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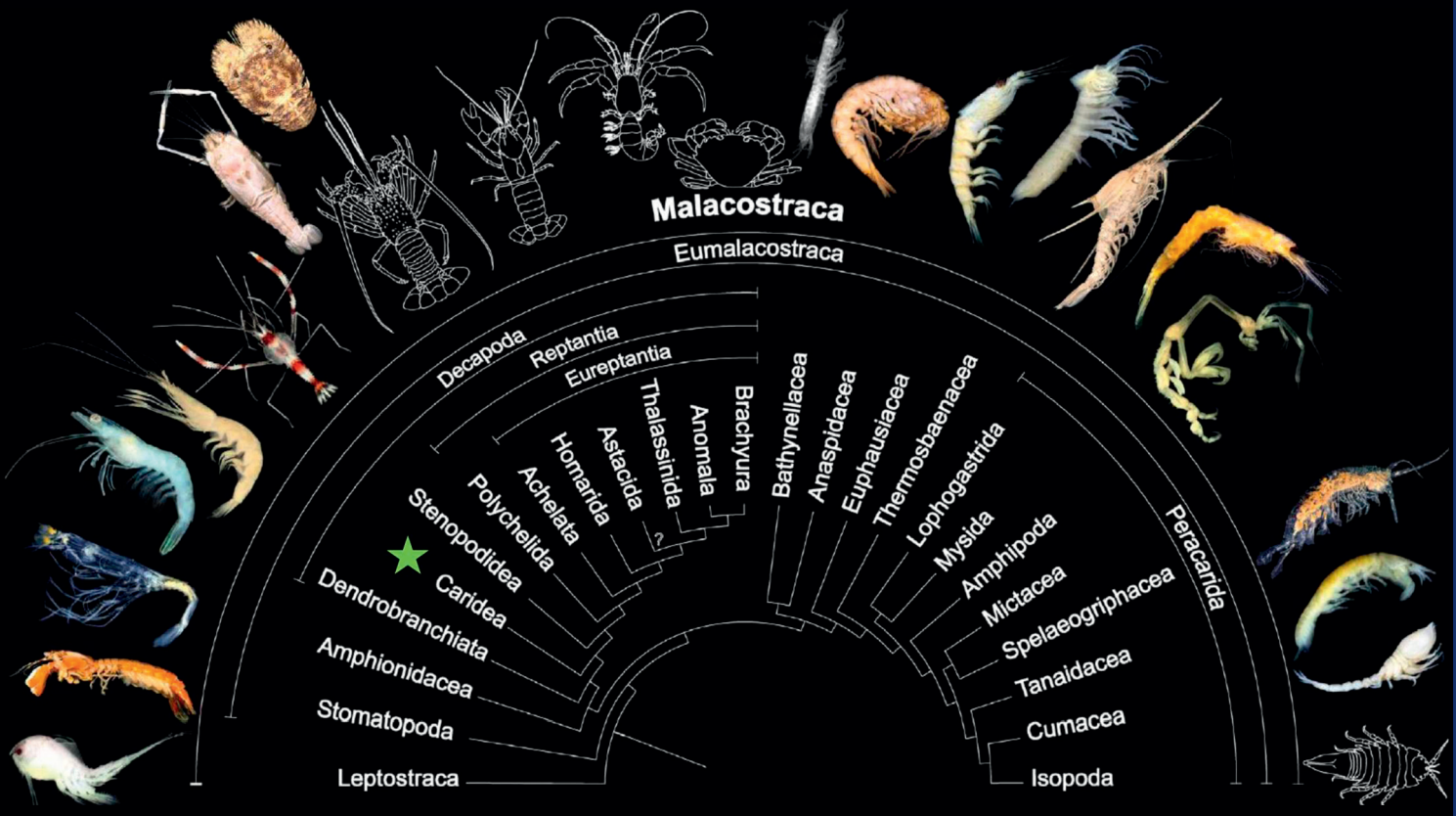

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HERAUSGEBER Neurowissenschaftliche Gesellschaft e.V. (NWG), Kontakt: Meino Alexandra Gibson, Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Straße 10, 13092 Berlin, Tel.: +49 (0)30 9406 3336, gibson@mdc-berlin.de, www.nwg-info.de

CHEFREDAKTEURIN Petra Wahle, AG Entwicklungsneurobiologie, Fakultät für Biologie & Biotechnologie, Ruhr-Universität, ND 6/72, 44780 Bochum, wahle@neurobiologie.ruhr-uni-bochum.de

REDAKTION Susanne Hannig, Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Str. 10, 13092 Berlin (Germany), Tel.: +49 (0)30 9406 3336, susanne.hannig@mdc-berlin.de

VERLAG Walter de Gruyter GmbH, Berlin/Boston, Genthiner Straße 13, 10785 Berlin, Germany

JOURNAL MANAGER Torsten Krüger, De Gruyter, Genthiner Straße 13, 10785 Berlin, Germany. Tel.: +49 (0)30 260 05-173, Fax: +49 (0)30 260 05-250, E-Mail: Neuroforum.Editorial@degruyter.com

ANZEIGENVERANTWORTLICHE top-ad Bernd Beutel, Schlossergäßchen 10, 69469 Weinheim, Tel.: +49 (0)6201 290 92-0, Fax +49 (0)6201 290 92-20 20, disposition@top-ad-online.de

COVER ILLUSTRATION Phylogenetic relationships of malacostracan crustaceans (modified from Harzsch and Krieger, 2018; compiled after Richter and Scholtz, 2001; and Wirkner and Richter, 2010). The green asterisk show the phylogenetic position of the deep vent shrimp Rimicaris exoculata among Caridea. Cover figure provided by Julia Machon et al., Exploring brain diversity in crustaceans: sensory systems of deep vent shrimps (nf-2020-0009, pp. 73–84 in this issue).

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VORSTAND DER AMTSPERIODE 2019–2021

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Review article

Finja Grospietsch* and Jürgen Mayer

Misconceptions about neuroscience – prevalence and persistence of neuromyths in education

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Abstract: *Brain-friendly learning* is a new catchphrase in school and university instructional practice. However, it often escapes the notice of the teachers and learners involved that *neurodidactics* is not simply a plausible concept – it can also be a myth if applied incorrectly. Numerous international studies show that both pre-service and in-service teachers as well as university educators endorse misconceptions on the topic of learning and the brain and orient their didactic conception on so-called neuromyths. This paper presents nine neuromyths on the topic of learning and memory. Based on a review of the current research, we discuss what determines their emergence and prevalence, to what extent neuromyths pose a problem for practice, and why and how both neurodidactics and neuromyths should be made an object of university instruction.

Keywords: brain-friendly learning; neurodidactics; neuromyths; (pre-service) teachers; university educators

Zusammenfassung: *Gehirngerechtes Lernen* gilt als neues Schlagwort in der schulischen und hochschuldidaktischen Praxis. Was dabei häufig aus dem Blick der engagierten Lehrenden und Lernenden gerät, ist, dass *Neurodidaktik* nicht nur ein plausibles Konzept, sondern falsch angewendet auch ein Mythos sein kann. Zahlreiche internationale Studien zeigen, dass sowohl angehende und praktizierende Lehrkräfte als auch Hochschullehrende Fehlvorstellungen zum Thema Gehirn und Lernen Glauben schenken und ihre didaktische Konzeption an sogenannten Neuromythen ausrichten. In diesem Beitrag werden neun Neuromythen zum Thema Lernen und Gedächtnis vorgestellt. Auf Basis einer Darstellung des aktuellen Forschungsstands wird erläutert, was ihre Entstehung und Verbreitung bedingt, inwiefern Neuromythen ein Problem für die Praxis darstellen und warum bzw. wie sowohl Neurodidaktik als auch Neuromythen zum Gegenstand universitärer Lehre gemacht werden sollten.

Schlüsselwörter: (angehende) Lehrkräfte; Gehirngerechtes Lernen; Hochschullehrende; Neurodidaktik; Neuromythen

Introduction and objectives

In recent years, insights from the field of brain research have launched a downright *neuro-boom* reflected not only in numerous publications but also in transfer attempts such as *neuromarketing*, *neuroarchitecture, neuromanagement*, and *neurodidactics* (cf. e. g., Häusel, 2008; Herreros, 2012; Herrmann, 2009; Metzger, 2018). Teachers especially show great interest in neuroscientific research findings and consider it useful to incorporate them when designing their instruction (Dekker et al., 2012). *Brain-friendly learning*[1] is seen as a new magic spell, not only in schools but also in university instruction (Folta-Schoofs and Ostermann, 2019). Nevertheless, (pre-service) teachers and university instructors, the alleged experts on learning, still endorse numerous neuroscientific misconceptions and partially orient their instructional practice on so-called neuromyths (e. g., Dekker et al., 2012; Gleichgerrcht et al., 2015). The term *neuromyths* can be traced back to the neurosurgeon Alan Crockard, who used it in the 1980s to refer to scientifically inappropriate understandings of the brain in medical

***Corresponding author: Finja Grospietsch**, Department of Biology Education, Faculty of Mathematics and Science, University of Kassel, Heinrich-Plett-Straße 40, Kassel, 34132, Germany, E-mail: finja.grospietsch@uni-kassel.de. https://orcid.org/0000-0002-4141-9671

Jürgen Mayer: Department of Biology Education, Faculty of Mathematics and Science, University of Kassel, Heinrich-Plett-Straße 40, Kassel, 34132, Germany, E-mail: jmayer@uni-kassel.de. https://orcid.org/0000-0003-4150-0688

1 The terms *brain-friendly* or *brain-based* learning are used in education science as synonyms for neurodidactics. The authors note that these terms are not neuroscientifically justifiable, because all learning is based on neural changes in the brain and can never be *non-brain-friendly* or *non-brain-based*. The authors recommend using the term "neuro-didactics" in future discourse.

culture (Howard-Jones, 2010). The Organization for Economic Co-operation and Development (OECD, 2002) defines neuromyths as "misconception[s] generated by a misunderstanding, a misreading, or a misquoting of facts scientifically established (by brain research) to make a case for use of brain research in education and other contexts" (p. 111). Neuromyths have been identified with respect to numerous topics, including indications of specific learning difficulties such as dyslexia (Macdonald et al., 2017) or the influence of nutrition (Dekker et al., 2012) and music (Düvel et al., 2017) on the brain. This paper focuses on neuromyths concerning the topic of learning and memory. We discuss what research findings on neuromyths currently exist and what problems and objects for university instruction result from them.

Neuromyths on the topic of learning and memory

Grospietsch and Mayer (2019) identified 11 neuromyths on the topic of learning and memory. The study's scientific content analysis showed that each of these misconceptions is based on a *kernel of truth* (= scientific term/research finding) and morphs over a chain of erroneous conclusions into a no-longer-scientifically-correct implication for teaching and learning (= neuromyth). Insights from neuroscience and cognitive psychology form the starting points for each fallacious line of argument. Table 1 compares the kernel of *truth* and neuromyth for the nine

Table 1: Scientific *kernels of truth* and the neuromyths resulting from them.

Scientific *kernel of truth*	Neuromyth
Perception preferences Learners exhibit preferences for receiving information in a specific mode.	*Existence of learning styles* Individuals learn better when they receive information corresponding to their learning style (e. g., auditory, visual, haptic, or intellectual).
Crossover in neural pathways Neural pathways link the left brain hemisphere to the right side of the body and vice versa.	*Effectiveness of Brain Gym* Coordination exercises (e. g., cross-body movements) can improve the interaction between the left and right brain hemispheres and thus learning and/or intelligence.
Existence of cortical regions The cerebrum is made up of different cortical regions subject to a functional division of labor.	*Specific storage locations (hard drive)* The brain works like a hard drive. Information is stored in specific locations (e. g., in *the* center for math).
Hemispheric dominance One brain hemisphere is more strongly involved in a certain cognitive process than the other.	*Differences due to hemispheric use* Each person uses their left and right brain hemispheres to different degrees, which explains differences between learners. This hemispheric dominance needs to be taken into account.
Brain development Neural cell connections enormously increase in the first years of life.	*Best learning before age 3* Learners are most receptive to learning processes from birth until age 3.
Hemispheric asymmetry Two cerebral hemispheres exist that are not completely identical, both anatomically and functionally.	*Logic on the left/creativity on the right* Creative thought processes engage the right brain hemisphere, while logical thought processes engage the left. Deliberate effort must be made to equally engage both brain hemispheres.
Sensitive phases in child development There are sensitive phases in childhood during which certain things can be learned more easily and in which isolation from stimuli can lead to irreversible damage (e. g., language acquisition).	*Critical time periods for learning* Children must be presented with as many *good* stimuli as possible during this time window that then closes irrevocably so that their learning will not be impaired throughout their life, as this cannot be corrected through education.
Brain activity Imaging techniques make it possible to measure which brain regions are involved in a mental or physical activity.	*Only use 10% of the brain* We only use the 10% of our brain regions highlighted in images (e. g., fMRI) and thus only a fraction of our mental capacity.
Consolidation Nighttime restructuring processes can lead to the gaining of new insights during sleep.	*Learning while you sleep* Completely new content can be learned during sleep via the acoustic channel (e. g., audio recordings of vocabulary lists).

Note: This table was created based on a summary of the current state of theory on neuromyths as well as supplementary literature research: Bear et al. (2018), Biswal et al. (2010), Carter (2014), Dekker et al. (2012), de Lussanet and Osse (2012), Gais and Born (2004), Grospietsch and Mayer (2019); Höffler et al. (2017), Jäncke (2013), OECD (2002).

neuromyths addressed in this paper to provide an overview of the problem of neuroscientific research findings being inaccurately transferred to teaching and learning. Three concrete examples of the individual errors in transfer involved as well as the scientific refutation of the neuromyths *learning while you sleep*, *logic in the left hemisphere/ creativity in the right*, and *that we only use 10% of the brain* can be found in Grospietsch and Mayer (2019).

Numerous studies (cf. Figure 1) show that pre-service and in-service teachers as well as university educators exhibit great interest in neuroscience but are simultaneously unable to differentiate between neuromyths and neurofacts. There is a general tendency to endorse neuroscientific statements about the topic of learning and memory – regardless of whether or not they are neuromyths (Grospietsch and Mayer, 2019). University professors and instructors who train future teachers endorse neuromyths at slightly lower rates than (pre-service) teachers (Gleichgerrcht et al., 2015; van Dijk and Lane, 2018). In-service teachers, in turn, endorse neuromyths a little less frequently than pre-service teachers (Canbulat and Kiriktas, 2017). However, Zhang et al. (2019) and Horvath et al. (2018) demonstrate that even headmasters and award-winning teachers endorse neuromyths at high levels.

Research on the prevalence of neuromyths can be summarized as mostly consistent, with the exception of a few cultural differences between countries. Many neuromyths on learning and memory are endorsed to a high degree. Myths concerning the *effectiveness of Brain Gym* and *existence of learning styles* are particularly widespread and have found their way into learning guides and educational programs (Grospietsch and Mayer, 2019; Pasquinelli, 2012). Research findings on the factors determining the endorsement of neuromyths are more diverse. Ferrero et al. (2016) conclude that reading educational magazines increases beliefs in neuromyths. Conversely, Düvel et al. (2017) show that reading a large number of educational books, magazines, and websites reduces endorsement of neuromyths. Research by Macdonald et al. (2017) and Ferrero et al. (2016) indicates that reading scientific journals reduces beliefs in neuromyths. In contrast, Gleichgerrcht et al. (2015) determine that neither neuroscientific nor popular science articles sufficiently reduce endorsement of neuromyths. Macdonald et al. (2017) could show that people with high levels of neuroscientific knowledge endorse neuromyths to a lesser degree than teachers and the general public. Papadatou-Pastou et al. (2017) emphasize that general knowledge about the brain is the best "safeguard against believing in neuromyths" (p. 1). This result is corroborated by van Dijk and Lane (2018). However, in numerous studies, teachers with high levels of

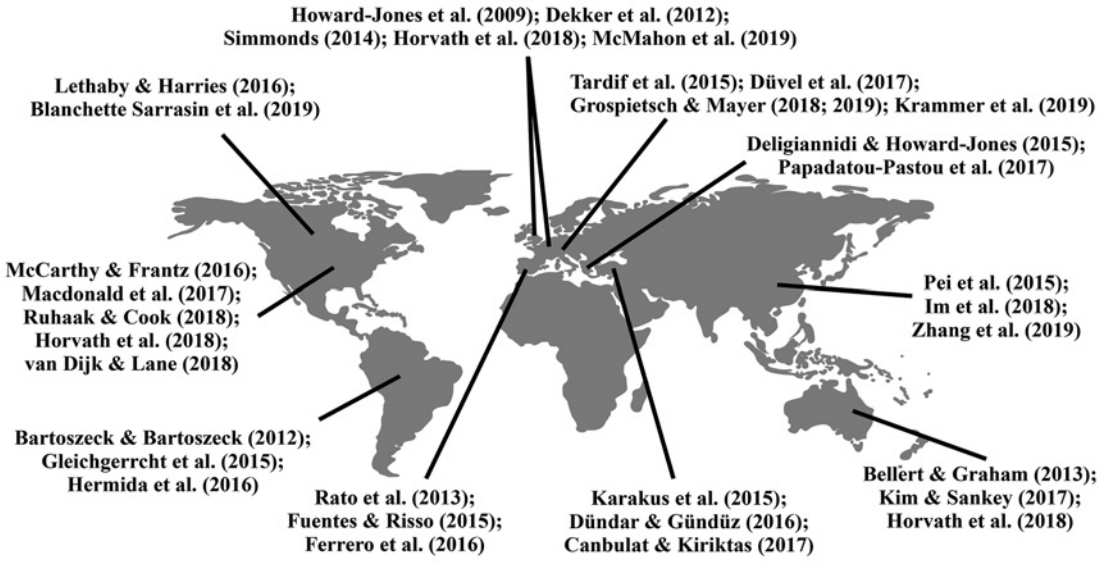

Figure 1: Overview of existing studies on neuromyths among pre-service teachers, in-service teachers, and university educators.

scientifically appropriate conceptions of the brain prove to be more susceptible to neuromyths (e. g., Dekker et al., 2012; Ferrero et al., 2016; Papadatou-Pastou et al., 2017). Research findings concerning personal characteristics are also inconsistent. The majority of studies show that age, gender, professional experience, teaching subject, school type, school location (urban/rural) and participation in professional development courses are not correlated with the endorsement of either neuromyths or scientifically appropriate conceptions about the brain (e. g., Dekker et al., 2012; Karakus et al., 2015; Papadatou-Pastou et al., 2017; Rato et al., 2013). Macdonald et al. (2017) conclude that being younger, having a university degree, and attending neuroscience courses reduce but do not eliminate endorsement of neuromyths. The latter result is corroborated by Canbulat and Kiriktas (2017) as well as Ruhaak and Cook (2018). Four studies have found an association between endorsement of neuromyths and gender. In two studies, female teachers are more likely to endorse neuromyths (Dündar and Gündüz, 2016; Ferrero et al., 2016), but they outperform male subjects in two other studies (Canbulat and Kiriktas, 2017; Macdonald et al., 2017). In sum, we primarily know one thing: pre-service and in-service teachers as well as university educators endorse numerous

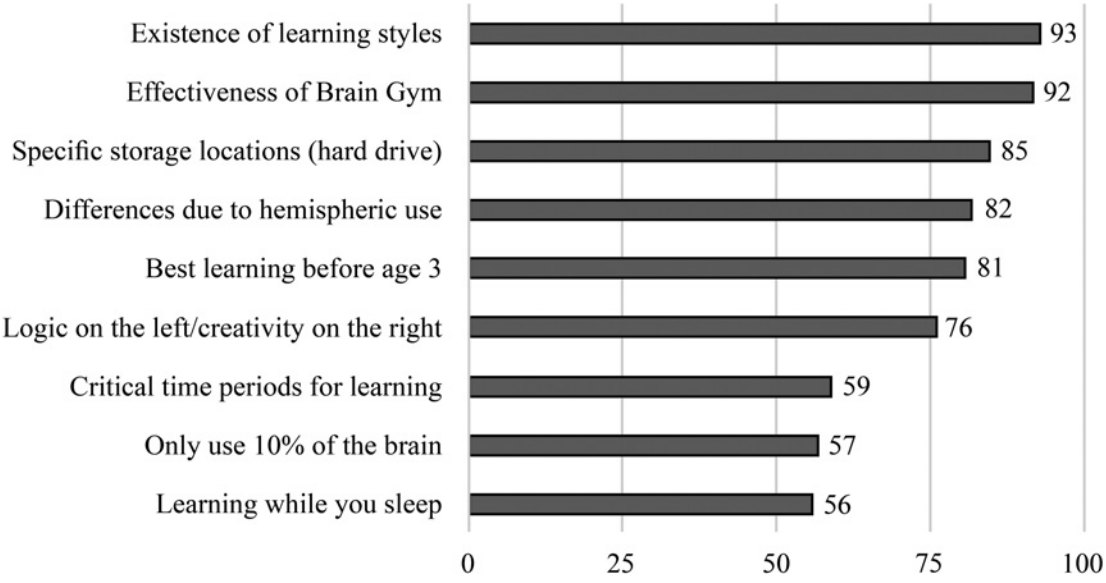

Figure 2: Pre-service biology teachers' (N = 550) endorsement of nine neuromyths on the topic of learning and memory (Grospietsch and Mayer, 2019).

neuromyths on the topic of learning and memory. The leading determinants of these beliefs and how they can be effectively reduced still remain open questions.

Neuromyths' resistance as a problem of university instruction

Although the current research literature on neuromyths calls for integrating more neuroscience into teacher training (e. g., Howard-Jones, 2014), this alone does not seem to be sufficient to professionalize pre-service teachers' misconceptions on the topic of learning and memory. According to Dündar and Gündüz (2016), pre-service science teachers significantly outperform pre-service teachers of other subjects, whereas studies by Macdonald et al. (2017) and Im et al. (2018) indicate that mere enrollment in neuroscience or psychology courses at university does not sufficiently reduce endorsement of neuromyths. A study by Grospietsch and Mayer (2019) showed that even pre-service biology teachers, who receive instruction in neuroscientific content during their studies (e. g., courses in human biology and animal physiology), endorse neuromyths to a great extent. As shown in Figure 2, all nine misconceptions on the topic of learning and memory were endorsed by more than half of pre-service biology teachers. Participants at different stages of their training (first-year students, more advanced students, and graduates enrolled in practical teacher preparation) differed only with respect to their endorsement of scientifically appropriate conceptions, but not in their endorsement of neuromyths (Grospietsch and Mayer, 2019).

Given that biology teachers need to not only address the topic of learning and the brain as instructional content but also use it to guide their students' learning processes, the conceptions of pre-service biology teachers – up until the end of their practical training phase – must be described as deficient. The results of another study (Grospietsch and Mayer, 2018) show that even a university course conveying and closely interlinking professional knowledge from the fields of cognitive psychology, neuroscience, and biology didactics on the topic of learning and the brain is insufficient for students to critically engage with neuromyths. The results of Grospietsch and Mayer's (2019) study indicate that neuromyths exist in parallel to accurate professional knowledge and beliefs about neuroscience and learning and can prove to be resistant to conventional teacher education. This means that even after acquiring professional knowledge, university students are released into practice with misconceptions. According to Horvath et al. (2018), there is still a lack of studies proving that endorsement of neuromyths negatively

affects teachers' effectiveness, students' learning performance or their perceived self-efficacy. However, Lethaby and Harries (2016) and Blanchette Sarrasin et al. (2019) highlight that many teachers who endorse neuromyths employ practices linked to these misconceptions in their instruction (with pre-school teachers doing so most frequently, followed by primary school teachers and then secondary school teachers). This is problematic on the one hand because it could lead teachers to pass on incorrect cognitive psychology/neuroscience content and/or ineffective learning strategies to their students. On the other hand, the education system's "money, time and effort" (Dekker et al., 2012, p. 1) could be wasted and both teachers and learners are deprived of the opportunity to expend these resources on more effective theories and methods (e. g., teaching learning strategies or cognitive activation) (Grospietsch and Mayer, 2019). Ruhaak and Cook (2018) show that accurate beliefs about neuromyths are associated with a higher probability of employing effective, rather than ineffective, neuromyth-based instructional practices. Hence, developing university instruction programs for pre-service teachers as well as professional development opportunities for in-service teachers that clarify neuromyths scientifically and professionalize them sustainably is of great relevance.

Brain-friendly learning and neuromyths as an object of university instruction

Related to the goal of lifelong learning in a rapidly developing society, a professional understanding of learning is important for both teachers and learners. University education should be capable of providing students with an appropriate conception of learning. Langfeldt and Nieder (2004) summarize, with respect to teacher education, that around one-third of students' learning concepts prove to be resistant to change over the course of their studies and cannot be sufficiently professionalized into more pedagogically-desirable concepts. Studies on neuromyths show that (pre-service) teachers encounter neuromyths and related practices in their academic and practical training and professional development (Blanchette Sarrasin et al., 2019; Howard-Jones et al., 2009; Lethaby and Harries, 2016; Ruhaak and Cook, 2018; Tardif et al., 2015). Although (pre-service) teachers primarily refer to TV, the internet and popular science magazines in their research (Ferrero et al., 2016; Rato et al., 2013), university instructors should view their teaching as a significant opportunity to build up accurate neuroscientific knowledge, a well-founded conception of neurodidactic, and an evidence-based understanding of learning (Grospietsch and Mayer, 2019). Thus, in light of the reported research findings, it seems necessary for the disciplines involved (neurobiology, cognitive psychology, education science, and subject and university didactics) not to leave the field to pop science but rather to actively counter the misunderstanding, misreading, or misquoting of facts scientifically established by brain research to make a case for use of brain research in education and other contexts (OECD, 2002, p. 111). Pithy yet empty promises, such as *learn vocabulary while you sleep* or *Brain Gym exercises make you smarter*, are becoming more and more common in everyday life. Advertised by companies as "low-cost and easily implemented classroom approaches" (Howard-Jones, 2014, p. 819) promising to improve learning and/or memory performance, neuromyths find their way into teachers' methodological repertoires, which they pass on to their students with the best of intentions (Simmonds, 2014). These findings suggest that the prevalence of neuromyths should be responded to not only with criticism but also by constructively addressing the problem. Previous studies on neuromyths (cf. Figure 1) all indicate that teachers and university students are highly interested in neuroscience but need help to correctly relate elements of knowledge from cognitive psychology, neuroscience, and subject didactics and to critically engage with information lacking a grounding in proper evidence. Grospietsch and Mayer (2018) developed a neurodidactic concept for times in which the catchphrase *brain-friendly learning* enjoys persistent popularity to clarify for (pre-service) teachers and university educators that although neurodidactics is certainly a plausible concept, it can become a myth when applied incorrectly. Moreover, Papadatou-Pastou et al. (2017) emphasize the importance of developing an understanding among (pre-service) teachers of how neuroscience research is conducted and presented (e. g., understanding images which show increased brain activity). Consequently, both neuroscience content/methods and an accurate foundation for neurodidactics approaches should be incorporated into teacher education and university didactics. A corresponding curriculum would be quite helpful.

