain year, we are looking for some new enthusiastic first-year Neuroscience and Cognition students that could take over this awesome job and turn the Mind the Brain symposium 2021 into a great experience as well! On behalf of the Mind the Brain committee, I would like to wish them good luck and we are looking forward to the symposium in 2021!

- Emma Eeltink, Chair

Best presentation

'The role of GFAP isoforms in glioma invasion'

by Dasha Fedorushkova

'I am incredibly happy to have won the best student presentation of the online Mind the Brain Symposium 2020. The cytoskeleton and especially the intermediate filaments are of great importance in glioma, and in the future I hope to continue my research in this field!'

Best abstract

'Whole Brain Connectivity Analysis of 22q11DS: A graph Theory Based Network Study of Resting fMRI Signal'

by Teuntje Pelgrim

'It was great to hear that I had won the award for best abstract at the Mind the Brain Symposium. I'm very intrigued by the mechanisms of neuropsychiatric disorders in the brain, and hopefully my abstract gives you a quick, but interesting insight into what neuroimaging research of these disorders can entail!'

Best poster and pitch

'Role of Prefrontal Cortical Output Pathways to the Lateral Hypothalamus in Stress Eating'

by Robin Haak

'I'm very grateful to have been awarded the prize for best poster presentation at this year's Mind the Brain Symposium. I think it's fascinating to explore how individual neurons process information within a circuit, and I hope to continue my research in this field.'

Role of Prefrontal Cortical Output Pathways to the Lateral Hypothalamus in Stress Eating

Robin Haak, Rogier B. Poorthuis, Frank J. Meye
Department of Translational Neuroscience, UMC Utrecht

Introduction

Stress enhances food intake and shifts dietary choices towards high-calorie and unhealthy foods (in both humans and animals);

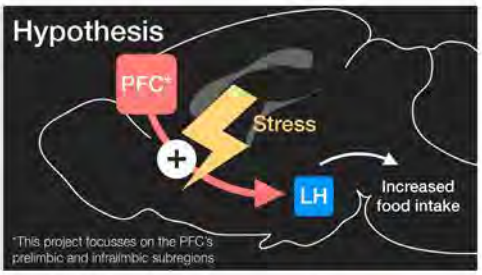
This 'comfort feeding' is a leading cause of obesity and associated comorbidities;

Human and animal studies point to a critical role for the prefrontal cortex (PFC) in stress eating;

The PFC robustly projects to the brain's feeding center: the lateral hypothalamus (LH), although the role of this connection in food intake remains enigmatic;

This project aims to elucidate whether the PFC→LH projection (I) is causally related to feeding and (II) drives increased food intake after stress in mice.

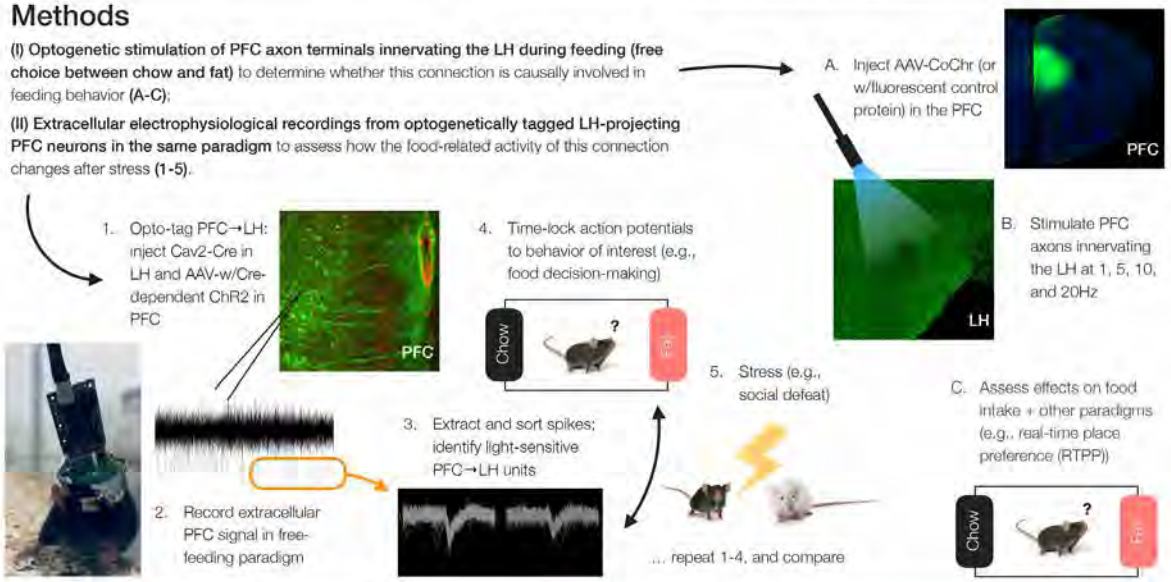
Hypothesis



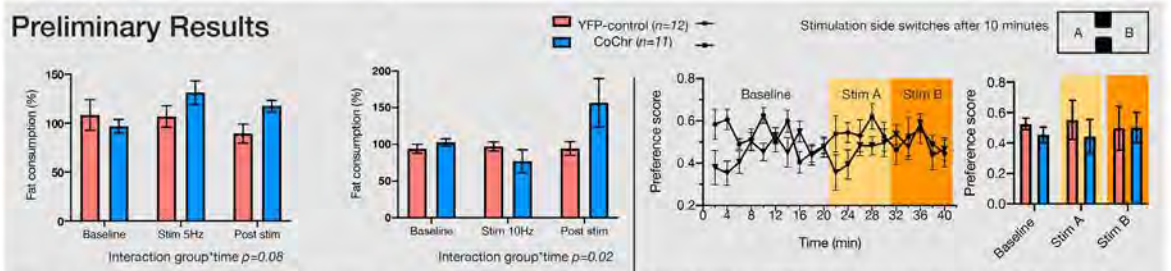
Methods

(I) Optogenetic stimulation of PFC axon terminals innervating the LH during feeding (free choice between chow and fat) to determine whether this connection is causally involved in feeding behavior **(A-C)**;

(II) Extracellular electrophysiological recordings from optogenetically tagged LH-projecting PFC neurons in the same paradigm to assess how the food-related activity of this connection changes after stress **(1-5)**.