One aspect that university instructors need to be aware of when addressing neuromyths is that these misconceptions can be deeply biographically anchored and difficult to change (Grospietsch and Mayer, 2019). Grospietsch and Mayer (2018) show that students argue not only scientifically in support of neuromyths (e. g., based on neuroscience and cognitive psychology) but also use biographical arguments (e. g., referring to personal experiences) and that refutation can even reinforce their

misconceptions (= backfire effect; cf. Cook and Lewandowsky, 2011). Work by Pettito and Dunbar (2004) has highlighted that students can stubbornly cling to their original beliefs despite empirical demonstrations and theoretical representations. Newton and Miah (2017) demonstrate this specifically for the neuromyth concerning the *existence of learning styles*. Moreover, based on a study by Kim and Sankey (2017), it must be acknowledged that pre-service teachers may have already learned neuromyths before beginning their university studies, that is, during their own school years. They can also be deeply convinced of their misconceptions due to their practical experiences or intuitively believe them to be true (cf. Blanchette Sarrasin et al., 2019). These are all potential reasons why few effective intervention approaches to combat neuromyths currently exist (Grospietsch and Mayer, 2018; McCarthy and Frantz, 2016; McMahon et al., 2019). Teaching strategies and methods that take up students' misconceptions, that deliberately bring them into a *cognitive conflict*, and that systematically expand them in the direction of scientifically appropriate conceptions have proven to be particularly effective at combating neuromyths (Grospietsch and Mayer, 2018). Based on this *conceptual change theory* (Vosniadou, 2013), a course was developed through an interdisciplinary collaboration at the University of Kassel (Grospietsch and Mayer, 2018). In contrast to merely imparting professional knowledge from cognitive psychology, neuroscience, and subject didactics, the instructional material used in this seminar, *conceptual change texts*, proved to be an effective and evidence-based means of translating neuroscientific content into the language of teachers. Moreover, both neuromyths and scientifically appropriate conceptions were sustainably professionalized with medium to large effect sizes (Grospietsch and Mayer, 2018). Based on our experiences, we can report that the quality of such learning programs can be improved by having neuroscientists and cognitive psychologists clear up neuromyths in a scientifically accurate way based on the most current research results. Precisely because disciplines have their unique methods and languages that are difficult to understand for experts in other areas, there is a need for cooperation among teacher educators, cognitive psychologists, and neuroscientists. Only by intensifying (existing) exchange networks can (pre-service) teachers' and university instructors' neuroscience literacy be improved and neuromyths related to *brain-friendly learning* be eliminated. In particular,

neuroscientists interested in a content-based collaboration are warmly invited to contact us.

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Bionotes

Finja Grospietsch
Department of Biology Education, Faculty of Mathematics and Science, University of Kassel, Heinrich-Plett-Straße 40, Kassel, 34132, Germany
finja.grospietsch@uni-kassel.de
https://orcid.org/0000-0002-4141-9671

Finja Grospietsch studied biology and German education for secondary schools at Kiel University (M. Ed. 2015) and obtained her PhD in biology didactics (Dr. rer. Nat.) at the University of Kassel in 2020. She works there as a research associate and coordinator for the project PRONET[2] (*Professionalisierung durch Vernetzung - Fortführung und Potenzierung*), which is part of the *Qualitätsoffensive Lehrerbildung*, a joint initiative of the German Federal Government and the Federal States.

Jürgen Mayer
Department of Biology Education, Faculty of Mathematics and Science, University of Kassel, Heinrich-Plett-Straße 40, Kassel, 34132, Germany
jmayer@uni-kassel.de
https://orcid.org/0000-0003-4150-0688

Jürgen Mayer researches in the field of biology didactics at the University of Kassel. He studied biology, chemistry, philosophy and education at the universities in Göttingen and Kiel and obtained his PhD in biology at Kiel University in 1992. Following several years of research in biology didactics at the Leibniz Institute for Science and Mathematics Education at Kiel University, he assumed a professorship for biology didactics at Giessen University in 1999. In 2009, he transferred to a position as Professor of Biology Didactics at the University of Kassel. His current research focuses on teaching and learning research with respect to subject didactics, modeling and assessment of scientific competence and science teacher education.

Review article

Julia Machon, Jakob Krieger, Magali Zbinden, Juliette Ravaux and Steffen Harzsch*

Exploring brain diversity in crustaceans: sensory systems of deep vent shrimps

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Abstract: The current report focuses on shrimps from deep hydrothermal vents of the Mid-Atlantic Ridge that live in an environment characterized by high hydrostatic pressure, lack of sunlight, and with hot and potentially toxic emissions of black smoker vents. Malacostracan crustaceans display a large diversity of lifestyles and life histories and a rich repertoire of complex behavioral patterns including sophisticated social interactions. These aspects promote this taxon as an interesting group of organisms for those neurobiologists interested in evolutionary transformation of brain structures and evolutionary diversification of neuronal circuits. Here, we explore how analyzing the nervous system of crustacean species from extreme habitats can provide deeper insights into the functional adaptations that drive the diversification of crustacean brain structure.

Keywords: brain evolution; hydrothermal vent; neuroanatomy; place memory; *Rimicaris exoculata*

Zusammenfassung: Innerhalb der höheren Krebstiere (Malacostraca) finden sich Vertreter mit einer großen morphologischen und ökologischen Diversität, die sich außerdem durch eine Vielfalt an unterschiedlichen Lebenszyklen und komplexen Verhaltensweisen auszeichnen. Diese Diversität bietet sehr gute Voraussetzungen, um evolutive Transformationen von Gehirnstrukturen und Sinnessystemen im Spannungsfeld zwischen funktionalen und phylogenetischen Zwängen zu analysieren. Insbesondere die Neurobiologie von Tieren aus extremen Lebensräumen kann uns neue Einblicke in funktionale Anpassungen ermöglichen, die die evolutive Diversifizierung von Gehirnstrukturen antreiben. In diesem Beitrag stellen wir die Sinnessysteme von Garnelen der Tiefsee vor, die eng assoziiert mit „black smoker“ hydrothermalen Quellen des Mittelatlantischen Rückens leben, einem lichtlosen und auf den ersten Blick lebensfeindlichem Habitat, dass durch hohen hydrostatischen Druck und die toxischen Emissionen der heißen Quellen geprägt ist.

Schlüsselwörter: Evolution des Gehirns; hydrothermale Quelle; Neuroanatomie; räumliches Gedächtnis; *Rimicaris exoculata*

***Corresponding author: Steffen Harzsch,** Department of Cytology and Evolutionary Biology, Zoological Institute and Museum, University of Greifswald, D-17498, Greifswald, Germany,
E-mail: steffen.harzsch@uni-greifswald.de. https://orcid.org/0000-0002-8645-3320
Julia Machon, Magali Zbinden and Juliette Ravaux: UMR CNRS MNHN 7208 Biologie des Organismes et Écosystèmes Aquatiques (BOREA), Equipe Adaptation aux Milieux Extrêmes, Sorbonne Université, Paris, France
Jakob Krieger: Department of Cytology and Evolutionary Biology, Zoological Institute and Museum, University of Greifswald, Greifswald, Germany

Exploring the diversity of crustacean brains

Among the arthropods, crustaceans represent an important subgroup that displays a large diversity of sizes, morphologies, lifestyles, and life histories (for a review, see Schram, 2013). Specifically, members of the malacostracan crustaceans (Figure 1) have colonized habitats extending from the deepest ocean trenches and hydrothermal vents, through coastal, estuarine, to freshwater ecosystems. They also display a rich repertoire of complex behavioral patterns related to finding food, shelter, and mating partners; kin recognition and brood care; and orientation and homing. Complex social interactions include the establishment of dominance hierarchies, communal defensive tactics, the occupation of common shelters, and cooperative behavior during long-distance, offshore seasonal migration (reviewed by Breithaupt and Thiel, 2011; Derby and Thiel, 2014; Duffy and Thiel, 2007; Thiel and Walting, 2015). Their striking morphological, behavioral, and ecological diversity promotes this taxon as an interesting group of organisms for those neurobiologists interested in evolutionary transformation of brain

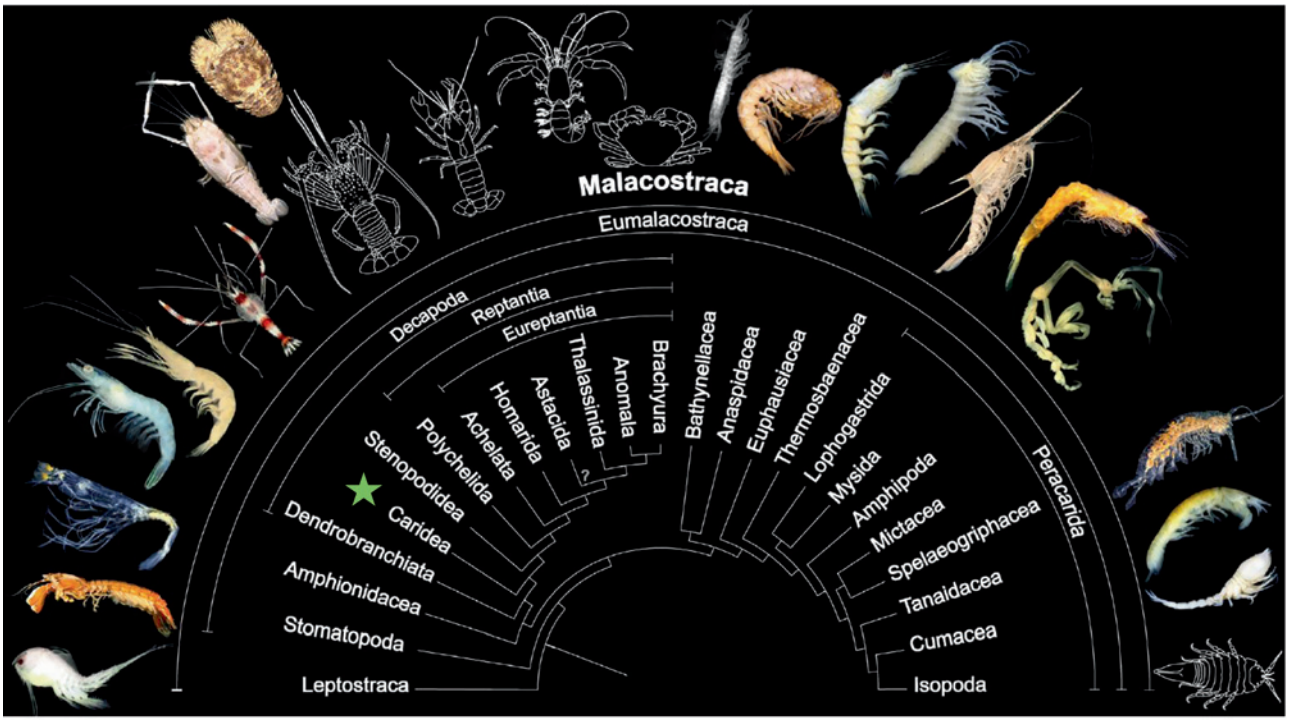

Figure 1: Phylogenetic relationships of malacostracan crustaceans (modified from Harzsch and Krieger, 2018; compiled after Richter and Scholtz, 2001; and Wirkner and Richter, 2010). The green asterisk show the phylogenetic position of the deep vent shrimp *Rimicaris exoculata* among Caridea.

structures and evolutionary diversification of neuronal circuits, specifically considering the antagonistic action of phylogenetic and functional constraints (Sandeman et al., 2014a, 2014b; Strausfeld, 2012). For example, crustaceans such as krill (*Euphausia superba*), which dominate the vast water bodies of the Southern oceans (Hempel, 1987), have evolved specific mechanisms of sensory-motor integration, which facilitates swimming in formation while schooling (Patria and Wiese, 2004). Cleaner shrimps, which are famous for their cleaning behavior of different species of coral reef fish and serve as iconic examples for communication across the vertebrate/invertebrate split (*Urocadirella sp*., Becker et al., 2005), display specific adaptation in their central mechanosensory pathways (*Stenopus hispidus*, Krieger et al., 2019). Fiddler crabs of the genus *Uca* are characteristic crustaceans of equatorial intertidal mud flats and serve as models to analyze crustacean visual ecology and the neuroethology of homing behaviors (Tomsic, 2016; Zeil and Hemmi, 2006).

We are interested in determining how the sensory landscape that the animals analyze in their various habitats is mirrored in the phenotype of the sensory systems and the morphology of the primary sensory brain areas, and against this background, we have previously studied the brains of a large diversity of malacostracan crustaceans (Figures 1 and 2). By describing the relative proportions of brain areas known to be involved in processing certain types of sensory input and comparing these proportions to other crustaceans' brains may reflect how important these senses are for the animals to analyze their environment (Sandeman et al., 2014a, 2014b). Specifically, analyzing the nervous system of crustacean species from extreme habitats may provide deeper insights into the functional adaptations, which drive the diversification of crustacean brain structure. One obvious example comes from crustacean living in lightless habitats, an environmental factor that promotes the simplification of visual systems (Ramm and Scholtz, 2017; Stegner et al., 2015). Representatives of the malacostracan crustaceans have also invaded terrestrial habitats multiple times independently (for a review, see Hansson et al., 2011) and representatives of isopod crustaceans survive and reproduce not only in our European forests and gardens but also in desert salt pans of North Africa. Neurobiological studies suggest that terrestrial isopod crustaceans have failed to evolve aerial olfaction during their evolutionary diversification from marine ancestors (Harzsch and Hansson, 2008), whereas representatives of terrestrial hermit crabs have invested

huge amounts of neuronal tissue in their olfactory pathway display a superb aerial sense of smell (Harzsch and Hansson, 2008; Krieger et al., 2010). Here, we explore what another example of crustaceans subjected to strong environmental constraints can teach us about the functional adaptations of crustacean sensory systems.

Introduction to vent shrimp sensory ecology

Since their discovery in the late 1980s, shrimps from deep hydrothermal vents have sparked interest among biologists because of their particular habitat and lifestyle. In addition to the high hydrostatic pressure and the absence of sunlight that characterize their environment, vent shrimps live in the surrounding areas of black smoker hydrothermal vents (Figure 3A, B). Hydrothermal fluids are hot (up to 350 °C) and rich in potentially toxic chemicals (Charlou et al., 2000, 2002, 2010). A key adaptation of the *Rimicaris exocualta* shrimps is their symbiotic association with chemoautotrophic bacteria that provide energy to their hosts by oxidizing the fluid chemicals. Hence, in light of their habitat, the fairly extreme conditions they must cope with, and their lifestyle, these vent shrimps are fascinating models to study biological adaptations to the environment.

An important question in vent shrimp ecology is to understand the sensory mechanisms that the animals use for orientation in their dark environment, to select a suitable habitat, to find food, to detect congeners, or to locate active smokers to supply their symbiotic bacteria with chemicals. Because they colonize the vicinity of black smokers, it has been suggested that they might exploit abiotic factors of the hydrothermal fluid as orientation cues (Sarrazin et al., 1999; Segonzac et al., 1993). The commonly considered factors are the fluid chemicals, temperature, and thermal radiation emitted by the hot fluids. The shrimp may have evolved specific abilities to detect these stimuli, and the related senses could play a major role in orientation within the habitat.

Therefore, analyzing the architecture of the peripheral and central sensory systems provides new insights into the sensory biology of vent shrimps (Figures 3 and 4), knowledge that is essential for learning their ecology and long-term evolutionary adaptations. Comparative studies with shallow water relatives are especially relevant in this context to reveal sensory adaptations to the habitat and related evolutionary processes (Derby and Weissburg, 2014).

A unique visual system

The first anatomical observations of vent shrimp specimens had led to the reassessment of the general belief that deep sea animals were entirely blind (Van Dover et al., 1989). Vent shrimps possess highly modified eyes, which, in *Rimicaris exoculata*, consist of large ocular plates located at the anterodorsal region of the cephalothorax (Figures 3C and 5A). Their retinal structure consists of a smooth cornea that covers a layer of photoreceptive rhabdoms, under which reflective cells of the tapetum are located (Figure 5B). Several authors have shown that the rhabdoms are hypertrophied, which would maximize the absorption of light (Chamberlain, 2000; Jinks et al., 1998; Machon et al., 2019; Nuckley et al., 1996; O'Neill et al., 1995). However, in the specimen presented here, the rhabdoms are degenerated, which appears to be a consequence of a dramatic deterioration of the retina following the intense light exposure during sampling, as shown by Herring et al. (1999). In addition, the dioptric apparatus, which is a characteristic of typical crustacean compound eyes, is lacking, indicating that these eyes cannot form images. Functional rhodopsin-like visual pigments were also identified in high quantities in the retina of *R. exoculata* (Van Dover et al., 1989) (Figure 5C). At the central nervous level, as in other malacostracans, vent shrimp present a suite of retinotopic visual neuropils (lamina, medulla, and lamina) (Figure 5D) that are strongly reduced in size and that are fused with the median brain (Figures 4 and 5A), coinciding with the absence of eyestalks (Machon et al., 2019).

Because selective pressure favors the reduction of unsolicited nervous tissues (Klaus et al., 2013; Moran et al., 2015; Niven and Laughlin, 2008), the presence of visual neuropils, together with a seemingly functional retina, is indicative of an effective visual system in vent shrimp, which implies that a light signal does occur in their environment. The prominent hypothesis is that vent shrimp might have evolved a highly sensitive retina, to the detriment of spatial resolution, to detect the very dim light of the thermal radiation emitted by the hot (up to 350 °C) hydrothermal fluids as they exit the black smoker (Pelli and Chamberlain, 1989; Van Dover and Fry, 1994; Van Dover et al., 1988, 1996).

A common olfactory system

Asking how the shrimps can detect active vent areas, biologists had previously proposed that they locate black smokers by chemotaxis, reacting to specific chemical compounds of the hydrothermal fluids (Segonzac et al.,

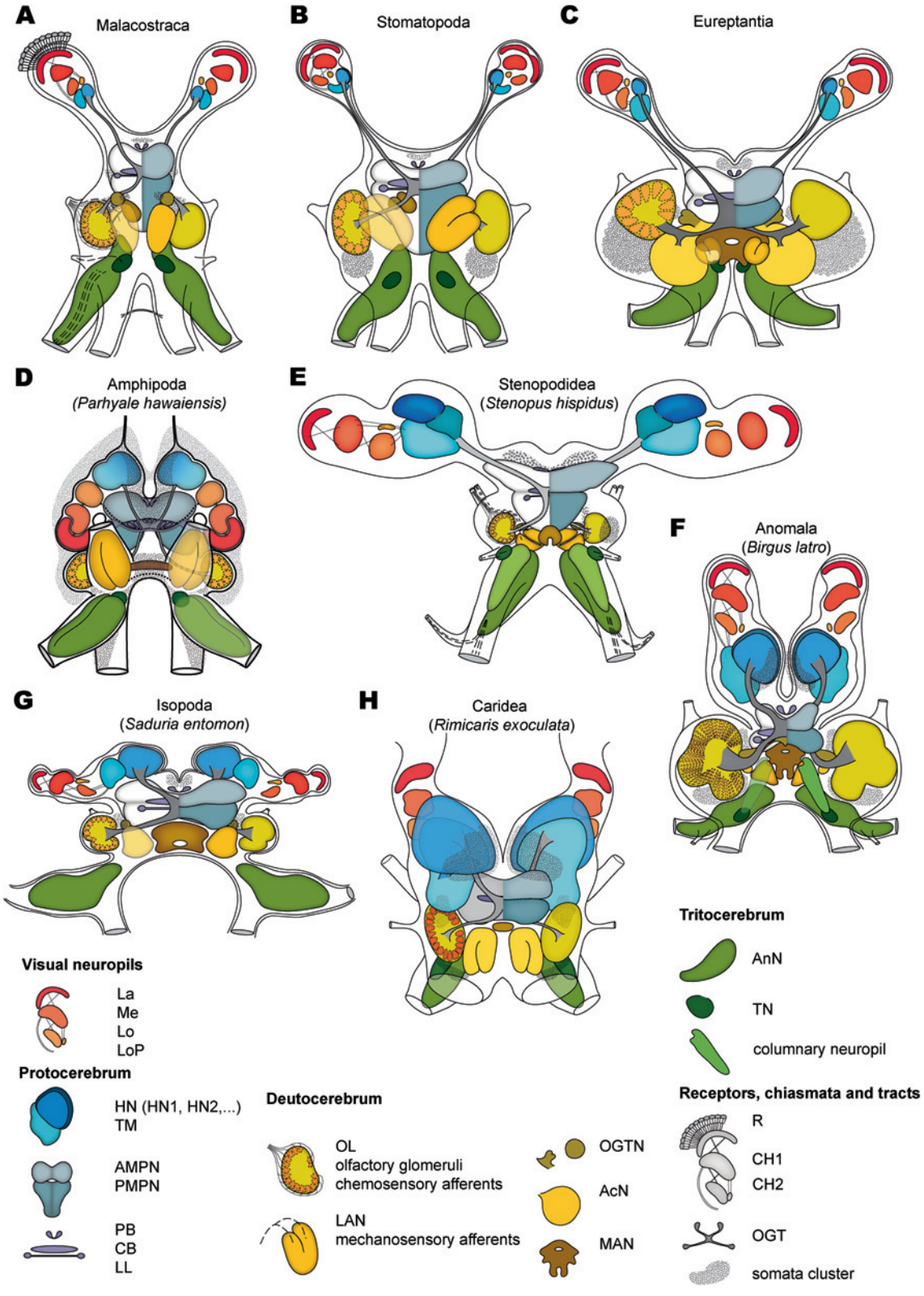

Figure 2: Brain anatomy of malacostracans (modified from Sandeman et al., 2014a, 2014b). The subdivisions of the proto-, deuto-, and tritocerebrum can be identified by their shape and color provided in the key. (A) Malacostraca. (B) Stomatopoda. (C) Eureptantia. (D) Amphipoda (*Parhyale hawaiensis*; from Wittfoth et al. 2019). (E) Stenopodidea (*Stenopus hispidus*; from Krieger et al., 2019). (F) Anomala (*Birgus latro*). (G) Isopoda (*Saduria entomon*). (H) Caridea (*Rimicaris exoculata*; from Machon et al., 2019).

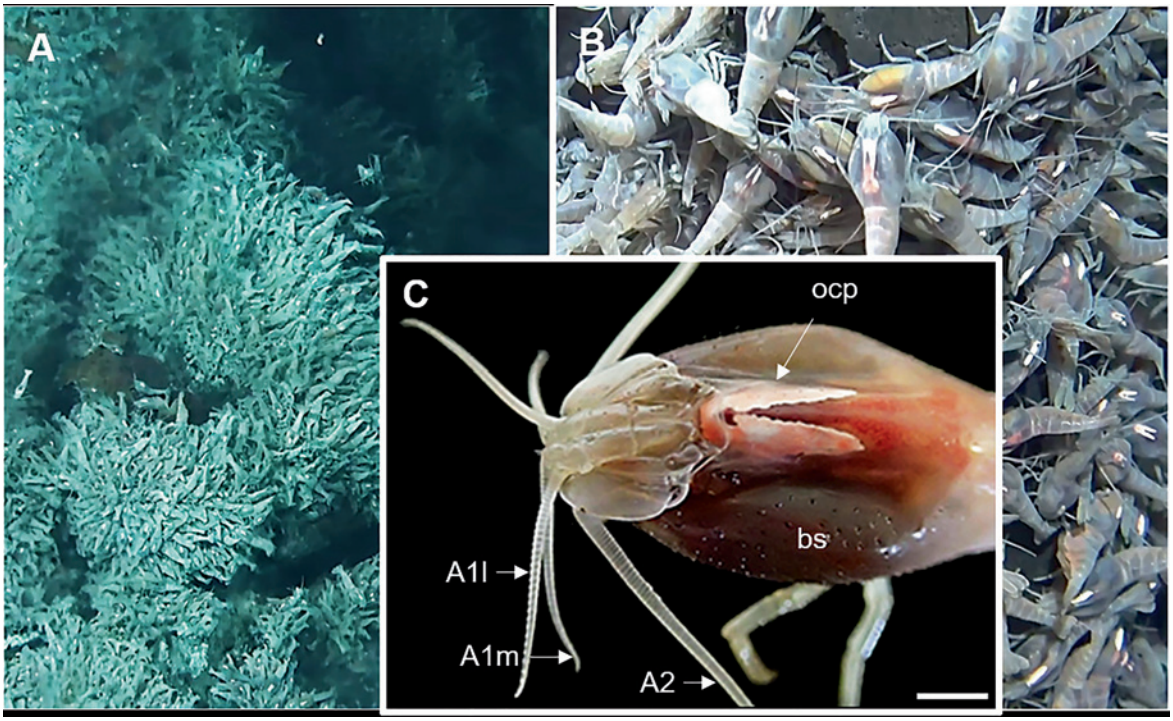

Figure 3: Swarms and morphology of the deep vent shrimp *Rimicaris exoculata*. A,B: Swarms of thousands of shrimp are surrounding the walls of black smoker hydrothermal vents at the TAG vent site (3600 m depth), Mid-Atlantic Ridge (©IFREMER/Nautile6000, BICOSE 2018 cruise). C: Dorsal view of the cephalothorax showing voluminous gill chambers covered by the branchiostegites, dorsal eyes (namely the ocular plate) with two elongated retinae fused in the anterior region, and sensory appendages (antennae 1 and 2). A1l, lateral flagellum of antenna 1; A1m, medial flagellum of antenna 1; A2, antenna 2; bs, branchiostegite; ocp, ocular plate. Scale bar = 5 mm. Modified from Machon et al. (2019).

1993). This hypothesis was soon supported by electrophysiological recordings of concentration-dependent sulfide sensitivity from the first antenna nerves of *R. exoculata* (Figure 6A), and the authors suggested that these shrimp may present enhanced olfactory abilities to detect naturally occurring sulfide gradients in the near field of the vents (Jinks et al., 1998; Renninger et al., 1995). The complexity of the animal's peripheral and central olfactory pathways may reflect these functional demands in that their chemical senses evolved to compensate for the underperformance of the visual system.

However, a comparative study of the first antenna and the specialized olfactory aesthetasc sensilla (Figure 6B, C) of *R. exoculata*, three other vent shrimp species, and a closely related shallow-water species revealed no specific adaptation between hydrothermal and coastal species regarding the chemosensory organs morphology (Zbinden et al., 2017). Additionally, recent electrophysiological recordings from the antennae in the vent shrimp *Mirocaris fortunata* and a related member of the Caridea, the shallow-water shrimp *Palaemon elegans*, showed that sulfide detection is not specific to the vent species (Machon et al., 2018). Hence, no features indicative of a more sophisticated olfactory performance have been identified from the peripheral system of vent shrimp thus far. Nevertheless, a noticeable aspect of vent shrimp is the dense coverage of their antennae and aesthetasc sensilla by bacteria (Figure 6D), which were identified to be similar to known chemoautotrophic sulfur oxidizers and may thus influence the chemosensory system in several ways, but their specific roles are unknown yet (Zbinden et al., 2018).

Regarding the sensory centers, a thorough description of the brain neuroanatomy in *R. exoculata*

Abbreviations: Visual neuropils: La, lamina; Me, medulla; Lo, lobula; LoP, lobula plate neuropil. Protocerebrum: HN (HN1, HN2), hemiellipsoid body (subdivisions thereof); TM, terminal medulla; AMPN, anterior medial protocerebral neuropil; PMPN, posterior medial protocerebral neuropil; PB, protocerebral bridge; CB, central body; LL, lateral lobe. Deutocerebrum: OL, olfactory lobe; LAN, lateral antennular (antenna 1) neuropil; OGTN, olfactory globular tract neuropil; AcN, accessory lobe; MAN, median antennular (antenna 1) neuropil. Tritocerebrum: AnN, antennal (antenna 2) neuropil; TN, tegumentary neuropil; Receptors, chiasmata and tracts: R, retina; CH1, first visual chiasm; CH2, second visual chiasm; OGT, olfactory globular tract. Note that brain schemes are aligned according to their neuroaxis (not to their arrangement within the cephalothorax) and are not equally scaled.

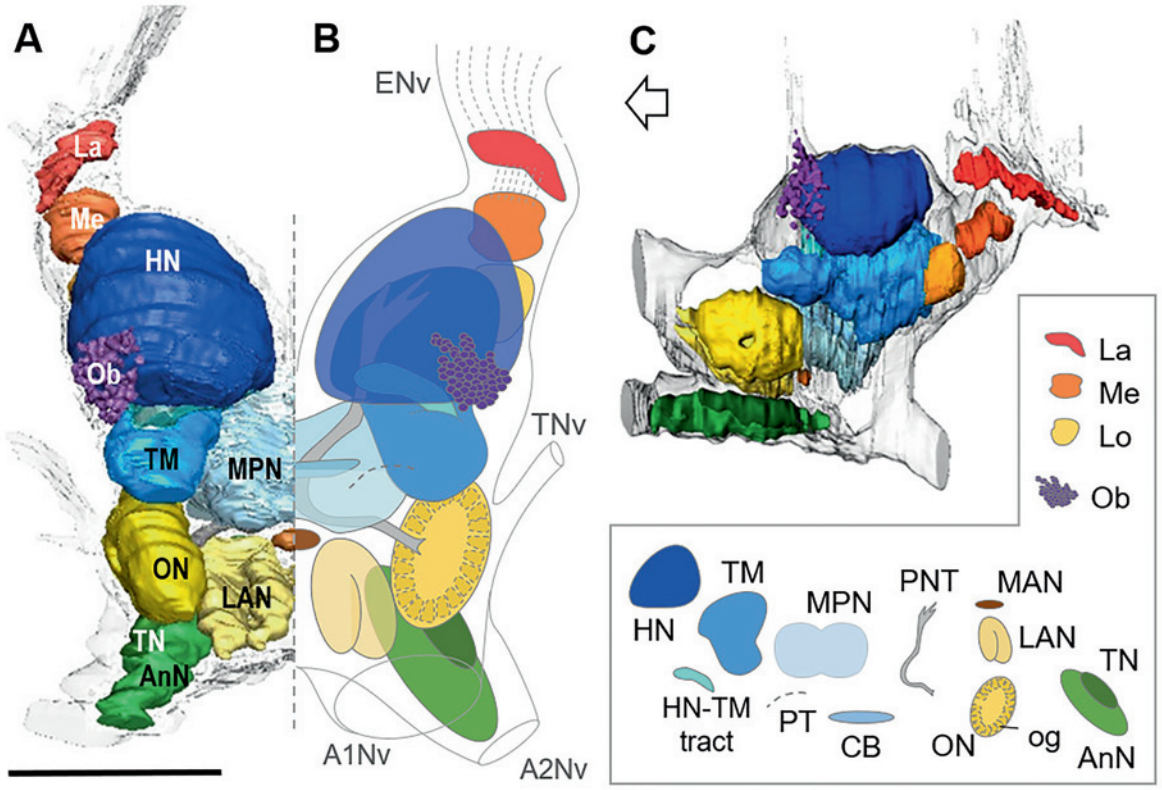

Figure 4: Brain architecture of the deep vent shrimp *Rimicaris exoculata*. 3D Reconstructions (A, C) and schematic representation (B) of the brain and neuropils, viewed from a dorsal, slightly anterior direction (A, B) and from the left (C). In C, the open white arrow point towards anterior of the body axis. The clusters of cell somata are not shown. The 3D reconstructions are based on an image stack obtained by serial sectioning of paraffin-embedded material. A1Nv, antenna 1 nerve; A2Nv, antenna 2 nerve; AnN, antenna 2 neuropil; CB, central body; ENv, eye nerve; HN, hemiellipsoid body neuropil; La, lamina; LAN, lateral antenna 1 neuropil; Lo, lobula; MAN, median antenna 1 neuropil; Me, medula; MPN, median protocerebral neuropil; Ob, onion bodies; og, olfactory glomerulus; ON, olfactory neuropil; PT, protocerebral tract; PNT, projection neuron tract; TM, terminal medulla neuropil; TN, tegumentary neuropil; TNv, tegumentary nerve. Scale bar = 500 μm. Modified from Machon et al. (2019).

allowed to search for a differential investment in the olfactory neuropils that might reflect an enhanced olfactory performance (Machon et al., 2019). These neuropils are lobe-shaped and composed of olfactory glomeruli that are radially arranged around the periphery of a non-synaptic core (Figure 6E, F) and subdivided into three regions (Figure 6F) as observed in several decapod taxa (Harzsch and Krieger, 2018). Structural features that may be linked to the efficiency of the olfactory system were compared between *R. exoculata* and other malacostracan species, showing that the olfactory neuropils are not overly hypertrophied in the vent species. Overall, the structural complexity of the olfactory system does not suggest that *R. exoculata* presents adaptations to the specific chemosensory landscape at vents, and olfaction is probably not a particularly dominant sensory modality in vent shrimp.

The hemiellipsoid body, a higher integrative brain center

Higher integrative centers in the malacostracan brain provide the neuronal substrate for more sophisticated processing and receive input exclusively from second- or higher-order neurons but not from any primary sensory afferents. Interneurons within such centers typically respond to the stimulation of several different sensory systems (reviewed by Sandeman et al., 2014a, 2014b). In the malacostracan brain, the (bilaterally paired) complex of hemiellipsoid body and terminal medulla (HE/MT) is one of these higher integrative centers. It is targeted by the axons of the olfactory projection neurons as output pathway of the olfactory system and also receives input from the visual neuropils (for reviews, see Derby and Weissburg, 2014; Harzsch and Krieger, 2018; Schmidt, 2016). Substantial morphological modifications

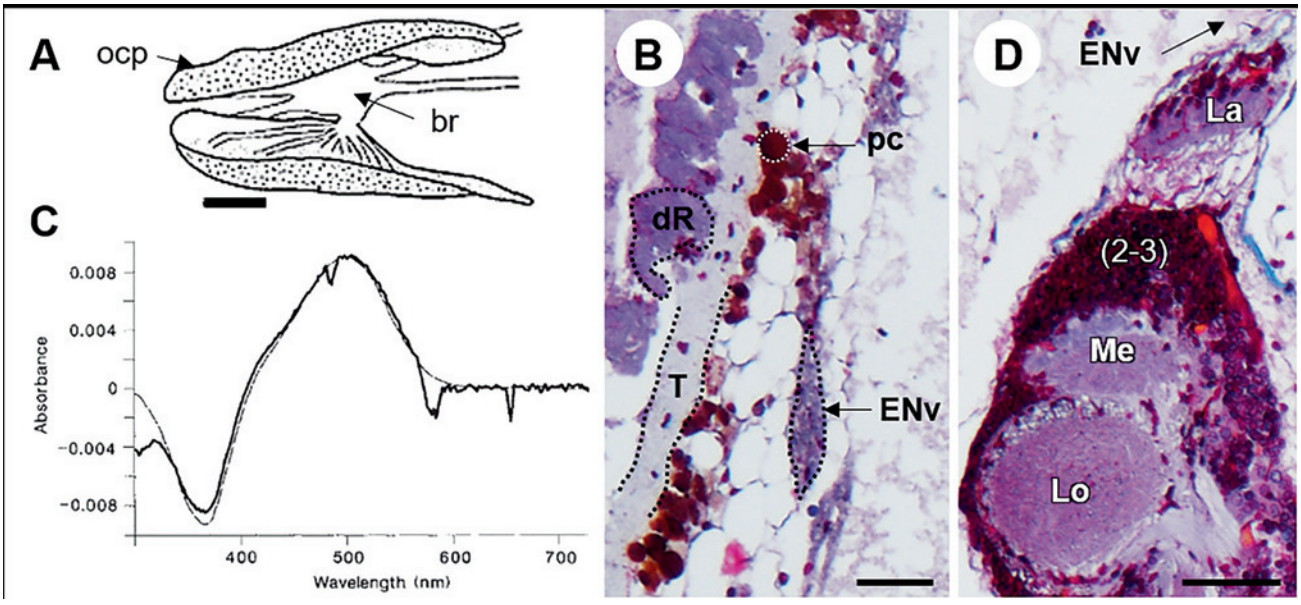

Figure 5: Visual system of the deep vent shrimp *Rimicaris exoculata*. A: Drawing showing the location of the visual organs and their connection to the brain (dorsal view; from Van Dover et al., 1989). B: Histological section of the retina. C: Bleaching difference spectrum of visual pigment in *R. exoculata* (solid trace) that maximally absorbs at 500 nm, typical of rhodopsin, and creates a new pigment at 367 nm, which corresponds to retinaloxime. The same features are seen in the difference spectrum of the frog rhodopsin (dashed line) (from Van Dover et al., 1989). D: Histological frontal section of the visual neuropils (from Machon et al., 2019). Scale bars = 100 μm. (2–3), cell cluster 2–3; br, brain; dR, degenerated rhabdoms; ENv, eye nerve; La, lamina; Lo, lobula; Me, medulla; ocp, ocular plate; pc, pigment cell; T, tapetum.

and changes related to the relative proportion of types of input occurred during the evolutionary elaboration of the HE/MT complex (reviewed, e. g., by Harzsch and Krieger, 2018; Machon et al. 2019). Nevertheless, recent studies suggest that, despite many morphological differences, the MT/HE complex of crustaceans and the iconic mushroom bodies of insects share common architectural, physiological, and neurochemical features, suggesting a homology of their very basic neuronal circuitry (e. g., Maza et al., 2016; Wolff et al., 2012, 2017; Wolff and Strausfeld, 2015; Strausfeld and Sayre et al. 2020, Sayre and Strausfeld 2019). Considering such basal anatomical similarities of the crustacean hemiellipsoid body and insect mushroom body, Wolff et al. (2017) suggested an involvement of both structures in place memory. Furthermore, because of its close anatomical association with the olfactory system as target of the projection neuron tract, evolutionary (Sullivan and Beltz, 2001, 2004), and functional considerations (Harzsch and Krieger, 2018; Sandeman et al., 2014a, 2014b; Strausfeld, 2012) have focused on the possible roles of these centers in higher-order olfactory processing and have suggested that the structural elaboration and size of hemiellipsoid bodies largely mirror the importance of the central olfactory pathway in a given brain (e. g., Harzsch and Hansson, 2008; Harzsch and Krieger, 2018; Krieger et al., 2010).

In the brain of *R. exoculata*, an inconspicuous and moderately developed olfactory neuropils (see previous section A common olfactory system) contrast with disproportionally large hemiellipsoid bodies (Figure 4). Because visual input also plays a minor role, Machon et al. (2019) suggested that the impressive hemiellipsoid body of *R. exoculata* may fulfill functions in addition to higher-order sensory processing in that they perhaps serve as the neuronal basis for a sophisticated place memory. For survival in the extreme, lightless habitat of *R. exoculata*, an excellent place memory may be essential for avoiding the dangerously hot vent chimneys and memorizing emission sites of hydrothermal fluids rich in those chemicals on which their endosymbiont bacteria depend (Machon et al. 2018). To test this hypothesis, other representatives of the taxon Alvinocarididae should serve as a model because behavioral experiments with *R. exoculata* are technically challenging because they need to be carried out in the pressurized aquariums. Other vent shrimp species such as *M. fortunata* also display pronounced hemiellipsoid bodies (see next section

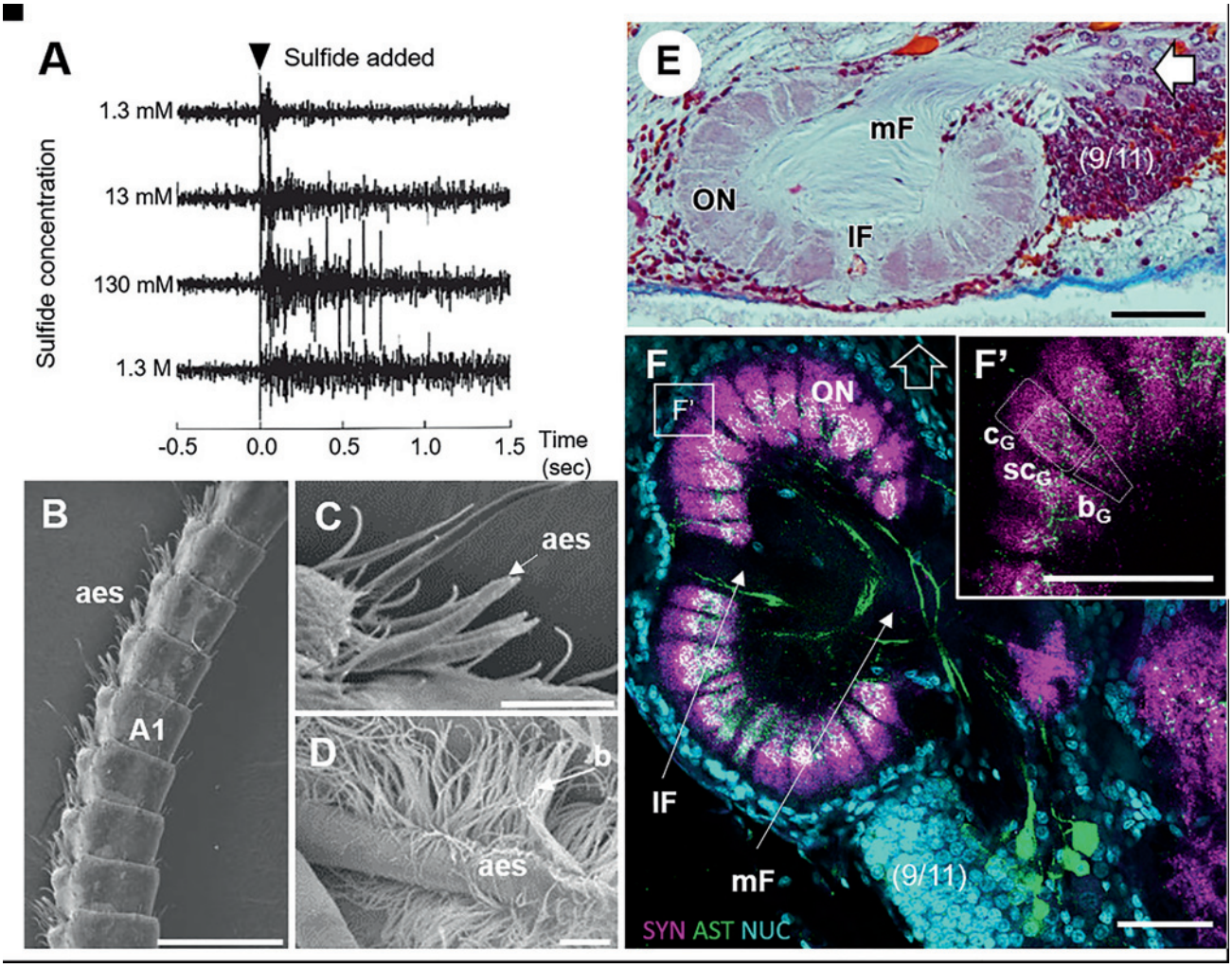

Figure 6: Olfactory system of the deep vent shrimp *Rimicaris exoculata*. A: Recordings from a bundle of nerve fibers in the lateral antenna 1 showing massed action potential generation in response to the application of different concentrations of sulphide (from Renninger et al., 1995). B–D: Electron microscopy images of the lateral flagellum of antenna 1 (B) bearing the aesthetasc olfactory sensilla (C), often associated with a bacterial coverage (D) (from Zbinden et al., 2017, 2018). E: Sagittal histological section of the olfactory neuropil. The white open arrow points towards anterior of the body axis. F: Horizontal immunohistological section of the olfactory neuropil, triple-labeled for synapsin immunoreactivity (SYN, magenta), allatostatin-like immunoreactivity (AST, green) and a nuclear marker (NUC, cyan). F' shows an enlargement view of the olfactory glomeruli (E and F from Machon et al., 2019). (9–11), cell cluster 9–11, A1, antenna 1; aes, aesthetasc; b, bacteria; bG, base region of the glomerulus; cG, cap region of the glomerulus; lF, lateral foramen; mF, medial foramen; ON, olfactory neuropil; scG, subcap region of the glomerulus. Scale bars B = 500 μm, C, E, F, F' = 100 μm, D = 20 μm.

Conclusions and perspectives), can be maintained at atmospheric pressure, and therefore are more suited for behavioral observations.

Conclusions and perspectives

The case of the vent shrimp discussed here provides new insights into aspects of the evolutionary transformation of crustacean brains and their associated sensory organs. Layered visual neuropils are present within *R. exoculata* brains with an arrangement similar to that of phylogenetically related shallow water shrimps with fully developed compound eyes, although much smaller. The compound eyes of *R. exoculata* ancestors, during the evolutionary diversification of this group, were transformed to a flattened but seemingly functional retina without notable spatial resolution but most likely can detect the very dim light of the thermal radiation (Pelli and Chamberlain, 1989; Van Dover and Fry, 1994; Van Dover et al., 1988, 1996). The fact that, despite these major modifications of sensory input, the number and

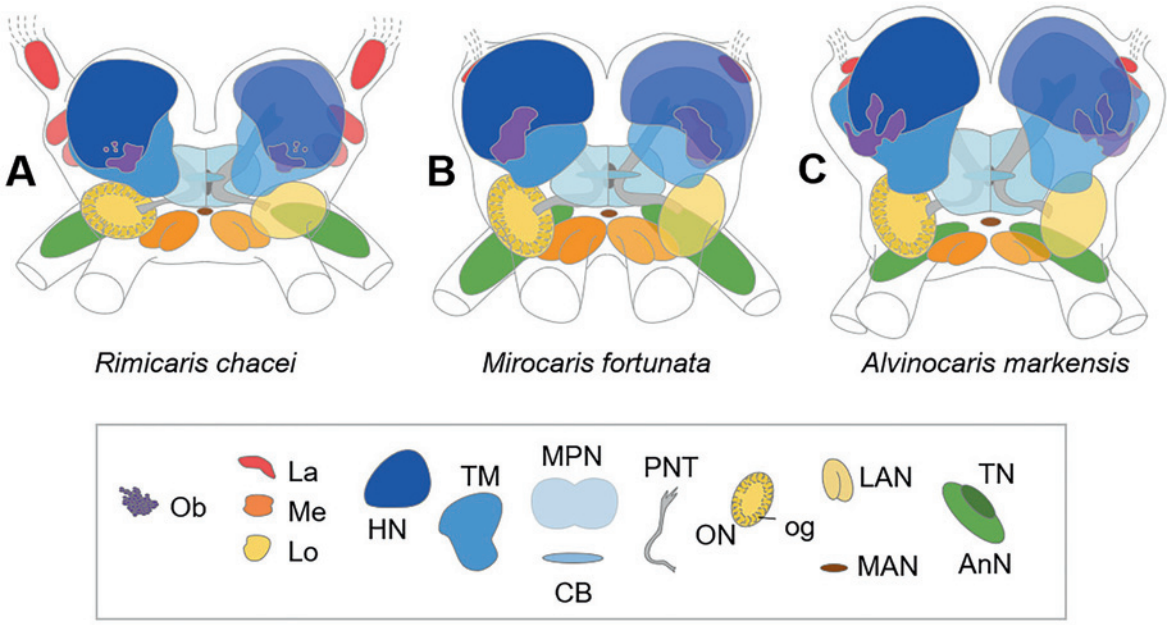

Figure 7: Brain architecture of other Alvinocarididae vent shrimp species. A: *Rimicaris chacei*. B: *Mirocaris fortunata*. C: *Alvinocaris markensis*. Abbreviations: Ob, organ of Bellonci. Visual neuropils: La, lamina; Me, medulla; Lo, lobula. Protocerebrum: HN hemiellipsoid body; TM, terminal medulla; MPN, medial protocerebral neuropil; CB, central body. Deutocerebrum: OL, olfactory lobe; og, olfactory glomerulus; LAN, lateral antennular (antenna 1) neuropil; MAN, median antennular (antenna 1) neuropil. Tritocerebrum: AnN, antennal (antenna 2) neuropil; TN, tegumentary neuropil. Note that brain schemes are aligned according to the neuroaxis (not to their arrangement within the cephalothorax) and are not equally scaled.

principal arrangement of the three visual neuropils remain conserved is remarkable and suggest a selective pressure acting to retain this arrangement. Concerning the HE/MT complex, the brain of *R. exoculata* has taught us that the structural elaboration of these neuropils may be less dependent on olfactory input than we previously thought and instead points to other functions that were previously less in the focus of crustacean neurobiologists (Machon et al., 2019).

Future studies in the field of research exposed in this paper should, in addition to qualitative aspects of brain structure, pay more attention to numerical and volumetric aspects. For example, determining across-species variations in numbers of olfactory sensory neurons and olfactory interneurons as well as glomerular numbers and volumes can be instructive for discussing functional aspects of crustacean olfactory systems such as the wiring logic from receptor to glomerulus (Harzsch and Krieger, 2018). We expect that determining neuropil volumes and neuronal numbers of other sensory systems and comparing these across species will be essential as a basis for new insights into brain adaptations related to ecological complexity of the habitat, lifestyle, and locomotion, and perhaps also biological processes such as invasiveness, sexual dimorphisms, sociality, and aging. As for crustaceans from extreme habitats, the related vent shrimp species from the Mid-Atlantic Ridge within the Alvinocarididae, which include *Rimicaris chacei*, *M. fortunata*, and *Alvinocaris markensis* (Gebruk et al., 2000), should be suitable for such comparisons, although they are less numerous than *R. exoculata* and are, therefore, even more difficult to sample. Preliminary results suggest that all these species present an overall similar brain pattern as in *R. exoculata* (Figure 7). Other vent crustaceans, such as the hydrothermal crab *Segonzacia mesatlantica*, could be also good models for future investigations (Charmantier-Daures and Segonzac, 1998; Matabos et al., 2015).

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Bionotes

Julia Machon
UMR CNRS MNHN 7208 Biologie des Organismes et Écosystèmes Aquatiques (BOREA), Equipe Adaptation aux Milieux Extrêmes, Sorbonne Université, Paris, France

Julia Machon studied Biology at the Sorbonne University (Paris, France). She obtained her Ph.D. degree in 2018 in the team "Adaptation to Extreme Environments", under the supervision of Dr. Magali Zbinden and Dr. Juliette Ravaux. Her research focused on sensory adaptations -mainly chemoreception- in shrimp from deep-sea hydrothermal vents, using various approaches such as morphology, behavior experiments and electrophysiology. She later worked with the team of Pr. Steffen Harzsch, at the University of

Greifswald (Germany) for a collaborative research project to investigate the brain architecture in these rare deep-sea invertebrates.

Jakob Krieger
Department of Cytology and Evolutionary Biology, Zoological Institute and Museum, University of Greifswald, Greifswald, Germany

Jakob Krieger is a scientific assistant and post doc in Steffen Harzsch's Lab at the Zoological Institute and Museum of the University of Greifswald. He studied Marine Biology in Rostock and received his Diploma in Biology and his PhD from the University of Greifswald. He worked on crustacean neuroanatomy with emphasis on the olfactory system as well as on behavioral aspects in different malacostracan crustaceans such as coconut crabs, brachyurans, or cleaner shrimps. He is currently analysing the neuroethology of terrestrial hermit crabs related to shell-choice behavior.

Magali Zbinden
UMR CNRS MNHN 7208 Biologie des Organismes et Écosystèmes Aquatiques (BOREA), Equipe Adaptation aux Milieux Extrêmes, Sorbonne Université, Paris, France

Magali Zbinden studied Biology at the University Pierre and Marie Curie (Paris, France) and obtained her Ph.D degree in Physiology in 2001. Since 2004: Associate professor at Sorbonne University, doing her research on deep-sea biology, and her teaching at the Zoology Department. 2017: French Scientific Habilitation in Life and Health Sciences. She carried out her postdoctoral research at the University of Liège, Belgium (European Ventox and FNRS grant). M. Zbinden works on the biology and physiology of animals associated with chemosynthetic ecosystems, in particular deep hydrothermal vents. Her main research field are: i) study of symbiosis with chemoautotrophic bacteria, ii) sensory perception in hydrothermal shrimp, using morphological, molecular and behavioral approaches and*in vivo*experiments in prototype pressurized aquarium developed by our lab (B. Shillito) dedicated to the study and maintenance of deep marine organisms.