Preliminary Results



Normalized fat intake (mean + SEM) during 1hr free feeding with 5 and 10Hz stimulation (total of three 10-minute stimulation epochs).

5Hz stimulation; RTPP preference score (mean + SEM; time spent at first stimulation side (A) divided by time spent in both compartments).

Conclusions

PFC→LH projection is involved in feeding behavior: 5Hz stimulation tends to increase fat intake, whereas 10Hz decreases food intake on the stimulation day;

This effect is frequency-dependent; 1 and 20Hz stimulation (data not shown) has little effect on fat intake;

PFC→LH stimulation at 1, 5, 10, or 20Hz is neither rewarding nor aversive (as assessed in the RTPP);

LH-projecting neurons mainly reside in layer 5 and 6 of the PFC (viral tracing using Cav2-Cre injection in LH and Cre-dependent fluorophore in the PFC).

What's next?

Extracellular electrophysiology experiment;

Repeat the optogenetics experiment with a new batch;

Identify and manipulate neuronal populations in the LH that are innervated by the PFC;

Further explore the PFC network: investigate the role of inhibitory interneurons in stress eating.

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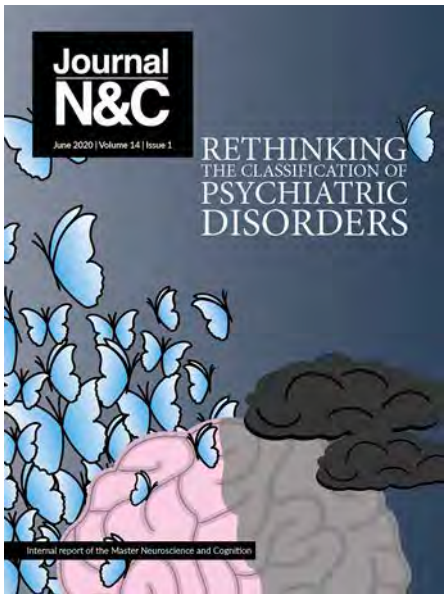
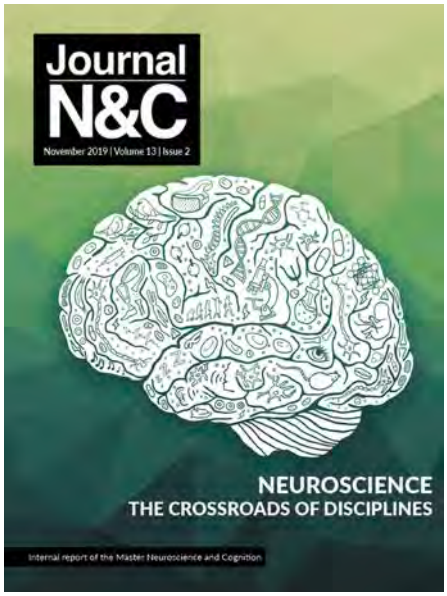
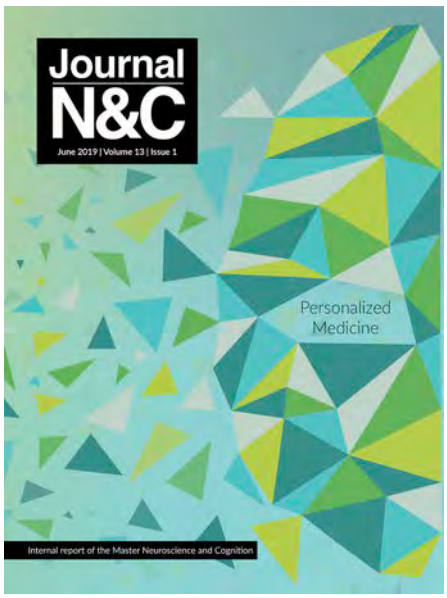
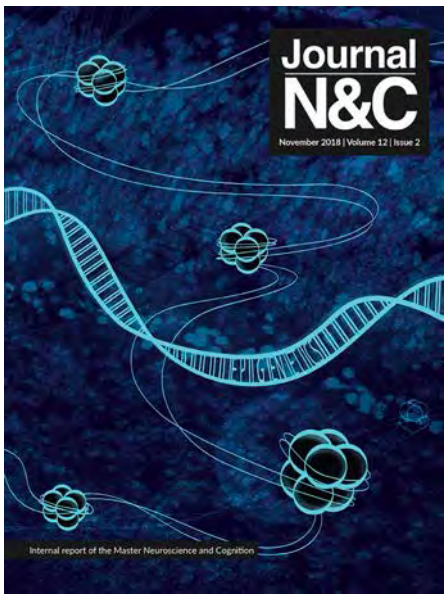
UMC Utrecht

Contribute to the next issue of the journal!









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