Juliette Ravaux
UMR CNRS MNHN 7208 Biologie des Organismes et Écosystèmes Aquatiques (BOREA), Equipe Adaptation aux Milieux Extrêmes, Sorbonne Université, Paris, France

Juliette Ravaux studied Biology at the University Pierre and Marie Curie (Paris, France) and obtained her Ph.D degree in Invertebrate Physiology in 1999. Since 2000: Associate professor at Sorbonne University, doing her research on deep-sea biology, and her teaching at the Zoology Department. 2014: French Scientific Habilitation in Life and Health Sciences. Her main research topics focus on understanding the adaptations to deep-sea vent environments, and more specifically the strategies to cope with highly variable thermal environments, the adaptation to high hydrostatic pressure, and more recently the sensory functions (as part of the european project Managing Impacts of Deep-sea resource exploitation, Work program ENV.2013.6.2-8 Sustainable management of Europe's deep sea and sub-sea floor resources, P.I. Pr. Philip Weaver, Southampton, UK).

Steffen Harzsch
Department of Cytology and Evolutionary Biology, Zoological Institute and Museum, University of Greifswald, Greifswald, Germany
steffen.harzsch@uni-greifswald.de
https://orcid.org/0000-0002-8645-3320

Steffen Harzsch obtained his PhD in 1995 from the University of Bielefeld/Germany with a thesis on the development of crustaceans and continued to work on crustacean neurogenesis as a PostDoc at Wellesley College/Massachusetts. He then moved to the University of Ulm/Germany for comparative analyses of arthropod brains and promoted the discipline "neurophylogeny", a synthesis of neurobiological studies and phylogenetic aspects. He obtained his habilitation in 2001 and continued to work in Ulm on a Heisenberg stipend funded by the Deutsche Forschungsgemeinschaft. In 2006, Steffen Harzsch moved to Bill Hansson's group "Evolutionary Neuroethology" at the Max Planck Institute for Chemical Ecology in Jena/Germany as group leader in neuroanatomy focussing on the evolution of arthropod olfactory systems. In 2008, Steffen Harzsch obtained tenure as professor in Cytology and Evolutionary Biology at the Zoological Institute of the University of Greifswald/Germany

Review article

Meltem Kiyar, Sarah Collet, Guy T'Sjoen and Sven C. Mueller*

Neuroscience in transgender people: an update

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Abstract: Transgender persons identify with a gender different from the one they were assigned at birth. Although describing oneself as transgender is not a new phenomenon, media attention has lately been increasing exponentially, thanks to progressive changes in laws and change in societal attitudes. These changes also allow more people nowadays to (openly) identify as transgender and/or seek gender-affirming treatment. However, simultaneously, not much is presently understood about the underlying neurobiology, and specifically the brain structure and brain function of transgender persons. One major question in neuroimaging and neuroscience has been to determine whether, at the brain level, transgender people resemble more their gender identity, their sex assigned at birth, or have a unique neural profile. Although the evidence is presently inconsistent, it suggests that while the brain structure, at least before hormonal treatment, is more similar to sex assigned at birth, it may shift with hormonal treatment. By contrast, on "sex-stereotypical tasks," brain function may already be more similar to gender identity in transgender persons, also before receiving gender-affirming hormone treatment. However, studies continue to be limited by small sample sizes and new initiatives are needed to further elucidate the neurobiology of a 'brain gender' (sex-dimorphic change according to one's gender).

Keywords: cross-sex hormones; gender; magnetic resonance imaging; neurobiology; trans

Zusammenfassung: Transgender-Personen identifizieren sich mit einem anderen Geschlecht als dem bei der Geburt zugewiesenen. Obwohl Menschen, die sich mit einem anderen Geschlecht identifizieren, kein neues Phänomen sind, so ist die mediale Aufmerksamkeit in den letzten Jahren diesbezüglich exponentiell gestiegen. Dies ist auch den gesetzlichen Verbesserungen und einer Veränderung in der gesellschaftlichen Einstellung zu dem Thema zu verdanken. Zur gleichen Zeit aber weiß man noch nicht viel über die Gehirnstruktur und Gehirnfunktion bei transgender Menschen. Eine Hauptfrage in den Neurowissenschaften ist es, ob die Gehirne von Transgender-Personen jenen ähneln des Geschlechtes, dem sie bei der Geburt zugewiesen wurden, des Geschlechtes mit dem sie sich identifizieren, oder ob sie ein unabhängiges neuronales Profil aufzeigen. Obwohl die Befunde derzeit widersprüchlich sind, zeigen sie in die Richtung, dass sich die Gehirnstruktur vor der hormonellen Behandlung nur unwesentlich verändert. Auf der anderen Seite gleicht die neuronale Aktivität bei "geschlechtstypischen Aufgaben" von Transgender-Personen der neuronalen Aktivität ihres identifizierten Geschlechts (auch schon vor der Hormonbehandlung). Trotzdem sind Studien weiterhin limitiert, da sie oft mit kleinen Stichproben auskommen müssen und neue Initiativen zur Bestätigung der ersten Befunde nötig sind.

Schlüsselwörter: Sexualhormone; Gender; Magnetresonanztomographie; Neurobiologie; trans

Introduction

An increasing interest in, and attention to, the life and experiences of transgender persons can be seen in a surge in movies, books, television shows, newspaper reports, and academic articles. *Transgender* persons experience an incongruence between the sex they were born with and their gender identity, which can be described as our internal experience and naming of gender. Some individuals are born as female, but identify as male, hereafter *transgender men*. Other individuals are born as male and identify as female, hereafter *transgender women*. The term "transgender" is broad and should be considered as an umbrella term for people whose gender identity differs from the sex they were born with. It includes a whole

Meltem Kiyar and Sarah Collet contributed equally and share first authorship.

***Corresponding author: Sven C. Mueller**, Department of Experimental Clinical and Health Psychology, Ghent University, Henri Dunantlaan 2, 9000, Ghent, Belgium, E-mail: sven.mueller@ugent.be
Meltem Kiyar: Department of Experimental Clinical and Health Psychology, Ghent University, Ghent, Belgium
Sarah Collet and Guy T'Sjoen: Department of Endocrinology and Center for Sexology and Gender, Ghent University Hospital, Ghent, Belgium

spectrum of genders: not only does it comprise people whose gender identity is the opposite of their sex at birth, it also characterizes people who are not exclusively masculine or feminine. This phenomenon is referred to as being *gender nonbinary* or *genderfluid*. In addition, some individuals can also identify themselves as *gender-nonconforming*. It is important to note that being transgender is independent of sexual orientation. Readers specifically interested in gender nonbinary or genderfluid persons are referred to the review of Richards et al. (2016). Contrary, *cisgender people* do not experience gender incongruence, implying that their gender identity or expression matches the sex they were born with. Although transgender does not exclusively include transgender men and women as mentioned before, we will focus in this paper on these two groups for the sake of clarity, knowing categorizing people always implies inaccuracies.

Clinical management of gender dysphoria and transgender persons

Recent population studies estimate prevalence rates at 4.6 transgender persons per 100,000 individuals (Arcelus et al., 2015). Some, but not all, transgender persons experience gender dysphoria, defined as the discomfort or distress that is caused by this discrepancy between gender identity and the sex they were born with (and the associated gender role and/or primary and secondary sex characteristics). Classification systems such as the International Classification of Diseases (ICD) and the Diagnostic Statistical Manual of Mental Disorders (DSM) are used to diagnose gender dysphoria. In ICD, see under "Conditions related to sexual health"; in DSM, see under "Gender dysphoria." Gender dysphoria may result in negative effects on one's psychological, physical, and social well-being. Psychological counseling and *gender-affirming medical treatment* are often wished for by a large proportion of transgender persons. Diagnosis and treatment of individuals suffering from gender dysphoria require a multidisciplinary approach, which might involve psychologists, endocrinologists, psychiatrists, urologists, gynecologists, dermatologists, surgeons, but also voice and communication therapists (i. e., to adapt their voice and manner of speaking to their identified gender, for example). Furthermore, treatment depends on individual needs: some persons only verbalize a wish for psychological support and guidance in the coming-out process, while others may require psychotherapeutic interventions to treat frequently occuring mental health problems such as mood and anxiety disorders (Branstrom and Pachankis, 2020; Heylens et al., 2014). Others may need gender-affirming hormone therapy (GAHT) and/or surgery to increase the match between their gender identity and expression. Guidelines for treatment are described by the World Professional Association for Transgender Health and the Endocrine Society. GAHT has been shown to effectively alleviate gender dysphoria and reduce anxiety, depression and suicide attempts of transgender persons (e. g., Branstrom and Pachankis, 2020).

The process of gender-affirming treatment is different for transgender women and transgender men. Transgender women's physical transition starts with high testosterone levels (i. e., the primary male sex hormone), and they need female sex hormones, i. e., estrogen in order to obtain feminization (breast development, fat and muscle distribution). Transgender men will need testosterone for masculinization of their physical appearance (voice deepening, masculine pattern of hair, fat and muscle distribution) as they start with testosterone levels in the female range.

Brief historical overview of transgender persons

Historically, although awareness for, and development of, the transgender community only started in the second half of the 20th century, the history of transgender identities goes back to various cases recorded in multiple ancient civilizations. In numerous cultures, multigender roles were recognized and documented with women who passed as men and vice versa by adopting clothes and roles of respective genders. The Chevalier d'Éon de Beaumont, a spy in the French king's service, is one of many examples. It was considered that d'Éon may have been a transgender woman, as he successfully infiltrated Russian court identifying as a female (Burrows, 2011). The first known European transgender person to undergo gender-affirming surgery was Dora Richter. She underwent genital surgery, first removing the testicles (i. e., orchiectomy) in 1922, followed by the removal of the penis and a vaginoplasty in 1931 in Berlin, Germany (Mancini, 2010). Lili Elbe (portrayed in the factually inaccurate movie *The Danish Girl*) underwent gender-affirming surgery in Germany in the 1930s. The surgeries of both transgender women were, at that time, highly experimental. with a high risk for adverse effects. Following infection-related complications, Lili Elbe died 3 months after surgery, whereas the fate of Dora Richter is unknown (Providentia, 2010). About 20 years later, Christine Jorgensen, an American transgender

woman, went to Denmark for gender-affirming surgery, a story that was covered by a *New York Daily News* front-page story (Bullough, 2009; Khan, 2016). Even though female sex hormones (i. e., estrogens) were first chemically synthesized in the 1920s and 1930s (Watkins, 2007b), the European pioneers probably did not use these, as the development of sex hormones started in the United States (Watkins, 2007a). The first professional association for gender dysphoria, now known as the World Professional Association for Transgender Health, was formed in 1979.

Neurobiology of transgender research

Surprisingly, relatively little is known regarding the underlying neurobiology of a transgender identity. Within the field of neuroscience, a landmark study in 1995 documented that part of the forebrain circuit (i. e., the bed nucleus of the stria terminalis [BNST] in transgender women was found to be similar in size as in cisgender women (Zhou et al., 1995). Moreover, differences in volume in the BNST have been found for cisgender men and women (Zhou et al., 1995). More specifically, the BNST was found to be larger in men relative to women showing a sexdimorphic pattern (Zhou et al., 1995). To measure this, post mortem brains were used. However, because the transgender women had received GAHT (i. e., estrogens), it was difficult to determine the impact that hormonal treatment may have had on the findings. Since the advent of the first *in vivo* magnetic resonance imaging (MRI) performed on humans (Belliveau et al., 1991), scientists nowadays are keen to examine the extent to which the brains of transgender persons show different brain *structural* properties (gray and white matter, cortical thickness, volume, and surface area) as well as brain *functionality* (functional MRI [fMRI]) and *connectivity* between brain areas (diffusion tensor imaging [DTI], functional and structural connectivity) (Figure 1). Although brain imaging studies have consistently documented brain differences between cisgender males and cisgender females, this has not been the case for transgender persons. Thus far, no clear explanation or neurobiological underpinning of being transgender has been identified (Mueller et al., 2017). Nonetheless, some progress has been made. Most neurobiological studies aim to investigate whether the brain of transgender persons resembles that of the sex they were born with, that of their gender identity, or a specific intermediate brain type. In terms of structural properties, total brain volume and the gray matter volume in the brains of

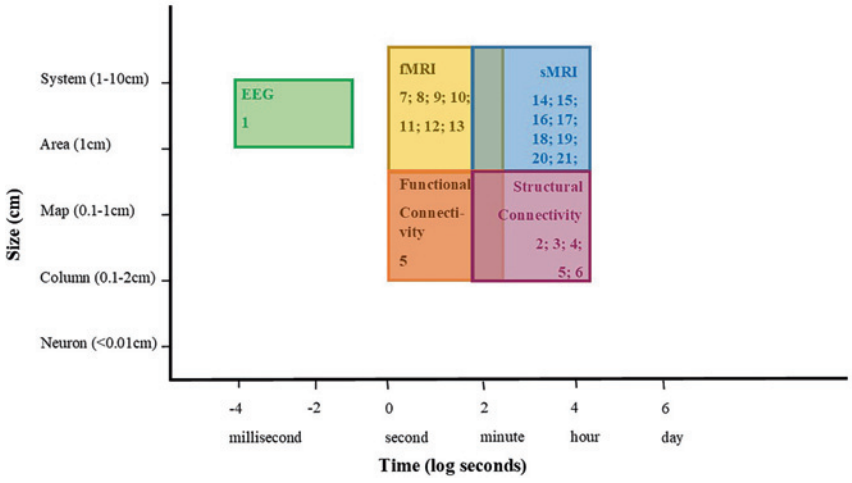

Figure 1: Graphical summary of some of the currently available neuroimaging techniques used in transgender research presented in the current review. This figure illustrates the sites of action in the brain on the vertical axis, indicating the smallest and largest sizes of the region from which the technique provides information. The horizontal axis represents the temporal dimensions over which information can be collected with each technique. The spatio-temporal capabilities are depicted by colored rectangles per neuroimaging technique. Reviewed papers are categorized per technique. Figure loosely modeled after Churchland and Sejnowski (1988). NB. sMRI and fMRI are acquired similarly; however, anatomical brain structure varies less in function of time compared to neuronal brain function. Electroencephalogram (EEG): (1) Künzel et al. (2010). Functional and structural connectivity: (2) Kranz et al. (2014); (3) Rametti et al. (2011a); (4) Rametti et al. (2011b); (5) Manzouri and Savic (2018); (6) Hahn et al. (2015). Functional MRI (fMRI): (7) Berglund et al. (2008); (8) Burke et al. (2014); (9) Carrillo et al. (2010); (10) Schöning et al. (2010); (11) Soleman et al. (2013); (12) Sommer et al. (2008); (13) Burke et al. (2016). Structural MRI (sMRI): (14) Zhou et al. (1995); (15) Savic and Arver (2011); (16) Luders et al. (2009); (17) Luders et al. (2012); (18) Zubiaurre-Elorza et al. (2013); (19) Hulshoff Pol et al. (2006); (20) Kim et al. (2015); (21) Zubiaurre-Elorza et al. (2014); (22) Seiger et al. (2016).

transgender persons who did not yet receive GAHT were similar relative to their sex assigned at birth (Savic and Arver, 2011). Although some studies show reduced gray matter volume in transgender women without GAHT relative to both cisgender groups (Luders et al., 2009), cortical thickness is increased in transgender women when compared to cisgender men, including both hemispheres, as well as the orbitofrontal cortex, and the insular and medial occipital cortices (Luders et al., 2012; Zubiaurre-Elorza et al., 2013). Furthermore, frontal and parietal cortical thickness was also larger in transgender men and cisgender women compared to cisgender men (Zubiaurre-Elorza et al., 2013).

Advances in software and computer technology have enabled to assess the intrinsic connections between brain regions. Connectivity refers to structural and functional links between different brain areas. Whereas structural connectivity describes how the white matter tracts physically connect distinct brain areas, functional connectivity represents the relationship between brain regions in how their neural activity varies (i. e., oscillates) across time together. Studies have revealed an intermediate white matter microstructure in transgender men and women relative to that of cisgender men and women. For example, the white matter tracts between the frontal lobe and the parietal lobe are less strongly connected in transgender persons relative to cisgender persons (Manzouri and Savic, 2018). Furthermore, trans people have been found to differ in their axonal organization of the white matter (Rametti et al., 2011a, b) and their rate of diffusion of molecules and water in the brain (Kranz et al., 2014) taking in-between positions between cisgender men and women. Another study showed a decreased structural connectivity between the left and right hemispheres in transgender relative to cisgender persons (Hahn et al., 2015). Presently, two hypotheses have been put forward why certain brain areas would evidence structural differences between transgender and cisgender persons. Although one theory focuses on neural developmental patterns and contributions of hormones and hormone receptors to brain development (Guillamon et al., 2016), the other theory centers around brain areas in which there is a discrepancy between the notion of one's gender identity and one's brain representation of one's body (Feusner et al., 2017). As of yet, it is too early to determine which theory might find more support, also in light of recent evidence suggesting compatibility between the two hypotheses (Uribe et al., 2020).

In comparison to studies on brain structure, research on brain activity in transgender persons is lagging behind. Several reasons may be responsible for this including: 1) the need to first establish anatomical differences before functionality can be addressed, 2) the relatively small number of laboratories doing neuroimaging research in this cohort, and 3) the need for highly interdisciplinary research teams requiring presence of teams with experience in functional MRI. Existing work has predominantly focused on tasks eliciting "sex-typical" brain responses and determining, similar to structural imaging work, whether the brain activity of transgender persons resembles those of their sex assigned at birth or their gender identity. As such, studies have focused on olfactory processing (Berglund et al., 2008; Burke et al., 2014), mental rotation (Carrillo et al., 2010; Schöningen et al., 2010), or verbal fluency (Soleman et al., 2013). Olfactory processing could be described as a primitive function that is essential to survival and procreation. Males and females are sensitive to steroid compounds acting as pheromones. These chemicals, which signal, among others, alarm, food, or sexual attraction, can influence the behavior of the receiving individual. As a consequence, it can lead to the activation of the hypothalamus. Surprisingly, transgender women's brains (hypothalamus) responded similar to cisgender women's brains when encountering a male pheromone (Berglund et al., 2008). However, sexual development appeared to play a role as this biological shift toward the identified gender appeared during the onset of puberty, when hormonal levels rise (Burke et al., 2014). Before the start of puberty, transgender youth resembled the sex assigned at birth on this olfactory task (Burke et al., 2014). Other, more abstract cognitive functions are also known to dissociate between males and females, i. e., being sex-stereotypical. For example, men tend to be, on average, slightly better than women to mentally rotate objects in space. Here, transgender women resembled their identified gender as they showed reduced brain activity in the parietal lobe, a brain region crucial for this skill, while mentally manipulating 2D and 3D objects in space (Carrillo et al., 2010). This was confirmed by Schöningen et al. (2010), who documented an increased parietal cortex activation in cisgender men relative to transgender women (with and without GAHT) during such a mental rotation task. Conversely, women tend to be better, on average, than men, when retrieving verbal information from memory (i. e., verbal fluency). During the verbal fluency task, transgender girls showed relatively better performance (i. e., retrieved more words) than cisgender boys, cisgender girls and transgender boys (Soleman et al., 2013). However, the participants' brain activation did not resemble either the gender identity or sex assigned at birth but had a unique neural profile (Soleman et al., 2013).

Even more scarce are studies that examine the effects of long-term GAHT on the brain. Limited data suggest that some structures of the brain develop toward the direction of the gender identity in transgender persons with hormonal treatment (Hulshoff Pol et al., 2006; Kim et al., 2015; for a review, see Smith et al., 2015). Although the hypothalamus in transgender women decreases in size after GAHT (Hulshoff Pol et al., 2006), it increases in size in transgender men (Kim et al., 2015). Additionally, proportionately, transgender women's brains decreased in size with GAHT, while transgender men's brains increased in total brain volume (Hulshoff Pol et al., 2006). This was not only true for overall volume but also cortical thickness, which increased after 6 months of hormonal treatment in transgender men, and decreased in transgender women (Zubiaurre-Elorza et al., 2014; Seiger et al., 2016). In a very small sample (transgender men, $n = 6$; transgender women, $n = 8$), Sommer et al. (2008) documented increases in brain activity after GAHT in a language task but not a mental rotation task. Yet, neural activation to the language task was related to post-treatment estradiol levels, while neural activation to mental rotation was related to post-treatment testosterone levels for both sexes (Sommer et al., 2008). Adding to these findings is a study in adolescents, in which brain activation during mental rotation increased with testosterone treatment in transgender girls (Burke et al., 2016). Even though studies aiming to confirm these initial findings are slowly increasing, more research is necessary.

Future directions

Although the number of transgender persons presenting to the gender clinics has increased, the understanding of the neurobiology is only slowly catching up. Whereas studies on structural brain properties are becoming available, work using functional MRI remains scarce. A continuing challenge will be the relatively small numbers in MRI studies (sample sizes are often limited to 10–25 individuals per group) (Schöning et al., 2010; Sommer et al., 2008), lack of cisgender control groups for comparisons (Kim et al., 2015), absence of pretreatment data (Carrillo et al., 2010), lack of taking into consideration confounding factors (e. g., age of onset of gender dysphoria, sexual orientation, non-sex-specific hormonal affects affecting both sexes), or the absence of studies on gender nonbinary persons. In addition, country- and culture-specific phenomena (for example, availability of type of GAHT, legal structures, through which transgender persons are recruited) might also affect study strategies and therefore their findings. As such, increasing the amount of studies, taking into account neuroimaging together with GAHT, is essential for future research. A first step in this direction is the ENIGMA Transgender Persons Working Group, which aims to share and pool neuroimaging data across countries and professionals to increase the study sample size (http://enigma.ini.usc.edu/ongoing/enigma-transgender-persons/). In addition, parallel neurobiological research has slowly begun to elucidate epigenetic influences on the brain (Cortes et al., 2019) as well as charting the genetic variation, presently mostly those involved in hormonal regulation (Fernandez et al., 2018), in transgender persons. Finally, translational animal work has begun to examine the influence of hormones on brain metabolites using a female rat model of androgenization (Perez-Laso et al., 2018). Thus, future synthesizing work will be able to inspect all of these lines of research to draw a neurobiological picture of a "brain

Conclusions

This review sought to provide a brief overview on the current knowledge in neuroimaging studies in transgender persons. Much progress has been made over the past 25 years trying to detect the neurobiological underpinnings of gender dysphoria and identifying the existence of a brain gender. Nonetheless, many findings remain inconsistent. As such, increased collaboration strategies are essential for further research validation.

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Glossary

Cisgender person: A person whose gender identity is congruent with his or her sex assigned at birth, for example, a person assigned female at birth with a female gender identity.

Gender-affirming medical treatment: An inclusive term for a number of gender-affirming medical interventions including gender-affirming hormone therapy and gender-affirming surgery.

Gender-affirming hormone therapy (GAHT): The primary medical intervention sought by transgender persons inducing the acquisition of secondary sex characteristics more aligned with the experienced gender identity. For example, a transgender man will receive testosterone treatment, whereas a transgender woman will receive estrogens and testosterone blockers.

Gender-affirming surgery: A range of surgical interventions available to transgender persons who wish to alter physical appearance or function to reduce gender incongruence.
Gender nonconformity: An individual who does not conform to a given gender role in a particular culture.
Gray matter: Contains the neuronal cell bodies and unmyelinated axons, which play a key role in controlling sensory and muscular activity.
In vivo: In a living organism.
Orchiectomy: A surgical procedure in which one or both testicles are removed.
Phalloplasty: A surgical (re)construction of the penis.
Pheromone: A chemical substance that serves as a stimulus for eliciting behavioral responses.
Post mortem: Occurring after death.
Transgender: An umbrella term to describe individuals whose gender identity varies from their sex assigned at birth.
Transgender man: A person whose sex was assigned female at birth, but identifies as male.
Transgender woman: A person whose sex was assigned male at birth, but identifies as female.
Steroids: Biologically active compounds functioning as signaling molecules.
Vaginoplasty: A surgical (re)construction of the vagina.
Verbal fluency: Cognitive function facilitating retrieval of information from memory. This can be measured by a psychological test in which participants have to produce as many words as possible from a category in a given time. For example, finding words beginning with the letter L.

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Bionotes

Meltem Kiyar
Department of Experimental Clinical and Health Psychology, Ghent University, Ghent, Belgium

Meltem Kiyar is a PhD student in the Department of Experimental Clinical and Health Psychology at Ghent University, Ghent, Belgium under the supervision of Sven Mueller and Guy T'Sjoen. Her PhD examines the impact of social exclusion on underlying psychophysiology and neurobiology in transgender persons before and during gender-affirming hormone treatment.

Sarah Collet
Department of Endocrinology and Center for Sexology and Gender, Ghent University Hospital, Ghent, Belgium

Sarah Collet is an MD and presently a PhD student in the Faculty of Medicine and Health Science in the Department of Internal Medicine & Pediatrics, Endocrinology at Ghent University Hospital, Ghent, Belgium under the supervision of Guy T'Sjoen and Sven Mueller. Her PhD examines the effect of minority stress on inflammation status and brain structure & function in transgender persons

Guy T'Sjoen
Department of Endocrinology and Center for Sexology and Gender, Ghent University Hospital, Ghent, Belgium

Guy T'Sjoen is an MD and Professor of Endocrinology in the Faculty of Medicine and Health Science in the Department of Internal Medicine & Pediatrics, Endocrinology. He is a founding member of the European Professional Association for Transgender Health (EPATH) and his researchers examines all aspects of transgender health and care.

Sven C. Mueller
Department of Experimental Clinical and Health Psychology, Ghent University, Henri Dunantlaan 2, 9000, Ghent, Belgium
sven.mueller@ugent.be

Sven C. Mueller is a psychologist and Associate Professor at the Department of Experimental Clinical and Health Psychology at Ghent University, Ghent, Belgium and a researcher at the Department of Personality, Psychological Assessment and Treatment, University of Deusto,Bilbao,Spain. His research centers on the effects of stress (social exclusion) on the mental health and underlying neurobiology of transgender persons. In addition, he examines the effect of childhood maltreatment on social and cognitive control processes.

Review article

Maryam Ghorbani and Lisa Marshall*

Manipulating neural activity and sleep-dependent memory consolidation

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Abstract: Sleep contributes actively to the consolidation of many forms of memory. This review describes the neural oscillations of non-rapid eye movement (NREM) sleep, the structures underlying these oscillations and their relation to hippocampus-dependent memory consolidation. A main focus lies on the relation between inter- and intraregional interactions and their electrophysiological representation. Methods for modulating neural oscillations with the intent of affecting memory consolidation are presented.

Keywords: behavior; brain rhythms; human; rodent; stimulation

Zusammenfassung: Schlaf unterstützt aktiv die Konsolidierung vieler Arten von Gedächtnisprozessen. Dieser Übersichtsartikel beschreibt die neuronalen Oszillationen während des non-rapid eye movement (NREM) (Non-rapid eye movement)-Schlafs, die diesen Oszillationen zugrunde liegenden Hirnstrukturen und ihre Beziehungen zur hippocampusabhängigen Gedächtniskonsolidierung. Ein Schwerpunkt liegt hierbei in der Beziehung zwischen inter- und intraregionalen Interaktionen und deren elektrophysiologische Repräsentation. Es werden Methoden zur Modulation neuronaler Oszillationen mit der Absicht, die Gedächtniskonsolidierung zu beeinflussen, vorgestellt.

Schlüsselwörter: Verhalten; Hirnrhythmen; Stimulation; Human; Nager

Introduction

During our active period, sensory systems take up information, which is typically processed and stored across varying time periods. Although sensory memory is stored up to hundreds of milliseconds, information in short-term working memory reflects activity sustained within neural circuits (e. g., within the prefrontal cortex) and lasting into the minute range; and long-term memory may last a lifetime. This latter form of memory is associated with persistent molecular and cellular changes in synaptic structure and neural circuits. Types of long-term memory are distinguished dependent upon the modified brain structures. A major distinction is made between hippocampus- and non-hippocampus-dependent memories. The formation of long-term memory consists of at least three processes: encoding (the uptake of information), consolidation (i. e., storage), and recall (i. e., retrieval of memory contents from storage). Processes involved in memory consolidation occur both at the cellular (and molecular) and systems levels. Systems consolidation refers to the transfer across time and among neuronal networks or brain regions of memory representations, their reorganization, and concurrent stabilization. According to the two-stage model of memory consolidation after encoding using a fast information storage system (as the hippocampus), a subsequent (offline) transfer to a long-term storage site (neocortex) occurs. This concept has been extended to non-hippocampus-dependent memory consolidation (Buzsáki, 2015; Diekelmann and Born, 2010). Studies across the last decades have shown that system consolidation benefits from sleep: a simple schematized experiment would reveal enhanced recall performance after a period of sleep as compared to wakefulness. Moreover, the discovery of neuronal ensemble reactivations, occurring most frequently during nonrapid eye movement (NREM) sleep, and the neuronal activity associated with the sleep slow oscillation (SO) indicated an active role of sleep in memory consolidation. (For comprehensive reviews on historical background, theories, and mechanisms of systems consolidation, the reader is referred to Diekelmann and Born, 2010; Klinzing et al., 2019; Marshall and Born, 2007; Rasch and Born, 2013.)

***Corresponding author: Lisa Marshall**, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Lübeck, Germany, E-mail: lisa.marshall@uni-luebeck.de
Maryam Ghorbani: Department of Electrical Engineering, Rayan Center for Neuroscience and Behavior, Ferdowsi University of Mashhad, Mashhad, Razavi Khorasan, Iran

This review focuses on the neural oscillations of the brain during NREM sleep and their relationship to sleep-associated hippocampus-dependent memory consolidation. Brain oscillations during NREM sleep are most closely linked to hippocampus-dependent memory consolidation. First, the neural oscillations characterizing NREM sleep and associated with memory consolidation are introduced: the cortical slow oscillation (SO), thalamocortical sleep spindles, and hippocampal sharp wave ripples (SPWRs). As these neural oscillations reflect activity within specific brain regions involved in the presumed transfer of memory representations, the endogenous temporal coordination of these rhythms is presented next. The field of sleep-associated memory consolidation is rapidly expanding. In the last part of this review, some directions of future research are presented from the perspective of our own findings.

Neural oscillations of NREM sleep

Neocortical SO

In the electroencephalogram (EEG) or cortical local field potential, NREM sleep is characterized by large-amplitude SOs of ~1 Hz. The sleep SO, first described by Steriade and colleagues in 1993, is a cortically generated biphasic rhythm consisting of widespread synchronized membrane potential fluctuations alternating between hyperpolarization, during the "down state," and depolarization with firing of excitatory and inhibitory cells, during the "up state" (Steriade, 2006; Figure 1). The relatively clear association of neuronal activity patterns to local field potentials and to the superficial EEG raised great interest in this oscillation. Recently, distinct sequences of excitatory pyramidal, and inhibitory parvalbumin- and somatostatin-positive interneuron activity within a SO cycle were revealed. In fact, the activity of these different neuron populations differed dependent upon the occurrence of either an isolated SO, a SO conjointly with a sleep spindle during the SO up state, or an isolated spindle. The activity of a pyramidal cell and parvalbumin-positive interneurons (producing perisomatic inhibition of a pyramidal cell) were several-fold higher when a sleep spindle occurred conjointly with a SO, whereby the activity of somatostatin-positive interneurons (producing dendritic inhibition of a pyramidal cell) was decreased. This constellation of inhibitory inputs onto pyramidal neurons is believed to facilitate dendritic synaptic plasticity (Contreras et al., 1997; Niethard et al., 2018; Zucca et al., 2019).

Thalamocortical spindles and hippocampal SPWRs

Thalamocortical sleep spindles of NREM sleep commence at a lighter NREM sleep stage than SOs. Sleep spindles result from shifts in the membrane potential of thalamic reticular neurons as sleep deepens, enabling an emergent interaction between neurons in the GABAergic reticular thalamic nuclei and thalamocortical and cortical neurons (details of spindle generation can be found in Fernandez and Lüthi, 2019). Sleep spindle events in the EEG or cortical local field potential last 0.5–3 s and oscillate between about 9 and 15 Hz. Their amplitude, frequency, and spatial distribution across the cortex or scalp can vary. These variations are suggested to reflect divergent inputs and properties of network synchronization (Andrillon et al., 2011; Gretenkord et al., 2017; Kim et al., 2015; Klinzing et al., 2016). Most importantly, these properties are not static, but they change across the sleep period and with depth of NREM sleep, indicating dynamic changes in differential thalamocortical processing (Ayoub et al., 2013; Mölle et al., 2011; Nir et al., 2011).

A hippocampal SPWR is associated with a strong depolarization and is therefore a candidate event for information transfer from the hippocampus to the neocortex, as required for systems consolidation. A SPWR results from sequential activity in hippocampal subfields (termed "Cornu Ammonis" [CA]): pyramidal cell population bursting in CA3 (~100 ms) produce a strong depolarization in CA1 pyramidal cell apical dendrites in conjunction with high-frequency firing in these cells at a frequency of 150–200 Hz in rodents and at ~100 Hz in humans (Buzsáki, 2015).

Coupling of rhythms in sleep and memory consolidation

Historically, the discovery of hippocampal place cell firing conjointly with hippocampal theta rhythms when performing a spatial task (i. e., encoding information) and the reactivation of this activity during subsequent sleep contributed strongly to the field of sleep-associated memory consolidation. Studies on postlearning modulations of sleep brain rhythms and their distinct events (e. g., density, amplitude, duration of SPWRs, spindles or SOs), as compared to activity after nonlearning conditions, revealed temporally coordinated network and cellular activity. Concepts on the relevance for memory consolidation of temporally fine-tuned communication between brain regions were boosted by findings on the time- and phase-dependent occurrence (phase-amplitude coupling) of SPWRs and

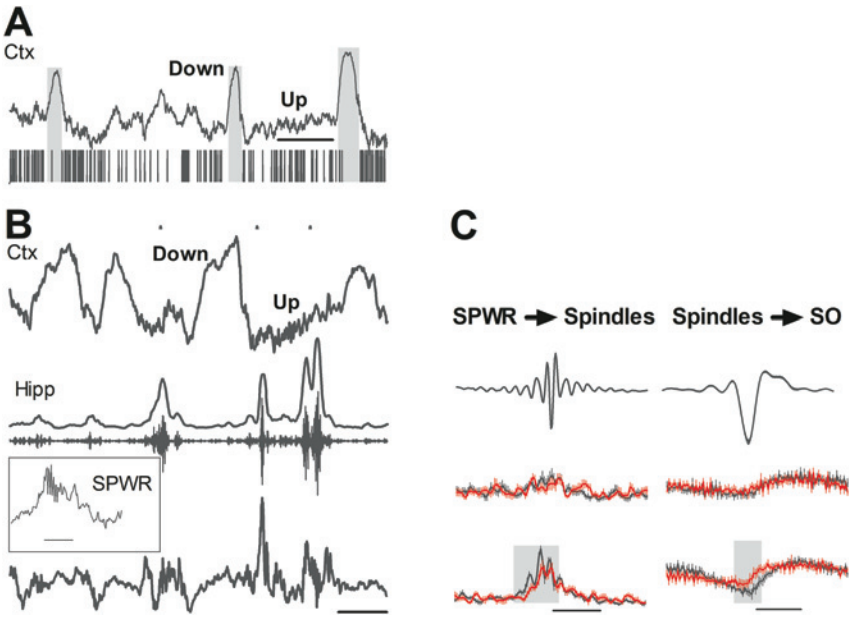

Figure 1: Neocortical and hippocampal recordings schematizing the prominent neural oscillations of NREM sleep and their coupling characteristics. (A) Depolarizing up state and hyperpolarizing down state of the cortical slow oscillation (SO) (depth local field potential, parietal cortex) and below cortical multiunit firing. The majority of units, but not all, fire during the up state (rat data from M. Mehta, UCLA). (B) Comparison of SOs in the cortex and sharp wave ripples (SPWRs) in the hippocampus reveals their temporal coupling. (Bottom) Local field potential from the dorsal region of the hippocampus (subfield Cornu Ammonis 1). (Row 3) Corresponding bandpass filtered signal (150–250 Hz) for ripple detection. (Row 2) Root mean square of the filtered signal. (Row 1) Cortical local field potential recording (medial prefrontal cortex). The dots indicate time points of detected hippocampal SPWRs. The inset indicates a SPWR with a scaling bar for 100 ms. All other scaling bars (A–C) represent 500 ms. (C) Coupling of the hippocampal SPWRs to spindles (left) and spindles (9–15 Hz) to SOs (SO, right) after learning on the Barnes maze. (Row 1) Averaged hippocampal SPWR activity time-locked to the deepest spindle trough (left), and averaged spindle activity time-locked to the negative peak (down state) of the SO. (Row 2) Event correlation histograms of hippocampal SPWR activity (number of peaks and troughs) with reference to the spindle trough at baseline (left) and of spindle activity with reference to the negative SO peak. (Row 3) Same as row 2 after learning on the Barnes maze for controls (black) and for mice after optoinhibition of monosynaptic hippocampal-prefrontal activity (red). Note: Significant decoupling (left) and decreased modulation (right) after optoinhibition (gray area; adapted from Binder et al., 2019). Amplitudes are z-scored. Ctx, cortical recording, Hipp, hippocampal recording.

spindle activity relative to slower neural events (for reviews, see Girardeau and Zugaro, 2011; Marshall et al., 2020).

Regarding phase of coupling, it is broadly consistent that (fast) spindles as well as SPWRs occur during or shortly preceding the SO up state (for reviews, see Skelin et al., 2019; Todorova and Zugaro, 2020; Figure 1). A major goal of research on neural oscillations in sleep is to identify which information is reflected in phase-dependent activity (or consistency of coupling). Timing during the SO phase may well reflect the nature of presumed information transfer. For example, SO-phase-dependent firing preferences differed for thalamocortical nuclei and for thalamic nuclei receiving major inputs from the cerebellum (ventrolateral nuclei) versus the basal ganglia (ventral anterior/ventromedial nuclei; Ushimaru et al., 2012). The locus ceruleus of the brainstem in rats also revealed transient SO-phase-dependent firing during the down- to up-state transition in postlearning NREM sleep (Eschenko et al., 2012). In relation to behavior, the locus ceruleus selectively increased firing during postlearning sleep (Eschenko and Sara, 2008). Together, different brain structures become active at preferred phases of the SO, which may be indicative of specific processing steps during memory consolidation. The preferred phase of thalamocortical spindle activity with reference to the SO is most intensely studied and can differ strongly between subjects, but it is highly stable intra-individually. This spindle-to-SO coupling in humans also changes between light (stage N2) and deep (stage N3) NREM sleep, with (fast) spindles occurring at a slightly earlier phase in N3 than N2 (Cox et al., 2018).

Memory consolidation – recent neuromodulatory approaches and research perspectives

Especially in humans, noninvasive brain stimulation, such as transcranial electric stimulation or sensory stimulation,

enable neural oscillations and their coupling to be targeted. Weak electric stimulation has the advantage of essentially targeting subthreshold activity. Sensory stimulation is typically suprathreshold and induces a temporally precise neural response. Thus, it has the advantage that delivery can target a specific phase of the ongoing oscillation. The phase-dependent occurrence of sleep events relative to the SO gave rise to the working hypothesis that facilitating SOs affects synaptic plasticity and the memory consolidation function of NREM sleep. Initial studies revealing that transcranial electric stimulation oscillating at the frequency of the SO or SO - auditory closed loop stimulation induce endogenous SO activity supported this postulate (Marshall et al., 2006; Ngo et al., 2013). However, the story is not so simple (for reviews of divergent findings, see Campos-Beltrán and Marshall, 2017; Malkani and Zee, 2020). Recently, evidence that weak electric stimulation as typically applied in humans can indeed influence the timing of spiking activity in a frequency- and location-dependent manner was provided by an investigation in nonhuman primates (Krause et al., 2019; however, see also the review of Liu et al., 2018). Thus, what could underlie the frequent variability in results? We, as well as other investigators, suggest that deviant study outcomes should be taken as important indicators for interactions between undisclosed overt and covert confounding factors. Such confounding factors that can affect neural responses and memory consolidation are the precise phase of sensory stimulation relative to the endogenous coupling of rhythms (Wei et al., 2020; Weigenand et al., 2016), a different length in time

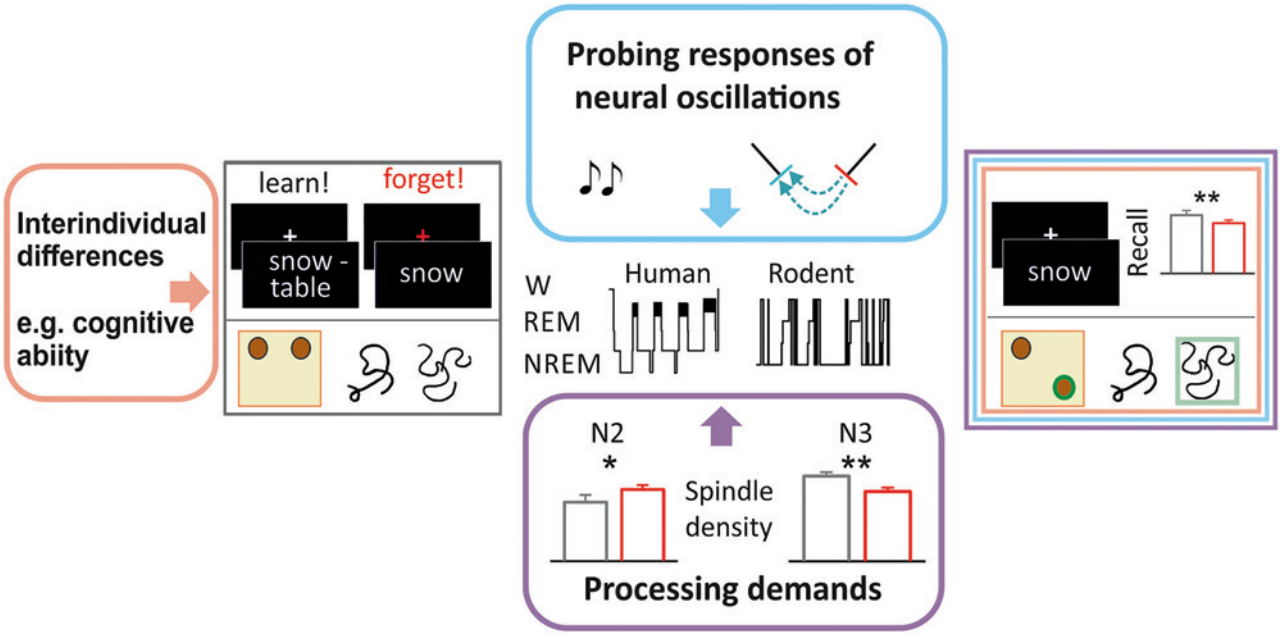

Figure 2: Potential levels of interaction during neural processing in sleep. The square boxes indicate learning and recall performance on three tasks: motivated forgetting (top), object-place recognition task (bottom left), and figural paired associate task (bottom right). In the motivated forgetting protocol, human subjects first learn word pairs (e. g., snow-table). In a subsequent phase, subjects are instructed to suppress memory for red-cued words (e. g., snow). In the recall box (tri-colored frame), in the condition of motivated forgetting, recall performance is suppressed (red) compared to a control condition (gray). In the figural paired-associate task, human subjects have to learn that the two figures belong together. At recall, the correct match (indicated by the small green square) is to be selected from a set of five other figures. In the object-place recognition task, rodents are placed in an open field with two objects for 10 min. After an interim period, subjects are introduced again to the open field wherein one object has been displaced. Due to novelty preference the displaced object, if its location is recognized as novel, is explored more intensely (indicated by the green circle). Thus, indicating the subject has remembered the objects' initial locations. Orange, blue and purple frames indicate endogenous and exogenous factors affecting neural processing in sleep and subsequent recall performance. The motivated forgetting task is associated with differential spindle density in light NREM sleep stage N2 and deep NREM sleep stage N3 as compared to sleep after a control condition. Thus, reflecting discrepant processing demands during both sleep states (control, gray; motivated forgetting, red; adapted from Dehnavi et al. 2019). Stimulation procedures reveal often weak effect sizes: In addition to stimulation efficacy depending on parameters of the stimulation and neuroanatomical features, induced neural responses depend on interactions between interindividual factors and processing demands. Tasks affected by stimulation are schematized for rodents by the object place recognition task and for humans by the figural paired-associate task (reviewed in Campos-Beltrán and Marshall, 2017; Koo et al., 2018). Hypnograms (human, 7 h; rodent, 1 h) schematize an interim period of sleep. W, Wake. The asterisks indicate significant differences: *, $p < 0.05$; **$p < 0.01$.

between learning and stimulation (Lu et al., 2018; Miyamoto et al., 2016), subjects' cognitive ability (Koo et al., 2018), different processing demands of the learning material, and/or differences in NREM sleep stage composition at the time of stimulation (Dehnavi et al., 2019; Jiang et al., 2019; Lerner et al., 2019; Figure 2). For example, when, after learning a list of word pairs, subjects are instructed to forget correspondingly cued words (motivated forgetting), the relation of post-task spindle density during light versus deep NREM sleep differed as compared to a control condition (Figure 2). We found higher spindle density in N2 during sleep subsequent to motivated forgetting leading to the notion that spindles during N2 enhance the erasure of unwanted memories (Dehnavi et al., 2019; Figure 2). Regardless of the specific function, it is well conceivable that stimulation during post-task N2 versus N3 would differentially affect the ongoing consolidation process.

In rodents, optogenetic tools can be employed to interact with brain rhythms at high temporal precision. For instance, optogenetic stimulation with temporal resolution in the millisecond range was used to drive sleep spindles. Increased memory consolidation and increased coupling of SOs, sleep spindles, and ripples indicated the functional relevance of this manipulation (Latchoumane et al., 2017). In our study, we employed optogenetic inhibition of the monosynaptic connection from the hippocampus (ventral region) to the neocortex (medial prefrontal cortex) to investigate its role for both ongoing neural oscillations and behavior after systems consolidation (Figure 2). We showed that inhibition of this pathway during NREM sleep subsequent to consecutive days of learning on the Barnes maze reduced phase-specific coupling of SPWR-to-spindle and spindle-to-SO events as well as memory performance (Binder et al., 2019; Figure 1).

In summary, investigations into the underpinnings of sleep-associated memory consolidation have made major gains in the last few decades. It will be the task of future research to disentangle the relevance of activity at different hierarchical time and spatial scales and correlate it with behavioral output to discover functional relevance.

Glossary

NREM: Nonrapid eye movement (sleep) is composed of different brain oscillations (or rhythms). In humans, light N1 to deep N3 stages differ. Sleep spindles commence in N2. Delta waves (1–4 Hz) and SOs (~1 Hz) are most pronounced in N3. In rodents, NREM sleep periods are shorter and the depth of sleep fluctuates more frequently; thus, a differentiation of sleep depths is not made.

Phase-amplitude coupling: This is a method to describe how oscillations in various frequency bands interact. It characterizes the modulation in the amplitude (or power) of one oscillation by the phase of a slower oscillation. A more general description is cross-frequency coupling, wherein the interaction of other signal parameters may interact (see also Jensen and Colgin, 2007).

Reactivation: This refers to the re-occurrence of neurons and/or neuronal networks that were active during encoding (learning) during postlearning sleep. Direct measurements assess cellular activity. Reactivation may be inferred indirectly from measurements assessing activity of larger networks (see also Klinzing et al., 2019).

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Bionotes

Maryam Ghorbani
Department of Electrical Engineering, Rayan Center for Neuroscience and Behavior, Ferdowsi University of Mashhad, Mashhad, Razavi Khorasan, Iran

Maryam Ghorbani received her doctoral degree in biological physics at the Institute for Advanced Studies, Zanjan, Iran, in 2009. She started her research on sleep at the University of California, Los Angeles, CA, USA, as a postdoctoral scholar working under supervision of Prof. Robijn Bruinsma and Prof. Mayank Mehta. Since 2013, she has been an assistant professor at the Biomedical Engineering Department and the Rayan Center for Neuroscience at Ferdowsi University of Mashhad, Mashhad, Iran. Her present research focuses on neuronal modeling in addition to analysis of human EEG and rodent extracellular recordings to understand the mechanisms underlying the generation of sleep rhythms and their functional importance. She is also interested in understanding the effect of both external and endogenous electric fields on neuronal activity.

Lisa Marshall
Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Lübeck, Germany
lisa.marshall@uni-luebeck.de

Lisa Marshall studied biology and received her doctoral degree at the Institute of Physiology, Humboldt-University of Berlin, Charité (Peter Bartsch). At the Institute of Neuroendocrinology of the University of Lübeck (Jan Born), she began her research on sleep. During a habilitation scholarship in 2000, she was a research fellow at the Center for Molecular and Behavioral Neuroscience, Newark, NJ, USA (György Buzsáki). In 2009 she became Professor of Behavioral Neurobiology at the Department of Neuroendocrinology and in 2014 at the Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck (Markus Schwaninger). As the head of the Research Group Neuroplasticity and Rhythms, her present research on mice and humans focuses on the factors and mechanisms through which sleep-associated memory consolidation is modulated.

Review article

Sandra Pérez-Domínguez, Shruti G. Kulkarni, Carmela Rianna and Manfred Radmacher*

Atomic force microscopy for cell mechanics and diseases

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Abstract: Atomic Force Microscopy (AFM) is a powerful technique widely employed in biophysics, for instance to study topography of living cells and cell mechanics. Cell mechanics is a very interesting, biophysical parameter of cells, because it is strongly changed by various cellular processes, for example during cell division, cell movement, differentiation, aging, and also various diseases. Since cancer is a prominent example of changes in mechanical properties of diseases, the concept of the mechanical fingerprint has developed, which makes it possible to distinguish between healthy and diseased cells. In this article we report on various studies of cell mechanics with the AFM. We will first give a brief introduction on AFM principles and operational modes and then we will report on some applications of AFM in the field of cellular biophysics, like discriminating between healthy and cancer cells, as well as distinguishing cancer cells at different stages of malignancy. Overall, we will show that AFM has made a significant contribution in studying the biophysics of cancer and the concept of mechanical fingerprints could find a wide variety of applications in biomedicine and medical diagnostics.

Keywords: Atomic Force Microscope; biophysics; cancer; disease

Zusammenfassung: Das Kraftmikroskop (AFM für Atomic Force Microcope) ist ein vielfältiges Instrument, das immer häufiger in der Biophysik, insbesondere für topographische und mechanische Untersuchungen von Zellen, verwendet wird. Hier ist besonders hervorzuheben, dass das Kraftmikroskop es erlaubt zelluläre Prozesse in physiologischen Bedingungen zu verfolgen. Zellmechanik ist eine sehr interessante, biophysikalische Kenngröße, da sie stark durch verschiedenste zelluläre Prozesse verändert wird. Beispiele hierfür sind Zellteilung, Zellbewegung, Differentiation, Altern, und auch diverse Krankheiten. Da sich bei diversen Krankheiten, Krebs ist hier nur ein prominentes Beispiel, die mechanischen Eigenschaften ändern, hat sich das Konzept des mechanischen Fingerabdrucks entwickelt, also die Idee damit zwischen gesunden und kranken Zellen zu unterscheiden. Wir geben in diesem Artikel einen Überblick über Studien der Zellmechanik mit dem Kraftmikroskop. Zunächst werden wir in die Technik des Kraftmikroskops und einfache Betriebsmodi, etwa zur Abbildung, berichten, um dann über Anwendungen in der zellulären Biophysik zu berichten. Gerade bei Krebszellen hat sich gezeigt, dass man mit Hilfe der Zellmechanik nicht nur zwischen benign und malign, sondern sogar den Grad der Malignizität bestimmen kann. Dieses Beispiel zeigt sehr eindrücklich, dass das Studium der mechanischen Eigenschaften von Zellen und Geweben bereits jetzt hilft Zellen und deren Zustand zu charakterisieren. Das Konzept des mechanischen Fingerabdrucks könnte vielfältige Anwendungen in der Biomedizin und in der medizinischen Diagnostik finden.

Schlüsselwörter: Biophysik; Kraftmikroskopie; Krankheiten; Krebs; Zellmechanik

Introduction

Atomic Force Microscopy (AFM) is a powerful technique to measure topographical and mechanical properties of soft samples including live cells. AFM belongs to the family of scanning probe microscopes, which, as the name suggests, is based on the interaction between a scanning probe (like a tip) and the sample. The precursor of the AFM was the Scanning Tunneling Microscope (STM), developed in the early 1980's to study surface structures of conductive samples (Binnig and Rohrer, 1983). While STM is based on the tunneling current between a metallic tip and a conductive sample, AFM is based on the forces between the tip and the surface (Binnig et al., 1986). Initially, AFM had been mainly used to measure topography of non-biological

Sandra Pérez-Domínguez and Shruti G. Kulkarni contributed equally to the paper.

***Corresponding author: Manfred Radmacher,** Institute of Biophysics, University of Bremen, Otto-Hahn Allee 1, 28359 Bremen, Germany, E-mail: radmacher@uni-bremen.de. https://orcid.org/0000-0001-8744-4541

Sandra Pérez-Domínguez, Shruti G. Kulkarni and Carmela Rianna: Institute of Biophysics, University of Bremen, Bremen, Germany

samples; however, the high-resolution capability of AFM has been increasingly used for biological and biomedical applications as well (Drake et al., 1989). Nowadays, AFM is employed for other applications aside from studying topography, such as cell mechanics (Radmacher et al., 1995), single-molecule interaction force measurements (Florin et al., 1994), and cell-cell interaction studies (Grandbois et al., 2000; Viji Babu et al., 2018).

This review will focus on the study of cell mechanics and elastic properties of cells, which can be investigated with different techniques, like micropipette aspiration (Hochmuth, 2000), optical (Yousafzai et al., 2017; Zhang and Liu, 2008) or magnetic tweezers (Jakab et al., 2002), magnetic twisting cytometry (Laurent et al., 2002), optical stretching (Guck et al., 2001), real-time deformability cytometry (Mietke et al., 2015; Otto et al., 2015), and AFM (Rotsch et al., 1999). AFM has the advantage of allowing mechanical measurements on adherent cells, whereas the majority of other techniques can be applied to cells in suspension. In addition, AFM allows positioning the probe with high resolution for measuring the mechanical properties at different regions of the cell. Recently, a large interest in studying cell mechanics has been raised, mainly because it has a fundamental role in many cellular processes, including cell protrusion, division, migration, differentiation, and morphology (Bohnet et al., 2006; Chen et al., 2004; Lautenschläger et al., 2009; Matzke et al., 2001; McKenzie et al., 2018; Prass et al., 2006; Rehfeldt et al., 2007). Moreover, changes in cell mechanics are strongly related to many diseases, such as cancer, blood, and cardiovascular diseases (Rianna and Radmacher, 2016a). For mammalian cells, it has been shown that cell mechanical properties are mainly determined by the cell cytoskeleton network, where the density and arrangements of filaments, the number of cross-links, activity, and stress generation affect the elastic properties (Brückner and Janshoff, 2015; Moeendarbary and Harris, 2014; Pegoraro et al., 2017; Rotsch and Radmacher, 2000; Schäfer and Radmacher, 2005). The cytoskeleton is mostly made of actin, intermediate filaments, and microtubules. Currently, studies have demonstrated that disruption, disorganization, and deformability of the cytoskeleton play an important role in cancer, even allowing the differentiation between cells in distinct stage of the disease (Ochalek et al., 1988). These changes are commonly related to either a partial loss of actin filaments (Yamaguchi and Condeelis, 2007) or disorganization of microtubules (Pachenari et al., 2014) being the consequence of the lower density of the cellular scaffold.

Moreover, large differences between the mechanical properties of cancerous and healthy cells have been measured using AFM (Abidine et al., 2018; Cross et al., 2008; Lekka, 2016; Li et al., 2008; Prabhune et al., 2012). Lekka and co-workers were the first to use AFM to compare normal and cancer cells, and they found that bladder cancer cells were one order of magnitude softer than their normal counterparts (Lekka et al., 1999). Thereafter, several groups have compared the mechanical properties of cancerous and healthy cells, reaching the consensus that cancer cells are softer than normal (healthy) ones. However, more recent studies have shown that cell mechanics is also strongly affected by the cell's microenvironment, e. g., using materials of different stiffness as cell culture supports; thus, the general paradigm of cancer cells being softer than normal cells can even be inverted (Alibert et al., 2017; Rianna and Radmacher, 2017; Rianna et al., 2017).

In this review, we discuss the applications of AFM in diseases, with a special focus on cancer. We will first give a brief introduction on AFM principles and operational modes, with emphasis on using the AFM to study cell mechanics. Then, we will report the AFM's contribution in the field of cellular biophysics like discriminating between cell's physiological and pathological conditions as well as distinguishing cancer cells at different stages of malignancy. To conclude, we will discuss some future perspectives and possible directions for the widespread use of AFM in the field of biomedicine.

AFM principles

AFM can be used in various modes to image a surface or to study its mechanical properties. The main components of a typical AFM setup are a microfabricated cantilever with an integrated tip, a laser, a position-sensitive photodiode for measuring the cantilever's deflection, and an xyz piezo-scanner for positioning the sample or tip (Figure 1A). The cantilever, which is considered as a spring, is brought in contact with the sample (Figure 1B). The cantilever's deflection is measured using the optical lever scheme. A laser beam is reflected from the back of the cantilever, which is coated with a reflective material, like gold, and detected by a split photodiode. The difference in the photocurrents of the two segments of the photodiode is a measure of the deflection of the cantilever. The position of the cantilever (or of the sample) is accurately moved and controlled by the xyz piezo-ceramic transducer. For mechanical measurements (e. g., to study cell mechanics), the tip gradually approaches and indents the sample until a maximum pre-set loading force is reached. Then, the tip is retracted from the sample and stops at its original position off the surface. During the approach and retraction, the deflection of the cantilever is recorded and displayed in a force curve,

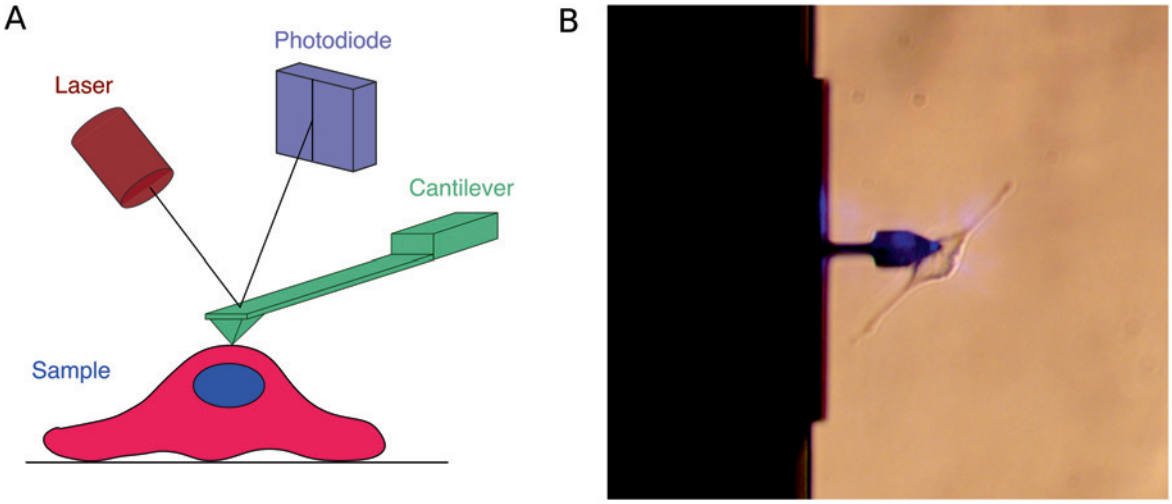

Figure 1: (A) Components of a typical AFM set-up: Laser, Split-photodiode, Cantilever and Sample. (B) Image of an AFM cantilever in contact with a live NIH-3T3 fibroblast in Dulbecco's Modified Eagle Medium with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin. Experiments were done in 5% CO_2.

which shows the deflection of the cantilever versus the z height (Figure 2).

The elastic properties of the sample can be obtained by analyzing the force, F, versus the indentation, δ. Both quantities can be derived from a force curve: the force, by multiplying the spring constant of the cantilever k with the measured deflection d, and the indentation, by subtracting the deflection from the sample height z:

$$F = kd \tag{1}$$

$$\delta = z - d \tag{2}$$

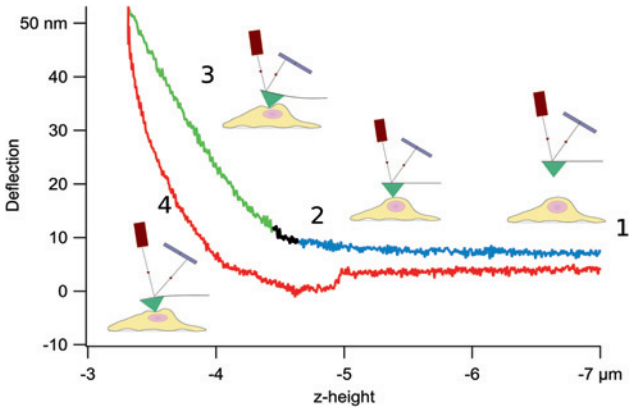

Figure 2: Schematic of an AFM force curve: (1) As the AFM tip approaches the cell, the laser's deflection remains constant, (2) when the tip comes in contact with the cell and starts to indent it, the laser is deflected and its position on the photodiode changes, (3) as the tip continues to indent, the deflection increases and (4) after the deflection trigger threshold is reached (50 nm in this example), the tip is retracted.

AFM of live cells requires certain conditions in order to keep them alive and intact. Biological conditions such as the liquid medium, pH, and temperature should also be maintained during the experiment. When compared to fixed or air-dried cells, live cells are much softer, and special attention must be paid to AFM experimental conditions such as choosing the appropriate cantilever (force constant and tip geometry) and calibrating these two parameters correctly to carry out accurate force measurements. A large variety of cantilevers, which offer many different force constants and tip geometries, are commercially available. The pressure exerted by the cantilever tip on the live cell should be minimized, and at the same time, appropriate force must be exerted to obtain an elastic response. AFM tips can be sharp (such as triangular or pyramidal shape) or relatively blunt (like spherical tips). A sharp probe has the advantage of higher resolution mapping of cells' topography (spatial resolution); however, it may also damage the cell due to higher pressures applied. Hence, when using sharp tips for cell investigations, the spring constant should be very low, to prevent cell damage during measurement. Blunter tips will reduce the possibility of damaging the cell but sacrifices lateral resolution.

Apart from choosing the appropriate cantilever for each measurement, knowing its spring constant k is also crucial. The manufacturer usually only claims a nominal spring constant of the cantilever with a large error margin. Therefore, the spring constant has to be calibrated by measuring the thermal fluctuations of the cantilever and employing Boltzmann's equipartition theorem (Hutter and Bechhoefer, 1993). According to this theorem, the energy in any degree of freedom of the system has to be equal to the

thermal energy due to the absolute temperature of the system.

$$\langle E \rangle = \frac{k_B T}{2}, \tag{3}$$

where E is the energy, k_B is the Boltzmann constant, and T is the temperature. Assuming that the cantilever undergoes mainly bending vibrations at the resonance frequency, we estimate the spring constant from the fluctuations in tip position:

$$\langle E \rangle = \frac{k_c \langle d^2 \rangle}{2}, \tag{4}$$

where E is the energy, k_c is the spring constant, and $\langle d^2 \rangle$ is the mean square displacement of the cantilever. By joining both equations, we can obtain the spring constant:

$$k_c = \frac{k_B T}{\langle d^2 \rangle}. \tag{5}$$

The value $\langle d^2 \rangle$ is measured from the fit of the power frequency spectrum. This can be done with the AFM itself or with a very accurate vibrometer. Some AFM cantilevers can be purchased already calibrated by the manufacturer. A procedure to reduce errors and to obtain mechanical data in a reproducible and standardized manner has been reported recently (Schillers et al., 2017).

Mechanical properties

To measure the mechanical properties of a soft sample, e. g., a cell, the force applied versus the achieved indentation has to be analyzed with an appropriate model from contact mechanics (Figure 3A). In contact mechanics, stress (force per area) and strain (relative length change) are related by the elastic (or Young's) modulus, E. Here, we use the force indentation relations (Figure 3B), which were initially derived by Heinrich Hertz in 1882 (Hertz, 1881), for the case of spherical indenters. The Hertz model can also be applied to parabolic indenters, which are occasionally used as well. Sneddon extended the former model to the case of conical indenters (Sneddon, 1965), whereas Rico and co workers extended it to pyramidal tips (Rico et al., 2005). The geometry of the indenter used during the experiment determines the relation among applied loading force, indentation, tip geometry, and the Young's modulus value of the sample:

$$\text{Parabolic or spherical indenters}: F = \frac{4}{3}\sqrt{R}\,\frac{E}{1-\nu^2}\delta^{3/2} \tag{6}$$

$$\text{Pyramidal indenter:} = \frac{1}{\sqrt{2}}\tan\alpha\,\frac{E}{1-\nu^2}\delta^2 \tag{7}$$

where F is the loading force, R is the radius of the curvature in spherical or parabolic probes, α is the half-opening angle of the cone or pyramidal face angle, and ν is the Poisson's ratio (which is considered 0.5 for cells, since the volume is conserved during compression). Finally, as previously mentioned, δ is the indentation depth and E is the elastic or Young's modulus of the sample.

The Hertz model is based on several assumptions: the sample is considered as an isotropic, homogeneous, and linear elastic material; moreover, it should be flat and infinitely thick. In a strict sense, none of these assumptions are fulfilled for cells; nevertheless, the Hertz model describes the mechanical data of cells surprisingly well and is therefore used widely. However, three points should always be

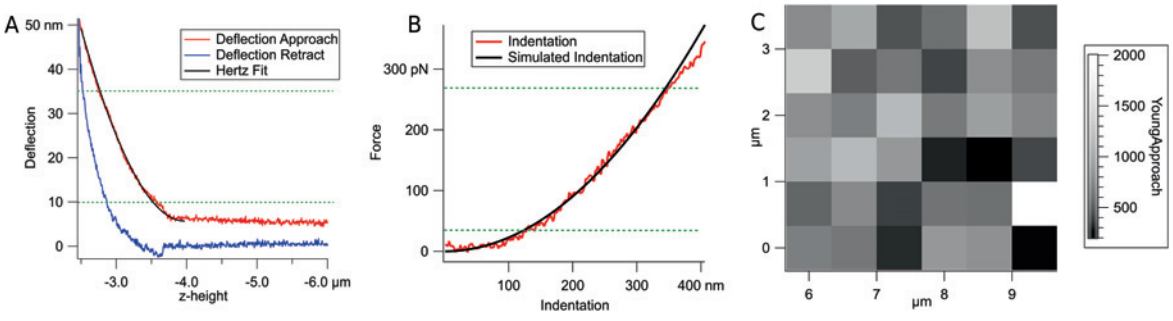

Figure 3: (A) Representation of an AFM force curve: deflection v/s z-height with approach and retract curves in red and blue, respectively. The Hertz fit on the approach curve is displayed in black with a fit range of 10–35 nm (green lines). (B) Representation of an AFM indentation curve: Force v/s indentation in red, where force is calculated using the relation $F = kd$, and then plotted against the indentation calculated using the formula $\delta = z - d$. Force range used to fit it corresponds to the deflection range used in the Hertz fit. (C) Force map, where each point is the Young's modulus calculated from the Hertz fit of the approach curve.

appreciated: the cell's internal structures, its finite thickness, and its viscous contributions. The latter can be easily seen in the hysteresis between the loading and unloading force curves (approach and retract) in the indentation part of the force curve (Kirmizis and Logothetidis, 2010) (Figure 3A).

Another critical parameter to bear in mind while measuring the cell's elastic properties is the indentation depth of the tip. Cells are living systems composed of a large number of different structures, such as organelles, cytoskeleton network, nucleus, etc. Depending on the indentation depth, different internal cellular structures may be compressed. Hence, the Young's modulus reflects the mechanical response originating from several cellular structures. Consequently, the correct choice of indentation depth can be crucial for the identification of pathologically modified cells (Lekka, 2016).

The velocity at which the measurement is performed is also an important parameter in the AFM experiment, which needs to be controlled. Large tip velocity results in high viscous response of the cell, whereas at very low speeds, the cell may undergo internal reorganization or movement, possibly in response to or induced by the indentation of the tip.

AFM applications: cell mechanics and cancer

Cell mechanics is a novel and very important biophysical property of cells, which is strongly connected to many cellular processes, such as migration, differentiation and aging (Bohnet et al., 2006; Chen et al., 2004; McKenzie et al., 2018). In diseases, including cancer, mechanical properties of cells often change, resulting in the notion of the mechanical fingerprint of diseases (Rianna and Radmacher, 2016a; Suresh, 2007).

Cancer is one of the most numerous causes of deaths worldwide. When this disease occurs, cancer cells acquire anomalous abilities, e. g., the formation of metastases (leaving local tissue to migrate through blood vessel to other secondary organs and invading them) and continuous proliferation. Another peculiar change happening during cancer is the change in the mechanical properties of cancer cells. Using different techniques, changes in cancer cell mechanics have been reported, leading to the notion of using cell mechanics as a parameter to discriminate between normal and cancer cells.

The study of cancer mechanics with the AFM has been introduced by Lekka and co workers (Lekka et al., 1999). In this study, they compared the elastic properties of cancer and normal cells and found that bladder and ureter cancer cells were one order of magnitude softer than normal cells. After this study, many more groups have used AFM to compare the mechanics of cancer cells and their normal counterparts.

Our group also contributed to these investigations. In 2012, Prabhune et al. employed AFM to compare the mechanical properties of primary thyroid cells to their malignant counterparts (Prabhune et al., 2012). AFM experiments were performed over three consecutive days after cell seeding to consider the effect of culture time on the elastic properties of both cell lines. It was found that cancer thyroid cells were softer than normal ones, and even over time, the differences between them increased (from ~1.5 to 5 kPa). Using the AFM in imaging mode, they also detected topographical differences between the two cell lines and found that cancer cells were higher than normal cells. Moreover, AFM measurements were complemented with fluorescence microscopy. Staining cell actin filaments with rhodamine-phalloidin, they observed that cancer cells presented fewer stress fibers and single filaments distributed throughout the cell body. Contrarily, primary thyroid cells exhibited thick bundles of actin filaments, running continuously at the basal and nuclear regions. This supports the hypothesis that changes in actin organization are connected to cell mechanics and cancer's mechanoadaptive phenomenon.

As mentioned above, many studies have reported that cancer cells are softer than normal cells (Lekka, 2016; Lekka et al., 1999; Li et al., 2008; Prabhune et al., 2012; Rebelo et al., 2013); however, in the majority of these experiments, conventional cell culture systems using Petri dishes as support, whose mechanical and topographical properties are very different from those in tissues, were used. In order to better mimic the natural cell microenvironment, the mechanics of cancer cells has also been investigated on different stiffness gels, for example, cancer cells have been plated on soft collagen matrices by Staunton et al. (Staunton et al., 2016), where they found that breast adenocarcinoma cells, which invaded the matrix, were stiffer than cells remaining on top of the collagen gel, suggesting that the contractility of actomyosin plays an important role in the first steps of metastatic invasion.

Attempting to further investigate differences in the mechanics of cancer and normal cells mimicking the mechanical properties of native tissues, Rianna et al. measured the viscoelastic properties of healthy and cancer thyroid cells on stiffness-tunable polyacrylamide gels (PA) (Rianna and Radmacher, 2016b). In this study, PA gels with two different stiffness values were used as cell culture supports and Petri dishes were used as control. They found that, in agreement with previous findings, when seeded on

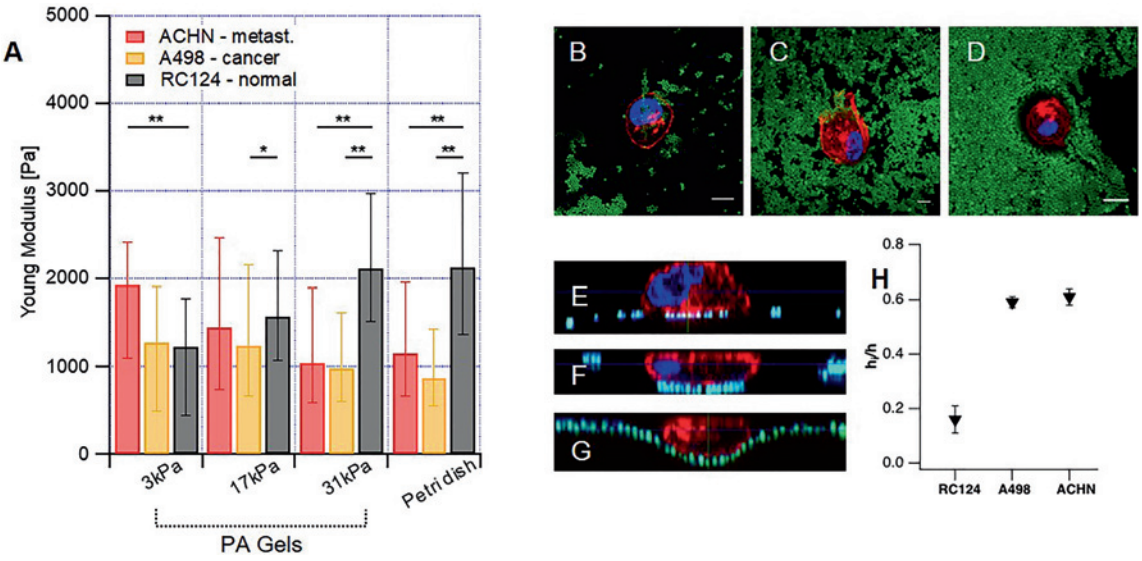

Figure 4: (A) Young's modulus values of normal and cancer cells on substrates with different mechanical properties: 3, 17 and 31 kPa stiffness PA gels and conventional Petri dishes, as a control. Red, orange and black bars stand for metastatic ACHN, cancer A498 and normal RC124 renal cells, respectively. Confocal images of fixed cells with color channels blue for nucleus, green for fluorescent bead and red for actin cytoskeleton are reported. Confocal images (B–D) and confocal side view (E–G) of normal RC124 (B and E), cancer A498 (C and F) and metastatic ACHN cells (D and G) on soft PA gels (3 kPa) with embedded 1 µm diameter green fluorescent beads Scale bars are 10 µm. The index of cell indentation (H) calculated as the ratio between the cell portion indenting the gel (h_i) and the total cell height (h); markers are median values and error bars are standard deviation. Reproduced from Rianna and Radmacher, 2017 with permission from the Royal Society of Chemistry.

Petri dishes, thyroid cancer cells were softer than their normal counterparts; however, the trend was reversed on soft gels, with cancer cells being stiffer than normal ones. Moreover, cancer cell mechanics were independent of substrate rigidity, showing constant values of Young's modulus (1.2–1.5 kPa) on gels with different stiffness, whereas the Young's modulus measured from normal cells increased with substrate stiffness (1.2–2.6 kPa). Thus, normal cells adapt their viscoelasticity to those of the underlying materials, whereas cancer cells are insensitive to the mechanics of the surrounding environment. In agreement with this result, Lin et al. found that most cancer cells did not alter their mechanical properties on matrices of varying stiffness, defining loss of stiffness sensing as a feature of the mechanical phenotype of cancer cells (Lin et al., 2015).

The stiffening of cancer cells on soft gels could be related to their function in metastasis, i. e., when in contact with soft and compliant matrices, cancer cells could attempt to invade and penetrate the gel, as they do with the local matrices in the process of metastatic invasion. This hypothesis is consistent with previous findings, showing that metastatic cancer cells apply high lateral forces on soft PA gels and that applied forces directly correlate with metastatic potential (Kristal-Muscal et al., 2015). Moreover, other studies showed that metastatic cancer cells increase their stiffness while invading into collagen I matrices (Staunton et al., 2016) and proved the role of traction stresses in regulating the mode with which cancer cells invade ECM networks to contribute to cancer metastasis (Aung et al., 2014).

To gain further insights in the relation between cancer cell mechanics on soft gels and metastatic invasive potential, Rianna et al. employed AFM and confocal microscopy to study the mechanical properties and invasive index of normal, cancer, and metastatic renal cells seeded on soft PA gels (Rianna and Radmacher, 2017). In accordance with their previous findings (Rianna and Radmacher, 2016b), they confirmed that cancer cells lost the ability to adapt their stiffness to the rigidity of the underlying support and that they were stiffer than normal cells when seeded on soft gels. Further studies on cancer cell invasion into PA gels by confocal microscopy revealed that the cell indentation index (calculated as the ratio between the cell portion indenting the gel and the total cell height) was higher in metastatic cells than in healthy cells (Figure 4).

Conclusions

The mechanical properties of cells, especially in diseased cells, are a marker of cell state and condition. Thus, studying cell mechanics will lead to an improved

understanding of cellular function and structure. Cell mechanics bridges the biochemical properties of living systems with their biophysical structure, as is becoming apparent in the case of actin cytoskeleton remodeling in cancer cells. In this review, we demonstrated that studying the mechanics of biological systems has vast potential in understanding health and disease.

AFM is an excellent tool to study cell mechanics, mainly for two broad reasons. First, with the combination of its high spatial resolution, including topographic imaging, with its great versatility in determining mechanical properties, mechanical measurements of biological samples can be performed fast and with high sensitivity. A large variety of relevant mechanical parameters can be obtained, and the mechanics of a cell's interaction with its environment can also be studied. There is also a variety of cantilevers to choose from, depending on the biological question and system, the mechanical parameters to be obtained, and the AFM system available. Second, it can be performed under biological conditions and for a wide range of samples, such as living samples in physiological environment. Application of drugs or other treatments during an experiment is also feasible as well as the combination with other broadly used microscopy techniques in biology, such as confocal microscopy.

Thus, AFM is a unique tool for determining the forces of, and between, cells and the mechanical properties of cells. It is entering its mature stage; therefore, AFM is moving from the field of basic research to biomedical applications, which opens up new and exciting applications. It may even pave the road to using the mechanical fingerprint of cells in diseases as a new diagnostic tool.

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Bionotes

Sandra Pérez-Domínguez
Institute of Biophysics, University of Bremen, Bremen, Germany

Sandra Pérez-Domínguez is a PhD student at the Institute of Biophysics, University of Bremen, Germany, funded by H2020-MSCA-ITN 2018. She obtained her Bachelor's degree in Chemistry in 2017 and her Master's degree in Molecular and Cellular Biology in 2018 at the University of Zaragoza, Spain.

Carmela Rianna
Institute of Biophysics, University of Bremen, Bremen, Germany

Dr. Carmela Rianna is a post-doctoral researcher at the Institute of Biophysics, University of Bremen, Germany. In 2015 she obtained a PhD at the Department of Chemical, Materials and Industrial Production Engineering, University of Naples Federico II, Italy. Previously, she obtained Bachelor's and Master's degrees in Biomedical Engineering at the University of Naples Federico II. In 2018 she was a post-doctoral researcher fellow for one year at the University of California, Berkeley, Department of Bioengineering, Berkeley, CA, USA.

Shruti G. Kulkarni
Institute of Biophysics, University of Bremen, Bremen, Germany

Shruti G. Kulkarni is an MSCA-ITN Early Stage Researcher and PhD student at Institute of Biophysics, University of Bremen. She obtained her joint Bachelor and Master of Science in Biological Sciences at the Indian Institute of Science Education and Research – Kolkata, West Bengal, India in 2019, with her MS thesis focusing on Biophysics.

Manfred Radmacher
Institute of Biophysics, University of Bremen, Bremen, Germany
radmacher@uni-bremen.de
https://orcid.org/0000-0001-8744-4541

Dr. Manfred Radmacher is a full professor in Biophysics at the University of Bremen. He studied physics at the Technical University in Munich where he also received his PhD in 1993 on applications of the Atomic Force Microscope for the investigation of soft samples. After two years as a Post-Doc at the University of California in Santa Barbara, he returned to the Ludwig-Maximilians University in Munich, where he received his habilitation in 1999. Then he moved to Göttingen to be Professor at the University, until he started his current full professor position in 2002 at the University of Bremen. His main interest are the mechanical properties of cells and the relation between cell mechanics and diseases.

Presentation of scientific institutions

Shuyan Liu, Anne Beck*, Michael N. Smolka, Christian Beste, Tanja Endrass, Michael A. Rapp, Falk Kiefer, Heike Tost, Rainer Spanagel and Andreas Heinz

Verlust und Wiedererlangen der Kontrolle über den Drogengebrauch

Zusammenfassung des CRC/TRR 265 (https://sfb-trr265.charite.de/)

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Hauptrisikofaktoren für Mortalität und Morbidität weltweit sind Alkohol- und Tabakkonsum. Während das Wissen über individuelle Faktoren, welche die Entstehung und Aufrechterhaltung des Substanzkonsums fördern, zunimmt, fehlt es immer noch an fundiertem Wissen über modulierende Faktoren und Mechanismen, die zum Verlust und zur Wiedererlangung der Kontrolle über den Drogenkonsum beitragen. Ein besseres Verständnis dieser Faktoren und Mechanismen wird entscheidend sein, um die Behandlung von Störungen des Substanzgebrauchs zu verbessern. Bei den Mechanismen, die zum Verlust und zur Wiedererlangung der Kontrolle über den Substanzgebrauch, insbesondere von Alkohol und Tabak, beitragen, lassen sich hier vier Aspekte unterscheiden (siehe Abbildung 1):

(I) Der Einfluss von Pawlowschen Mechanismen, die dazu führen, dass drogenassoziierte Reize als konditionierte Stimuli wirken und Drogenverlangen auslösen können. Einfluss auf komplexe Verhaltensweisen können solche Pawlowschen Stimuli dann nehmen, wenn sie beispielsweise im Rahmen des sogenannten „Pavlovian-to-Instrumental Transfer“ (PIT) als Hintergrundreize Einfluss auf die Ausführung instrumenteller Verhaltensweisen nehmen. So konnte gezeigt werden, dass positiv konditionierte Pawlowsche Reize ein Annäherungsverhalten fördern, auch wenn letzteres im Rahmen zielgerichteter Verhaltensweisen gar nicht hilfreich ist (Garbusow et al., 2014). Individuelle Unterschiede in der Ansprechbarkeit auf solche Pawlowschen Reize im Rahmen des PIT-Paradigmas waren mit verstärktem Alkoholkonsum bei jungen Erwachsenen und mit einem erhöhten Rückfallrisiko bei alkoholabhängigen Patienten assoziiert (Garbusow et al., 2015; Sommer et al., 2020).

(II) Zweitens tragen zum Verlust der Kontrolle über den Substanzgebrauch habituelle Konsummuster bei, die mehr oder weniger „automatisiert“ ablaufen und – anders als zielgerichtete Verhaltensweisen – nicht alle im Handlungsfeld möglichen Optionen bei der Handlungsplanung berücksichtigen (Dolan & Dayan, 2013). Erste Studien beim Menschen zeigen bei vielen Substanzgebrauchsstörungen eine Neigung zu gewohnheitsmäßigem anstelle von zielgerichtetem Verhalten (habitual versus goal-directed control), bezüglich des Alkoholkonsums müssen aber Alkoholerwartungen und damit kontextuelle Faktoren berücksichtigt werden (Voon et al., 2015; Sebold et al., 2017).

(III) Als dritter wesentlicher Faktor wird der Gebrauch alternativer Verstärker angesehen, der zur Wiedererlangung der Kontrolle über den Substanzgebrauch beitragen kann, oder eben dann zum Verlust der Kontrolle über den Drogenkonsum beiträgt, wenn alternative Verstärker gegenüber der Drogenwirkung an Bedeutung verlieren. Eine aktuelle Arbeit aus der Arbeitsgruppe von Markus Heilig zeigt hier, dass bezüglich des Alkoholkonsums Veränderungen in der GABAergen Inhibition im limbischen System, insbesondere in der Amygdala, zur relativen Bevorzugung beziehungsweise Vernachlässigung alternativer Verstärker gegenüber dem Drogenkonsum beitragen (Augier et al., 2018).

***Corresponding author: Anne Beck**, Klinik für Psychiatrie und Psychotherapie, Charité – Universitätsmedizin Berlin, Charité Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany, E-mail: anne.beck@charite.de

Shuyan Liu and Andreas Heinz: Klinik für Psychiatrie und Psychotherapie, Charité – Universitätsmedizin Berlin, Charité Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany
E-mail: siyan908@hotmail.com (S. Liu), andreas.heinz@charite.de (A. Heinz)

Michael N. Smolka, Christian Beste and Tanja Endrass: Technische Universität Dresden, 01062 Dresden, Germany

Michael A. Rapp: Universität Potsdam, Am Neuen Palais 10, 14469 Potsdam, Germany

Falk Kiefer, Heike Tost and Rainer Spanagel: Zentralinstitut für Seelische Gesundheit Mannheim (ZI Mannheim), J 5 68159 Mannheim, Germany

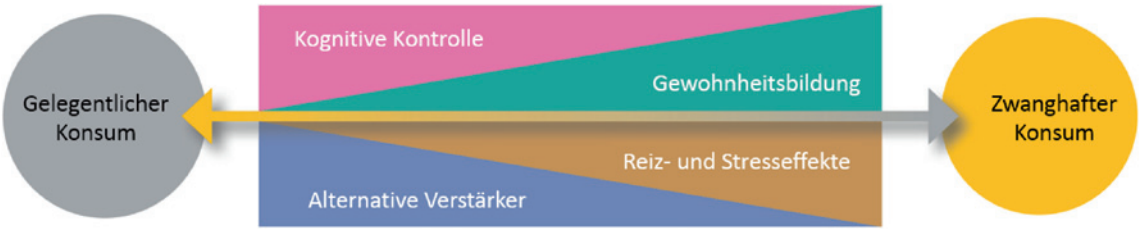

Abbildung 1: Vier Schlüsselkomponenten im Kontext: Verlust versus Wiedergewinnung der Kontrolle über den Drogengebrauch.

(IV) Viertens wird die Wiedererlangung der Kontrolle über den Substanzkonsum in aller Regel mit Inhibitionsmechanismen in Verbindung gebracht, die im Rahmen langfristiger Handlungsplanung als neurobiologisches Korrelat eine Aktivierung des frontalen Kortex voraussetzen (Volkow & Morales, 2015). Bei alkoholabhängigen Patienten konnte gezeigt werden, dass Exekutivfunktionen wie das Arbeitsgedächtnis bei vielen Patientinnen und Patienten nicht signifikant beeinträchtigt sind, was aber bezüglich der neurobiologischen Korrelate eine erhöhte und damit wahrscheinlich „ineffiziente" Aktivierung im frontalen Kortex gegeben ist, die wiederum mit dem Rückfallrisiko nach Entgiftung korreliert (Charlet et al., 2014). Umgekehrt gibt es Hinweise darauf, dass das Training von Exekutivfunktionen oder die indirekte Verbesserung durch sportliche Aktivität zur Senkung des Rückfallrisikos beitragen könnte (Zschucke et al., 2012).

Das Ziel unseres Forschungskonsortiums ist es, die Trajektorien des Verlusts und der Wiedererlangung der Kontrolle über den Drogenkonsum zu identifizieren, die zugrundeliegenden neurobiologischen und Lernmechanismen zu untersuchen und Mechanismen-basierte Therapien zu entwickeln. Diese Ziele sollen durch drei Ansätze erreicht werden:

(I) Die Nutzung innovativer sogenannter Mobile-Health-Tools um im Verlauf den Einfluss von Triggern (Drogenreizen, Stressexposition und kleine Drogenkonsummengen zu Beginn des Rückfalls) und der modifizierenden Faktoren (z.B. Alter, Gender, körperliche Aktivität und kognitive Funktionen) auf den Alkohol- und Tabakkonsum in der Lebenswelt und in Tiermodellen des Suchtverhaltens zu untersuchen.

(II) In eng-vernetzen Studien am Menschen und im Tiermodell (translationale Tandemprojekte) sollen die entscheidenden Mechanismen identifiziert und mathematisch modelliert werden, die den Einfluss solcher Trigger und modifizierender Faktoren auf zielgerichtete, habituelle und zwanghafte Aspekte des Drogenkonsums vermitteln.

(III) Darauf aufbauend sollen Interventionen entwickelt werden, die spezifisch auf diese Mechanismen zielen, um die Wiedergewinnung der Kontrolle über den Drogenkonsum zu unterstützen.

Das Innovationspotenzial unseres Forschungsansatzes basiert auf drei aktuellen Entwicklungen in der Suchtforschung:

(I) Ein dimensionaler Ansatz, der Suchterkrankungen als Kontinuum versteht, das vom zielgerichteten und genussorientierten Drogenkonsum bis hin zum habituellen und schließlich zwanghaften Konsum reicht.

(II) Die zunehmend verfeinerte Entwicklung komputationaler Verhaltensmodelle, die den Verlust und die Wiedergewinnung der Kontrolle über den Drogenkonsum modellieren und vorhersagen können und so entscheidende komputationale Schritte und ihre neurobiologischen Korrelate bezüglich belohnungsabhängigen Lernens, der Stressempfindlichkeit und kognitiven Kontrolle erklären kann.

(III) Technologische Fortschritte in sogenannten Mobile-Health-Tools inklusive des Ecological Momentary Assessments, der Geolokalisation und mobiler Sensoren, die Verhaltensbeobachtungen, kognitive und emotionale Zustände, Stressempfindlichkeit und die Exposition gegenüber verschiedenen Umweltfaktoren in der Lebenswelt direkt erfassen können.

Auf der Grundlage langandauernder Zusammenarbeit der Initiatoren dieser Initiative wollen wir diese neuen Möglichkeiten in dem Transregio CRC/TRR 265 (Heinz et al., 2020) verwirklichen, um in einer 12-Jahres Perspektive 1) die biologischen und Umweltfaktoren zu identifizieren, die den Krankheitsverlauf beeinflussen, 2) molekulare, neurokomputationale und neurokognitive Mechanismen identifizieren, die den Verlust und die Wiedergewinnung der Kontrolle über den Suchtmittelkonsum in der Lebenswelt steuern und 3) individuell angepasste Interventionen entwickeln, welche auf diese Mechanismen zielen.

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Presentation of scientific institutions

Anika Dirks and Daniela Christiane Dieterich*

Research on healthy aging mechanisms in Magdeburg by new DFG research training group 2413 SynAGE

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By 2050 the global number of elderly dependent people will supposedly have reached 277 million (Prince et al., 2013) with approximately every fourth Western citizen being over the age of 65 (Cracknell, 2010). This demographic change poses an increasing burden with incurred economic, infrastructural, and last but not least large social expenses – especially if it comes down to decline of cognitive function in the elderly. Thus, there is an urgent need for a better understanding of such cognitive decline in order to develop strategies for maintaining and improving mental health and quality of life in the elderly population. The DFG funded research program RTG 2413 SynAGE (http://www.synage.de) deals with the idea that cognitive decline in normal aging results from subtle synaptic alterations that impart an imbalance between stability and plastic properties of spine synapses and that is qualitatively different from neurodegeneration. This will further involve changes in the properties and functionality of the extracellular matrix, communication and interaction with glia cells and cells of the immune system, neuromodulation, and ultimately otherwise compensatory mechanisms. In total 13 PhD and 26 MD students aim to understand these processes of synaptic aging from a molecular, cellular, as well as behavioral angle by jointly addressing four transversal, intimately linked themes: (i) altered synaptic proteostasis and (ii) altered functionality of the multipartite synapse accompanied by (iii) dysfunctions of the immune system and (iv) changes in neuromodulation as core and key components for cognitive decline. The RTG 2413 puts special emphasis to include medical students into the program to foster the understanding of the importance of translational research and at the same time to improve the scientific training of MD students from an early stage onwards and allow them to develop into clinician scientists.

Current research in the field of aging focuses mainly on dementia and associated neurodegenerative diseases. Much less investigated are the consequences of normal aging as such for synaptic, cellular, and neuronal network properties. Normal aging is associated with a decline in sensory, motor, and cognitive function, in particular working memory, cognitive flexibility and multi-tasking capacity, and although relatively mild as compared to dementia, this negatively impacts on health and life quality. In fact, there is now ample evidence that not only genetic factors contribute to the course of aging but also individual lifestyle habits such as rich diet, little to no exercise, stress, provoked development of the metabolic syndrome, vascular alterations, all of which negatively impact on cognitive function in the elderly as well. Early work in non-human primates implicated neuronal cell loss as cause for cognitive decline, but studies in the human brain yielded conflicting results. An increasing number of studies now instead suggest that alterations in synaptic proteostasis and resulting impairments in synaptic neurotransmission and neuronal excitability might account for cognitive deficits in the elderly (Morrison and Baxter, 2012). Furthermore, as the brain ages glial cells become more activated, neurotransmitter levels are changed, proteins as well as pigments accumulate intracellularly, and neurovascular changes become more and more evident. The changes are subtle in nature and do not lead to gross morphological and structural defects although synapse loss in the range of 10–30% has been reported in humans, rodents and non-human primates (Morrison and Baxter, 2012). Thus, similar to early stages of neurodegenerative diseases, synapse alterations rather than neuronal cell death may cause neuronal dysfunction in normal aging. At present, however, very little is known about the mechanisms underlying mild age-related synaptic dysfunction and the cellular and molecular events

***Corresponding author: Daniela Christiane Dieterich**, Institute for Pharmacology and Toxicology, Medical Faculty, Otto von Guericke University, House 20, Leipziger Str. 44, Magdeburg, 39120, Germany; Center for Behavioral Brain Sciences, Magdeburg, Germany, E-mail: daniela.dieterich@med.ovgu.de. https://orcid.org/0000-0002-9880-1214

Anika Dirks: Institute for Pharmacology and Toxicology, Medical Faculty, Otto von Guericke University, House 20, Leipziger Str. 44, Magdeburg, 39120, Germany; Center for Behavioral Brain Sciences, Magdeburg, Germany, E-mail: anika.dirks@med.ovgu.de

responsible for this decline. Even fundamental questions concerning the change of cellular properties of neurons during normal aging have not been addressed systematically yet. This lack of knowledge greatly hampers every attempt to understand whether identified pathological hallmarks represent processes directly involved in neuropathology or protective/compensatory mechanisms that increase as the pathology progresses.

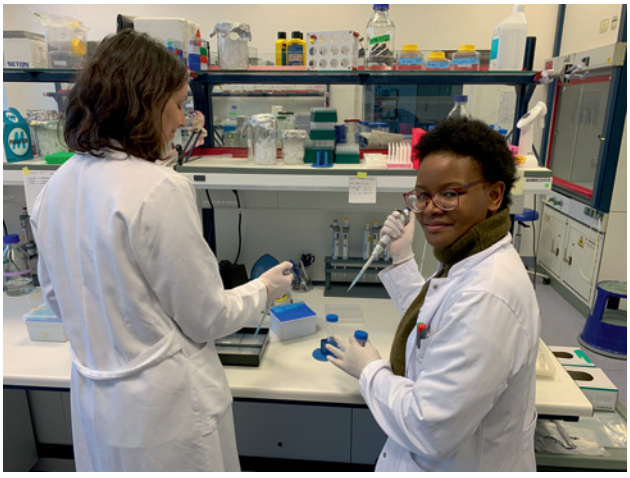

Fig. 1: Daily life in the lab. PhD student Danai and her local Postdoc mentor Anke. Photo: A. Dirks/SynAGE.

To approach this multifaceted field of research, international, interdisciplinary, highly motivated young scientists with excellent qualification in their respective studies were recruited. After preselection of the applicants based on their submitted documents, a two-day symposium at which the candidates presented their previous work was organized. Here they got the opportunity to interact with the faculty and their fellow doctoral students. Right after, MD students were attracted by a recruitment night to get in contact with SynAGE PIs and PhD students. All recruited students are given the opportunity to work with cutting-edge technologies and emergent techniques. Moreover, SynAGE promotes international visibility of their students at an early stage of their career and gives chances for intensive networking to promote career track development in- and outside of academia. Enhancing the career perspectives and employability of researchers and sustained contribution to their skills development is a core principle of that structured and individualized supervision and training PhD and MD program. To identify individual needs and, hence, implement measures accordingly, the students assess themselves with the help of an 'Individual Development Plan', provided by the local CBBS Graduate Program (CBBS GP, http://gp.cbbs.eu). Both, the neurospecific CBBS GP and the Otto von Guericke Graduate Academy, ensure high quality standards for education of graduate students serving as central service unit for doctoral students and postdocs. Another pillar that characterizes SynAGE is the strong involvement of postdocs striving to build up their own research from each lab in the experimental training program as well as in the lectures, seminars, workshops, retreats, summer/winter schools, and symposia. The organization of a Satellite Symposium on the occasion of the traditional international Learning and Memory Meeting in March 2020 in Magdeburg is representive for this. The postdoc network that is organized behind the RTG 2413 with the structural help of the cbbs graduate program is providing excellent cosupervision and at the same time career support through participation in general training courses as well as experimental courses focusing on individual needs of the postdoctoral researcher.

The RTG 2413 accomplished to combine basic experimental research on the molecular and cellular level with clinically relevant implications to understand mechanisms of cognitive decline and to develop therapeutic intervention strategies in healthy aging elderly. This endeavor relies on the joint collaborative, crossdisciplinary training of the RTG PhD students and medical doctorates providing them with the necessary skills for their own project and beyond. Through the tight collaborations of basic research groups working with animal models and even joined supervisions with clinics and human-based research, the students will gain invaluable insights into translational research. Moreover, SynAGE collaborates within the Center for Behavioral Brain Sciences CBBS, the local Health Campus: Immunology, Infectiology and Inflammation GC-I3, the Center Dynamic Systems and Biosystems Engineering CDS and the Research Training Group RTG 2408 "Maladaptive processes across physiological barriers in

Fig. 2: Public outreach activities of the RTG 2413. Long Night of Sciences 2019. Photo: A. Dirks/SynAGE

chronic diseases" as well as with international groups offering a competitive and instructive environment.

Besides conveying international conferences, summer and winter schools to Magdeburg, the SynAGE team of molecular/cellular and systems neurobiologists is involved in the organization of outreach activities like the annual Long Night of Sciences, the Company Relay and for the first time also bringing the Diversity Day to the Otto von Guericke University. Additionally, SynAGE students are proud of being part of the DFG campaign 2020 #DFG 2020 Für das Wissen entscheiden.

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Presentation of scientific institutions

Michael Denker, Alexandra Stein and Thomas Wachtler*

Better data – better science

NFDI Neuroscience: an initiative to promote efficient data management for neuroscience

https://doi.org/10.1515/nf-2020-0010

Information technology changes our lives with offers of novel tools and services at an unprecedented rate. Today, it is sometimes hard for us to understand how in the past we had managed to get by without such technologies at our fingertips – for example, a quick look at the street map on our mobile phone. Yet, many of these developments once were difficult to imagine. In science, the digital transformation has long begun and is bound to change our style of doing research in similarly radical ways. Through standardized interfaces, shared tools, and common vocabularies researchers will benefit from the advantages of professional research data management (RDM), such as comprehensive data descriptions, easy data exchange among collaborators, or the ability to navigate ever more complex and large data. In this way, modern expectations including reproducibility and practical reusability are met. However, the digital transformation requires researchers to adapt their data acquisition and analysis from custom "home-made" procedures towards standardized solutions co-designed by researchers and data scientists. As in the non-scientific world, the transformation requires expert guidance in choosing the right tools in a complex landscape of data management solutions.

To support the adoption of professional RDM, the DFG coordinates a funding program called the *Nationale Forschungsdateninfrastruktur* (NFDI, http://dfg.de/nfdi). In this context, the consortium NFDI Neuroscience formed in 2018 as an open community network. The aim of NFDI Neuroscience is to act as a platform that brings together solutions for RDM challenges emerging at national and international levels, making these solutions available to researchers on a large scale. Thus, neuroscience data should be handled in accordance with the FAIR principles (https://doi.org/10.1038/sdata.2016.18) by using appropriate and interoperable solutions for data storage, data annotation, data integration, and data processing. NFDI Neuroscience pursues a concept where the consortium acts as a direct point of contact for researchers regarding RDM issues. It will bring service providers and users together and push forward new developments based on needs identified by the neuroscientific community. As such, NFDI Neuroscience will build up a competence network interwoven with the neuroscientific community. The initiative is supported by the NWG and the Bernstein Network for Computational Neuroscience.

NFDI Neuroscience has held a series of community workshops (see also Ritzau-Jost & Seidenbecher (2019) Neuroforum 25(4):279–280) focusing on various groups involved in the process: individual researchers, large research consortia (such as CRCs), and providers of RDM services and expertise. Across all groups, the necessity for a more coherent approach to tackle RDM was recognized. Specific implementations and guidelines were identified as potential areas of NFDI Neuroscience involvement. In addition, NFDI Neuroscience could facilitate discussions in the community on overarching concerns, such as measures for ensuring the quality of curated data records. In particular, since broad training in RDM topics was often requested, NFDI Neuroscience is developing teaching activities including an introductory training workshop "Research Data Management in Neuroscience" (see https://nfdi-neuro.de for details).

Just like services such as online street maps once were science fiction, fully automated, standardized services for data acquisition and analysis may still seem like fantasy, given the daunting complexity and heterogeneity of the data. Nevertheless, more and more viable solutions appear that often inspire specifically the young generation of scientists to finally tackle the RDM challenges. After a recent RDM workshop for the Bernstein Network's SMART*START* program, one of the students confirms: "In the few months since the workshop I

***Corresponding author: Thomas Wachtler**, Ludwig-Maximilians-Universität München, Department of Biology II, Martinsried, Germany, E-mail: info@nfdi-neuro.de

Michael Denker: Institute of Neuroscience and Medicine (INM-6) and Institute for Advanced Simulation (IAS-6) and JARA-Institute Brain Structure-Function Relationships (INM-10), Jülich Research Centre, Jülich, Germany

Alexandra Stein: Bernstein Coordination Site, Bernstein Network Computational Neuroscience, Freiburg und Jülich, Germany

could already profit from the presented content, be it through minor things like references to data-analysis packages or more long-lasting aspects like taking the extra time to produce well-documented code and relevant meta-data."

Research data is at the core of scientific progress, and we as neuroscientists have a responsibility to maximize its impact now and in the future. Thus, research data needs to be handled in a rigorous, fully documented fashion to make it sustainable.

Rezension

Andreas Nieder: A brain for numbers. The biology of number instinct

Reviewed by **Hermann Wagner**, RWTH Aachen University, Institute of Biology II, Worringerweg 3, 52074, Aachen, Germany, E-mail: wagner@bio2.rwth-aachen.de

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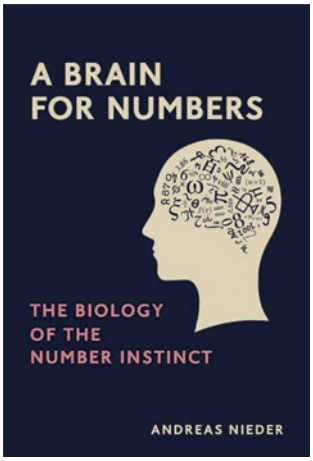

Figure: https://mitpress.mit.edu/books/brain-numbers

Have you ever thought that symbolic use of numbers is a cognitive achievement that discriminates us from animals as much or even more than our language? I certainly did not until I read *A Brain for numbers* by Andreas Nieder (chair of animal physiology at Tübingen University, Germany).

This book is special for two reasons. On the one hand, each of the 14 chapters starts from very basic knowledge and develops its contents step by step in a very clear language. On the other hand, it does not only include our human advanced number concepts but also the evolutionary basis of number competence and its adaptive value throughout the animal kingdom.

The book starts with the simple question "Where do numbers come from?". The answer is not easy, and the first two conceptualizing chapters spell out and define important basic terms and concepts. One of these important terms is "cardinality". Cardinality plays a central role in the understanding of number concepts in the animal kingdom. In Nieder's words, cardinality "refers to a quantitative assignment and thus applies to the empirical property 'number of elements in a set'." The next three chapters explain how numbers are rooted in the animal kingdom. Here, also methods are introduced on how to test numerical competence in behavior. The famous example of the horse "Clever Hans", who was erroneously thought to be able to count, reminds the reader on how difficult it may be to design, carry out and correctly interpret data obtained in animal studies. In the next part, the neuronal representation of numbers in the brain is introduced. An interim conclusion is – in the absence of symbols – animals and humans represent number in an approximate manner, consistent with the Weber quotient. In other words, numbers may be discriminated, if they are a certain percentage (the Weber quotient) apart: 3 may discriminated as well from 4 (Weber quotient: 0.33) as 30 from 40 (same Weber quotient). This holds for both behavior and the so-called 'number neurons' in the brain.

Humans, of course, have a higher numerical competence than animals, especially in that we are able to handle numbers in a symbolic way. Starting with part IV the books shifts to introducing more advanced numerical thinking. No animal is capable of representing numbers in a symbolic manner. Thus, advanced number competence discriminates us humans from animals as much as does our language of which also only rudimentary capacity exists in the animal kingdom. In the following chapters the book introduces, amongst others, brain areas dedicated to numerals, space and numbers in the brain, dissociated networks for calculation and language, the abstractness of number representation in the brain, the effect of developmental dyscalculia (a learning disability in math) on the life of the patients, and, finally, the special case of the number zero.

I very much enjoyed reading this book. The clear and plain language kept me going once I started to read. The different aspects of our number competence demonstrate an intriguing capacity rivaling that of language, but not as loved as the latter, especially if you are sitting in a mathematical exam. But this book is different. It invites you to use your language to understand what we can do with numbers. I recommend the book to everyone – from students via zoologists interested in animal behavior to cognitive scientists. Even the "educated layman" may profit from reading this book.

Andreas Nieder
A brain for numbers. The biology of the number instinct
The MIT Press, Cambridge Massachusetts (USA) 2019
392 pp, US$ 34.95
ISBN 978-0-262-04278-9

Nachruf

Jan Benda, Andreas Draguhn*, Frank Kirchhoff and Bernd Sutor

Dipl. Ing. univ. Hans Reiner Polder

(18. August 1957 – 19. Dezember 2019)

https://doi.org/10.1515/nf-2019-0037

Am 19. Dezember 2019 verstarb nach schwerer Krankheit Hans Reiner Polder, Gründer und Geschäftsführer der npi electronic GmbH. Er hat die elektrophysiologische Forschung in Deutschland und weit darüber hinaus mit seinen technischen Lösungen, seinem Rat und seinen wissenschaftlichen Zusammenarbeiten über fast vier Jahrzehnte begleitet und bereichert. Viele von uns verlieren mit Reiner einen Freund und Kollegen, der unser Denken und unsere Arbeitsweise als Neurophysiologen stark geprägt hat.

Bereits während des Studiums der Elektrotechnik an der TU München in den späten 1970er Jahren erwachte – durch einen Gastvortrag von Wolf Singer – Reiner Polders Interesse an der Neurophysiologie. Er begann daraufhin eine Tätigkeit als studentische Hilfskraft in der Abteilung von Hans-Dieter Lux am Max-Planck-Institut für Psychiatrie, wo er schließlich auch seine Diplomarbeit anfertigte. Sein Thema war der Aufbau eines sogenannten „single electrode voltage clamp amplifier“. Die Spannungsklemme („voltage clamp“) erlaubt, definierte Membranpotenziale einzustellen und aus der Höhe des jeweilig erforderlichen Korrekturstroms auf die Leitfähigkeit der Membran zu schließen. So lassen sich die biophysikalischen Mechanismen von Aktionspotenzialen, der synaptischen Übertragung und vieler weiterer Membranprozesse präzise charakterisieren.

Es ist sicher im Sinne Reiners, dass wir hier den theoretischen Hintergrund seiner Arbeit kurz beschreiben. Das Mitte des vorigen Jahrhunderts entwickelte „voltage clamp“-Verfahren wurde zu einer entscheidenden Grundlage der modernen zellulären Elektrophysiologie, blieb aber anfangs auf Messungen in großen und robusten Präparaten (insbesondere dem Riesenaxon des Tintenfischs) beschränkt. Nur hier ließen sich zwei getrennte Elektroden für Spannungsmessung und Strominjektion platzieren, um über einen Rückkopplungskreis Soll- und Ist-Potenzial abzugleichen. Für kleinere Zellen, insbesondere Neurone des Säugers, war es notwendig, Messungen mit einer einzelnen Elektrode zu realisieren. Dabei entsteht jedoch ein grundlegendes technisches Problem: aufgrund des geringen Durchmessers besteht an der Pipettenspitze ein nicht zu vernachlässigender elektrischer Widerstand, an dem ein Spannungsabfall erfolgt, sobald Strom fließt. Damit misst der Verstärker nicht mehr das korrekte Membranpotenzial der Zelle. Zur Beseitigung dieses bedeutenden Fehlers gibt es zwei praktikable Lösungen: entweder man korrigiert „im Voraus“ durch stromabhängige Anpassung des Sollpotenzials oder man entkoppelt die Spannungsmessung von der Strominjektion. Bei letzterer Variante erfolgt die Spannungsmessung in einer stromfreien Phase, in der kein Potentialabfall am Elektrodenwiderstand auftritt. Das entsprechende Verfahren wurde als ‚discontinuous single electrode voltage clamp‘ 1974 von Brennecke und Lindemann und wenig später von Wilson und Goldner sowie Finkel und Redman entwickelt und validiert. Dabei erfolgt die Strominjektion in Intervallen, zwischen denen jeweils

***Corresponding author: Andreas Draguhn**, Institute for Physiology and Pathophysiology, Heidelberg University, Heidelberg, Germany, E-mail: andreas.draguhn@physiologie.uni-heidelberg.de
Jan Benda: University of Tübingen, Neuroethology - Institute for Neurobiology, Tubingen, Baden-Württemberg, Germany
Frank Kirchhoff: University of Saarland, Molecular Physiology, CIPMM, Building 48, Homburg, Germany
Bernd Sutor: Institute of Physiology, Ludwig Maximilians University Munich, Munich, Bavaria, Germany

eine stromfreie (unverfälschte) Spannungsmessung liegt. Der gemessene Wert wird in einem ‚sample-and-hold' Verstärker gehalten, mit dem Sollwert verglichen und anschließend wieder ein Korrekturstrom injiziert. Die Frequenz der Zyklen aus Spannungsmessung und Strominjektion sollte hoch gegenüber der Geschwindigkeit der beobachteten Membranprozesse sein. Dazu ist es notwendig, die Kapazität der Elektrode soweit wie möglich zu kompensieren. Reiner Polder hat im Rahmen seiner Diplomarbeit mittels systemtheoretischer Modellbildung gezeigt, dass hierzu ein sogenannter Proportional-Integral-Regler besser geeignet ist als der sonst übliche Proportional-Differential-Regler. Die elektronische Umsetzung dieser theoretischen Überlegungen in einen funktionierenden Meßverstärker war eine außerordentliche, bravouröse Ingenieurleistung. Gleichzeitig eignete sich der Verstärker für verschiedene Meßverfahren (Voltage-Clamp, Current-Clamp oder Brückenmodus) und unterschiedliche Elektroden (hochohmige „scharfe" Mikroelektroden, niederohmige Patch-Clamp Pipetten).

Dieser Typ von Verstärker wurde zur Grundlage seiner weiteren Arbeit und seiner Firma. Dabei bot ihm das Lux'-sche Labor eine ideale Arbeitsumgebung, in der er eng mit ausgezeichneten jungen Elektrophysiologen zusammenarbeiten konnte. Die Nähe zu den wissenschaftlichen Anwendungen und zu den Wissenschaftlern selbst ist seither das prägende Charakteristikum von Reiner Polders Arbeitsweise geblieben. Er hat selbst nicht den Weg einer akademischen Laufbahn gewählt, sondern unmittelbar nach Abschluss der Diplomarbeit 1984 gemeinsam mit seiner Frau Hannelore in Tamm bei Stuttgart eine Firma gegründet, um maßgeschneiderte Lösungen für experimentell arbeitende Elektrophysiologen anzubieten: npi electronic. Der Name steht für „neurophysiology instruments", wird aber gerne mit der Reiner eigenen leisen Ironie als „nearly perfect instruments" übersetzt. Person und Firma blieben von den Ursprüngen in der physiologischen Forschung geprägt – dem Interesse an wissenschaftlichen Fragestellungen, dem intensiven Austausch mit den Wissenschaftlern und der Freude an der Vermittlung meßtechnischer Zusammenhänge. Diese Eigenschaften haben Reiner Polder zu einer zentralen Persönlichkeit der elektrophysiologischen „Szene" in Deutschland und weit darüber hinaus gemacht. Mit der Firma hat er, wie er einmal sagte, einen Lebenstraum verwirklicht – dass sie von seiner Frau Hannelore und seinem Sohn Bernd weitergeführt wird, war ihm in den letzten Jahren eine Freude und ein Trost.

Reiner Polder hat viele Wissenschaftler – gerade in frühen Karrierestadien – für die Besonderheiten und Fallstricke elektrophysiologischer Messungen sensibilisiert. Er war enorm belesen und zitierte mit erstaunlicher Detailkenntnis aus den verschiedensten Publikationen, samt einer Analyse der in den Abbildungen und Daten verborgenen messtechnischen Probleme. Ebenso kannte er für fast jede denkbare Anwendung Ansprechpartner, die bereits mit diesem oder einem ähnlichen Problem zu tun hatten. Reiner hatte den genuinen Wunsch, sein Wissen zu teilen und junge Wissenschaftler/-innen zu fördern. Viele heute etablierte Elektrophysiologen erinnern sich mit einem Schmunzeln daran, wie ihn seine Begeisterung gerade in den Anfangsjahren manchmal über das Ziel hinausschießen ließ – wer ratlos vor den vielen Knöpfen und Schaltern der frühen Verstärker saß, war mit den vorlesungsreifen elektrotechnischen und messtheoretischen Ausführungen von Reiner leicht überfordert. Aber eben nicht alleingelassen: Reiner begegnete allen Ansprechpartnern mit großem Ernst und machte ihre aktuellen methodischen Probleme zu seinen eigenen. Hier mag die eigene Erfahrung prägend gewesen sein – die Mutter war Lehrerin, der Vater Elektrotechniker und später ebenfalls als Lehrer tätig. So wurde Reiner früh an das Basteln und Bauen von Geräten herangeführt, aber auch an die Kultur der Wissensvermittlung. Nicht anders hat er es später mit seinen Söhnen gemacht.

Das Portfolio der Firma hat sich über die Jahrzehnte parallel zu den Erfordernissen einer modernen Neurophysiologie kontinuierlich erweitert und umfasst inzwischen verschiedenste Messverstärker, Stimulatoren, spezielle Geräte für die schnelle Applikation von Wirkstoffen, für optogenetische Verfahren und -zunehmend- für elektrophysiologische Messungen *in vivo*. Oft wurden Geräte in gemeinsamen Projekten mit den beteiligten Wissenschaftlern entwickelt oder optimiert, so dass Reiner Polder Autor mehrerer Publikationen war. Für viele spezielle Probleme hat er mit seinen Mitarbeitern eine technische Lösung entwickelt. Besonders stolz war er auf einen 64-Kanal-Verstärker, der im südamerikanischen Dschungel erfolgreich zum Einsatz kam, um das Kommunikationsverhalten von schwach elektrischen Fischen zu studieren. Er war aktiv an mehreren Forschungsverbünden beteiligt, z.B. dem Bernstein Center for Computational Neuroscience in München, in dem er seinen Verstärker für Dynamic Clamp Experimente weiterentwickelt hat. Mit Freude ergriff Reiner die Möglichkeit als „Beneficiary"-Partner in dem Horizon 2020 Netzwerk EU-GliaPhD mitzuarbeiten. Außerhalb des normalen Tagesgeschäftes konnte hier die Digitalisierung der bewährten ELC-Verstärker angegangen werden. Gemeinsam mit dem jungen indischen Doktoranden Chaitanya Jha brachte er nach wenigen Monaten das Projekt auf einen erfolgreichen Weg. Leider wird er den abschließenden Erfolg dieser Arbeiten nicht mehr miterleben.

Reiner war immer hoch motiviert, sein Wissen weiterzugeben, dabei eine methodenkritische, handwerklich saubere elektrophysiologische Kultur zu pflegen und vor

Abbildung 1: Hans Reiner Polder und sein Doktorand Chaitanya Jha diskutieren den ersten Prototypen zum neuen UniClamp-Verstärker, dem digitalen Pendant zum analogen ELX-03XS Verstärker, einem der „Arbeitspferde“ von npi electronic.

allem junge Wissenschaftler zu fördern. Die Tagungen der Fachgesellschaften (Neurowissenschaftliche Gesellschaft, Deutsche Physiologische Gesellschaft, FENS u.a.) hat er nicht nur als Aussteller unterstützt, sondern dort regelmäßig Symposien oder Workshops angeboten, in denen sowohl junge wie bereits profilierte Wissenschaftler ihre innovativen Methoden vorstellten. Über viele Jahre hinweg hat er Studierenden aus aller Welt die Grundlagen elektrophysiologischer Messverfahren und die ideale Verwendung der verschiedenen Messverstärker erklärt, sei es im „eigenen“ Horizon 2020 Projekt EU-GLiaPhD, auf dem bekannten Workshop ‚Microelectrode Techniques for Cell Physiology' in Plymouth oder bei den Sommerschulen an der Universität Sains Malaysia in Kota Bharu. Er hat diese Kurse mit nie nachlassender Konzentration, Engagement und Enthusiasmus begleitet.

Wer Reiner kennenlernte, kam schnell in Gespräche über die Elektrophysiologie hinaus. Seine reichen Geschichtskenntnisse und die anhaltende Verbindung zu seiner ursprünglichen Heimat in Siebenbürgen machten ihn zu einem aufmerksamen Beobachter des Zeitgeschehens, immer verbunden mit Geschichten über Menschen, die ihm nahestanden. Wir werden seine weltoffene, genuin menschenfreundliche Haltung, seinen Humor, seine Anekdoten, seine Fachkenntnisse und die gemeinsame Arbeit vermissen. Dankbar sind wir auch für sein großzügiges Wirken als Sponsor vieler kleiner und größerer Konferenzen. In seinen wissenschaftlichen Partnern und Freunden, seinen Mitarbeitern und vor allem seiner Familie wird viel von ihm weiterleben!

Prof. Jan Benda, Tübingen
Prof. Andreas Draguhn, Heidelberg
Prof. Frank Kirchhoff, Homburg
Prof. Bernd Sutor, München

Nachrichten aus der Gesellschaft

https://doi.org/10.1515/nf-2020-0012

Vorstandswahl für die Amtsperiode 2021 – 2023

Liebe NWG-Mitglieder,

laut Satzung ist im Januar 2021 die Wahl des NWG-Vorstandes für die Amtsperiode 2021 - 2023, die mit dem Ende der Göttinger Tagung am 27. März 2021 beginnen wird, fällig.

Alle Mitglieder sind aufgefordert, Vorschläge für die Positionen der Sektionssprecher*, des Schatzmeisters, des Generalsekretärs und des Vizepräsidenten bei der Geschäftsstelle einzureichen.

Das Amt des Präsidenten steht nicht zur Wahl, laut Satzung wird der Vizepräsident der vorangegangenen Amtsperiode automatisch Präsident der nächsten Amtsperiode.

Erstmals zur Wahl steht das Amt des Sektionssprechers für die neu gegründete Sektion „Junge NWG“ (jNWG). Die jNWG ist eine Gruppe junger Neurowissenschaftler, die das Interesse an einer stärkeren Repräsentation des wissenschaftlichen Nachwuchses innerhalb der NWG verbindet.

Es können nur Vorschläge berücksichtigt werden, die die komplette postalische Adresse, die Telefonnummer und die Email-Adresse des Kandidaten enthalten.

Der Stichtag für die Einsendung von Vorschlägen ist der 1. September 2020.

Bitte schicken Sie diese per E-Mail an:
Meino Alexandra Gibson
E-Mail: gibson@mdc-berlin.de

Die Vorschläge werden von der Wahlkommission der NWG für die endgültige Wahlliste gesichtet und bei Bedarf ergänzt.

*Für alle Personen- und Amtsbezeichnungen gilt selbstverständlich m/w/d.

Fortbildungsprogramme der Neurowissenschaftlichen Gesellschaft 2020/2021

Von Mitgliedern für Mitglieder

Es ist wieder Zeit, Vorschläge für die Methodenkurse und auch die Lehrerfortbildungen der NWG zu sammeln. Diese sind seit Langem eine feste Einrichtung und erfreuen sich großer Beliebtheit. Wir möchten die Mitglieder der NWG auffordern, derartige Kurse, für die die NWG eine finanzielle Unterstützung bereitstellt, im kommenden Jahr anzubieten.

Für die Methodenkurse stellt die NWG 125 € pro teilnehmendem NWG-Mitglied und 62,50 € pro teilnehmendem Nicht-Mitglied bis zu einer maximalen Höhe von 2.500 € pro Kurs zur Verfügung. Die Lehrerfortbildungsveranstaltungen werden mit einem Betrag in Höhe von maximal 250 € pro Veranstaltung unterstützt.

Beide Programme werden mit einem gedruckten Plakat bzw. gedruckten Flyern im Spätsommer des Vorjahres angekündigt. Das Lehrerfortbildungsprogramm erstreckt sich über ein Schuljahr, also von September 2020 bis Juli 2021, das Methodenkursprogramm über das Kalenderjahr 2021.

Einsendeschluss für Angebote ist Mittwoch, der 1. Juli 2020

Details können bei der Geschäftsstelle der NWG erfragt werden (E-Mail: gibson@mdc-berlin.de).

Weitere Informationen:
Methodenkurse 2020: https://nwg-info.de/aktivitaeten/kurse_workshops/2020
Lehrerfortbildungen 2020/2021: https://nwg-info.de/de/aktivitaeten/lehrerfortbildung/2020

NEU auf dasGehirn.info

dasGehirn.info hat sich zum Ziel gesetzt, das Gehirn, seine Funktionen und seine Bedeutung für unser Fühlen, Denken und Handeln darzustellen. Die Beiträge sind von Wissenschaftsjournalisten verfasst – umfassend, verständlich, attraktiv und anschaulich in Wort, Bild und Ton. Und mit der Garantie auf fachliche Richtigkeit, denn alle Inhalte werden von Wissenschaftlern, häufig NWG-Mitgliedern, begutachtet.

Hier werden Sie in Zukunft immer Hinweise auf die neuesten Beiträge finden. Aktuell sind das die folgenden Artikel zum Themenschwerpunkt **Tiergedanken**:

Schlau, schlauer, Schimpanse?

Der Ideenreichtum von Schimpansen – insbesondere, wenn es um Nahrungsbeschaffung geht – ist legendär. Doch ob sie anderen Affen grundsätzlich überlegen sind, ist fraglich.

Raben sind politische Talente

Vögel wurden lange für wenig intelligent gehalten. Dass sich das geändert hat, liegt nicht zuletzt an der Forschung des „Rabenvaters“ Thomas Bugnyar.

Möchten Sie eine Pressemeldung an “dasGehirn.info” weitergeben, wenden Sie sich bitte an Arvid Leyh (a.leyh@dasgehirn.info).

Vom Wolf zum Menschenversteher

Mehr als 30.000 Jahre lang haben Menschen und Hunde sich gemeinsam entwickelt. Dabei haben Hunde besser als jedes andere Haustier gelernt, uns zu durchschauen. Und zu manipulieren – die Erziehung ist hier wohl gegenseitig.

Im Monat April stand das Thema „**Das soziale Gehirn**“ im Mittelpunkt.

Außerdem erscheinen regelmäßig **Pressemeldungen** aus den Instituten – kurz und verständlich:

- Die **Netzhaut** sorgt nicht nur für scharfes, sondern auch für stabiles Sehen | Hertie-Institut für klinische Hirnforschung Tübingen (27.4.2020)
- Spezialisierte Nervenzellen machen **Lust auf fettreiche Nahrung** | Max Planck Institut für Stoffwechselstörungen Köln (25.4.2020)
- Wie die Erwartungshaltung das **Lernen** beeinflusst | Ruhr-Universität Bochum – (18.4.2020)

Neueintritte

Folgende Kolleginnen und Kollegen dürfen wir als Mitglieder der Neurowissenschaftlichen Gesellschaft begrüßen:

Enes Yagiz Akdas (Erlangen)
Muhammad Imran Arshaad (Bonn)
Elisabeta Balla (Aachen)
Thomas Johannes Baumgarten, Dr. (Düsseldorf)
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Rui Wang (Hamburg)
Jens Peter Weber, Dr. (Bochum)
Anna Wunderlich (Göttingen)
Hamed Yeganegi (Freising)
Stephanie Tanja Zeuch (Heidelberg)

Der Mitgliedsstand zum 23. März 2020 beträgt 2.192 Mitglieder.

Ausblick

Shira Meir-Drexler et al.
Stress modulation of fear and extinction in psychopathology and treatment

Martin Hadamitzky
How Learning Shapes Immunity

Sigrid Elsenbruch et al.
From Gut Feelings to Memories of Visceral Pain

Onur Guentuerkuen et al.
Beyond the Classic Extinction Network: A Wider, Comparative View

Armin Zlomuzica et al.
Clinical implications of fear extinction in anxiety disorders

Metin Uengoer et al.
Principles of extinction learning of non-aversive experience

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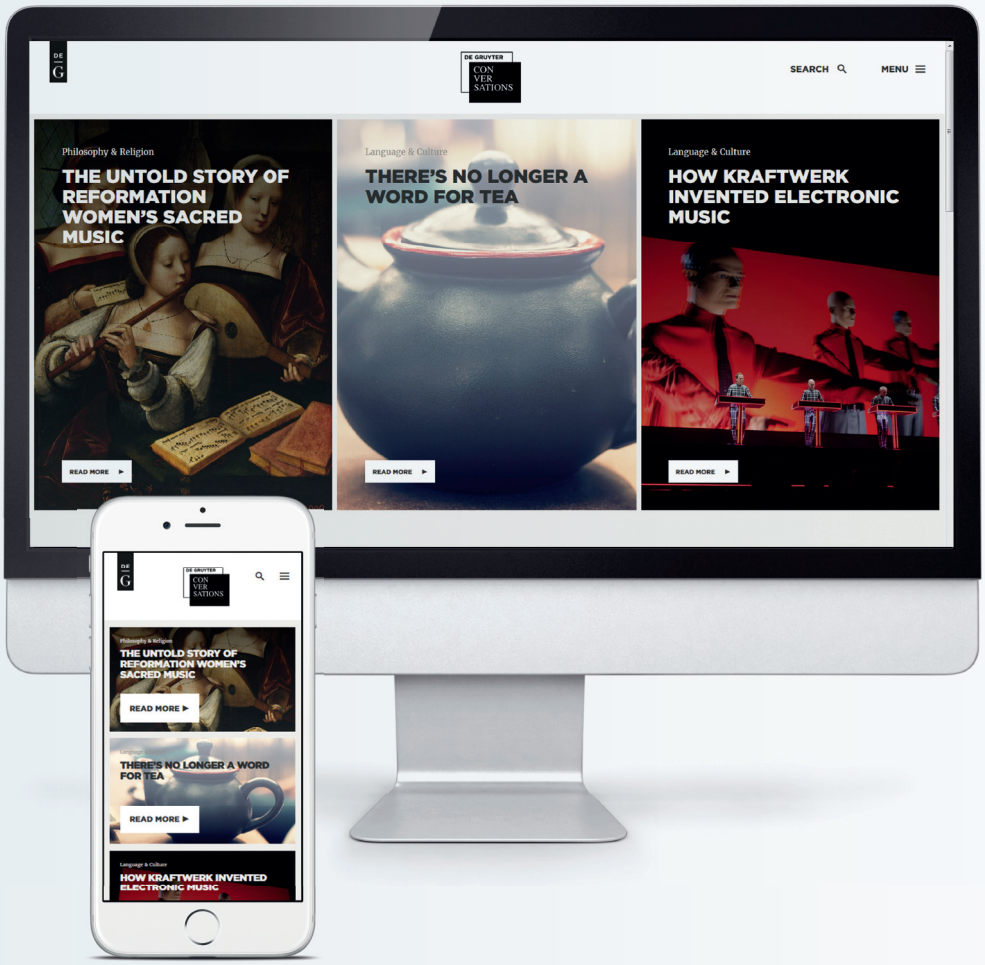

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NEUROWISSENSCHAFTLICHE GESELLSCHAFT
GERMAN NEUROSCIENCE SOCIETY

Neurowissenschaftliche Gesellschaft e.V. (NWG)

- Beitrittserklärung -

Hiermit erkläre ich meinen Beitritt zur Neurowissenschaftlichen Gesellschaft e.V. (NWG).

Eintrag in das Mitgliederverzeichnis:

Name

Vorname

Titel

Dienstadresse

Universität/Institut/Firma

Straße

PLZ/Ort Land

Telefon/Email

Privatadresse

Straße

PLZ/Ort

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Rechte und Pflichten der Mitgliedschaft siehe Satzung (nwg-info.de/de/ueber_uns/satzung). Mit meiner Unterschrift bestätige ich, dass ich die Satzung sowie die Datenschutzrichtlinie (nwg-info.de/de/datenschutz) zur Kenntnis genommen habe und diese anerkenne.

Datum/Unterschrift

Ich unterstütze den Antrag auf Beitritt zur NWG e.V.

Datum/Unterschrift des Mitglieds

Datum/Unterschrift des Mitglieds

Bitte senden Sie Ihren Antrag an die Geschäftsstelle der NWG:

Stefanie Korthals
Neurowissenschaftliche Gesellschaft e.V.
MDC
Robert-Rössle-Str. 10
13092 Berlin

Email: korthals@mdc-berlin.de
Tel.: +49 30 9406 3127

Ich optiere für folgende 2 Sektionen:

- ☐ Computational Neuroscience
- ☐ Entwicklungsneurobiologie/Neurogenetik
- ☐ junge NWG (jNWG)
- ☐ Klinische Neurowisschenschaften
- ☐ Kognitive Neurowissenschaften
- ☐ Molekulare Neurobiologie
- ☐ Neuropharmakologie und -toxikologie
- ☐ Systemneurobiologie
- ☐ Verhaltensneurowissenschaften
- ☐ Zelluläre Neurobiologie

Ich bin Student ☐ ja ☐ nein
(Bescheinigung anbei)

Ich bin ☐ weiblich ☐ männlich ☐ divers

☐ **Ich erkläre mich einverstanden**, dass meine Daten zum Zwecke wissenschaftlicher Informationsvermittlung (z.B. **FENS-Mitgliedschaft**) weitergegeben werden.
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Unterschrift: _____

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